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Akhlaq A. Farooqui
Department of Molecular
and Cellular Biochemistry
The Ohio State University
Columbus, OH 43221
USA
farooqui.1@osu.edu

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In the memory of my beloved uncle "late Mohd Ateeq Saheb" and grandmother "late Masoodi Begum," who brought me up, gave me their unyielding support, and taught me the difference between right and wrong.

– Akhlaq A. Farooqui

Preface

In neural membrane glycerophospholipids, essential polyunsaturated fatty acids, namely arachidonic acid (ARA) and docosahexaenoic acid (DHA), are exclusively located at the *sn*-2 position of glycerol moiety. Because humans do not possess desaturases that insert either the *n*-3 or the *n*-6 double bonds, these fatty acids are derived from diet. DHA can be obtained from fish or from the precursor α-linolenic acid (ALA), which is found in nuts. The common precursor for ARA is dietary linoleic acid (LA) from plant sources. The high intake of food enriched in vegetable oils elevates levels of ARA-derived eicosanoids and upregulates the expression of proinflammatory cytokines. ARA-derived eicosanoids have prothrombotic, proaggregatory, and proinflammatory properties. In contrast, a diet enriched in eicosapentaenoic acid (EPA) and DHA (fish and fish oil) generates docosanoids, which not only downregulate proinflammatory cytokines but also have antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory effects. Thus, levels of eicosanoids and docosanoids in neural and non-neural tissues are partly regulated by diet. The present Western diet is deficient in DHA, but has high amounts of ARA. The deficiency of *n*–3 fatty acids in diet results in a number of abnormalities in animals and human infants such as impaired vision, abnormal electroretinogram, and several behavioral abnormalities. In addition, inclusion of *n*–3 fatty acids in diet not only prevents cardiovascular disease but also protects from stroke, epilepsy, and other neurological and neurodegenerative diseases such as Alzheimer disease, Parkinson disease, and peroxisomal diseases.

There have been remarkable developments on DHA and ARA metabolism in brain in the past 20 years. The main objective of this book is to present readers with cutting-edge and comprehensive information on metabolism and roles of neural membrane DHA in a manner that is useful not only to students and teachers but also to researchers, dietitians, nutritionists, and physicians. This monograph has 11 chapters. Chapters 1 and 2 describe the importance of fish oil in human diet along with transport and importance of DHA in brain. Chapters 3 and 4 describe cutting-edge information on the release and catabolism of DHA in brain along with neurochemical effects of its lipid mediators. Chapter 5 describes roles of DHA in brain. Chapter 6 is devoted to alterations in DHA with aging and consequences of DHA deficiency in brain. Chapters 7

and 8 describe the status and therapeutic importance of DHA and EPA in acute metabolic trauma (stroke), neurotraumatic disorders (spinal cord and head injuries), and neurodegenerative diseases (Alzheimer disease, Parkinson disease, Huntington disease). Chapter 9 describes the status and therapeutic importance of DHA and EPA in neuropsychiatric disorders (schizophrenia, depression, bipolar disorders, and attention-deficit/hyperactivity disorder). Chapter 10 provides information on the importance of DHA and EPA in other neural (peroxisomal) and some non-neural (rheumatoid arthritis, cystic fibrosis, chronic obstructive pulmonary disease, dermatological conditions, and gastrointestinal disorders) diseases. Finally, Chapter 11 provides readers and researchers with perspective that will be important for future research work on DHA and EPA in brain tissue.

This monograph can be used as supplemental text for a range of neuroscience and nutrition courses. Neurologists and nutritionists will find this book useful for understanding molecular aspects of DHA and EPA metabolism in neurological disorders. To the best of my knowledge, no one has written a monograph on the role of DHA and EPA and their lipid mediators (docosanoids) in brain tissue, and this monograph is the first to provide a comprehensive description of docosanoids and their interactions with ARA-derived eicosanoids in normal brain and in brain tissue from patients with neurological disorders. This monograph not only provides background and refresher information on DHA metabolism in brain for readers not working in the field of fatty acid metabolism but also presents new knowledge on DHA-derived lipid mediators and therefore broadens our understanding of DHA function in health and neurological disorders. The monograph offers a thorough and unique overview of the neurobiology of *n*–3 and *n*–6 fatty acids and their association with neurological disorders. It is anticipated that nutritionists and clinicians may gain insight into problems associated with nutrition in the 21st century and resolve difficulties experienced in their research on fatty acid metabolism and DHA- and ARA-derived lipid mediators in their laboratories.

The choice of topics presented in this monograph is personal. It is based not only on my interest in DHA and ARA metabolism in neurological disorders but also in areas where major progress has been made. I have tried to ensure uniformity in mode of presentation as well as a logical progression of subject from one topic to another and have provided extensive bibliography. For the sake of simplicity and uniformity, a large number of figures and line diagrams of signal transduction pathways are also included. I hope that my attempt to integrate and consolidate the knowledge of DHA metabolism and signal transduction processes in normal and diseased brain will provide the basis of more dramatic advances and developments on the determination, characterization, and roles of DHA- and ARA-derived lipid mediators in neurological disorders.

Akhlaq A. Farooqui
Columbus, Ohio

Acknowledgments

I thank my wife, Tahira, for critical reading of this monograph, offering valuable advice, and evaluation of subject matter. Without her help and participation, this monograph neither could nor would have been completed. I also thank my son, Siraj, for drawing chemical structures of lipid mediators and signal transduction pathways associated with arachidonic acid and docosahexaenoic acid metabolism. I would also like to express my gratitude to Ann H. Avouris of Springer, New York, for her able and professional manuscript handling.

Akhlaq A. Farooqui

Contents

Abbreviations

Phosphatidylcholine	PtdCho
Phosphatidylethanolamine	PtdEtn
Choline plasmalogen	PlsCho
Ethanolamine plasmalogen	PlsEtn
Phosphatidylinositol	PtdIns
Phoshatidylinositol 4-phosphate	PtdIns4*P*
Phosphatidylinositol 4,5-bisphosphate	PtdIns(4,5)P_2
Inositol-1,4,5-trisphosphate	Ins-1,4,5-P_3
Phosphatidic acid	PtdH
Phosphatidylserine	PtdSer
Reactive oxygen species	ROS
Nuclear factor-kappaB	NF-κB
Arachidonic acid	ARA
Docosahexaenoic acid	DHA
Eicosapentaenoic acid	EPA
Linoleic acid	LA
α-Linolenic acid	ALA
Phospholipase A_2	PLA_2
Phospholipase C	PLC
Phospholipase D	PLD
Cyclooxygenase	COX
Lipoxygenase	LOX
Epoxygenase	EPOX
Protein kinase C	PKC
Protein kinase A	PKA
Prostaglandin	PG
Leukotriene	LT
Lipoxin	LX
Resolvin	Rv
Neuroprotectin D	NPD

About the Author

Akhlaq A. Farooqui is a leader in the field of brain phospholipases A_2, bioactive ether lipid metabolism, polyunsaturated fatty acid metabolism, glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators, and glutamate-induced neurotoxicity. Akhlaq A. Farooqui has discovered the stimulation of plasmalogen-selective phospholipase A_2 (PlsEtn-PLA_2) and diacyl- and monoacylglycerol lipases in brains from patients with Alzheimer disease. Stimulation of PlsEtn-PLA_2 produces plasmalogen deficiency and increases levels of eicosanoids that may be related to the loss of synapses in brains of patients with Alzheimer disease. Akhlaq A. Farooqui has published cutting edge research on the generation and identification of glycerophospholipid-, sphingolipid-, and cholesterol-derived lipid mediators in kainic acid mediated neurotoxicity by lipidomics. Akhlaq A. Farooqui has authored four monographs: *Glycerophospholipids in Brain: Phospholipase A_2 in Neurological Disorders* (2007); *Neurochemical Aspects of Excitotoxicity* (2008); *Metabolism and Functions of Bioactive Ether Lipids in Brain* (2008); and *Hot Topics in Neural Membrane Lipidology* (2009). All monographs are published by Springer, New York.

Chapter 1
Fish Oil and Importance of Its Ingredients in Human Diet

1.1 Introduction

Interest in the potential health benefits of fish oil increased immensely after epidemiological studies indicated a remarkably low incidence of death from ischemic heart disease in Greenland Eskimos, despite their consumption of a high-fat and cholesterol-enriched diet (Bang et al., 1976). Low incidences of ischemic heart disease have also been reported in coastal-dwelling Turkish and Japanese populations consuming fish and fish constituent-enriched diet. Soon it became apparent that fatty fish and marine oils, which were major components of the Eskimo, coastal Turkish, and Japanese diets, were responsible for the low incidence of ischemic heart disease (Kagawa et al., 1982). These studies are supported by hard data from several prevential studies such as GISSI Prevenzione, JELIS, DART, and RCT Trials, which indicated the usefulness of fish oil for the treatment of heart disease in patients (GISSI-Prevenzione investigators, 1999; Yokoyama et al., 2007; Burr et al., 1989; Nilsen et al., 2001; Bucher et al., 2002; Studer et al., 2005).

Fatty fish and marine oils are enriched in *n*–3 or ω–3 family of unsaturated fatty acids. This family of essential fatty acids includes α-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) (Fig. 1.1). They are not synthesized by humans and therefore must be obtained from dietary sources or derived from the 18-carbon precursor, ALA, through a series of enzymic reactions involving chain elongation, desaturation, and β-oxidation in liver (Voss et al., 1991). Human liver also synthesizes arachidonic acid (ARA) from linoleic acid (LA), another 18-carbon fatty acid that belongs to *n*–6 family of essential fatty acids (Schmidt, 1997).

Both *n*–3 and *n*–6 fatty acids have beneficial effects on human health. Neurons lack the ability to synthesize DHA and ARA from their precursors ALA and LA, respectively. Thus, DHA and ARA are obtained either directly from the diet or synthesized from ALA and LA in liver and transported to the brain through plasma lipoproteins (Scott and Bazan, 1989). As stated above, fish oil derived from fatty fishes (mackerel, salmon, herring, anchovies, albacore tuna, and sardines) is enriched in *n*–3 fatty acids (Fig. 1.2), whereas

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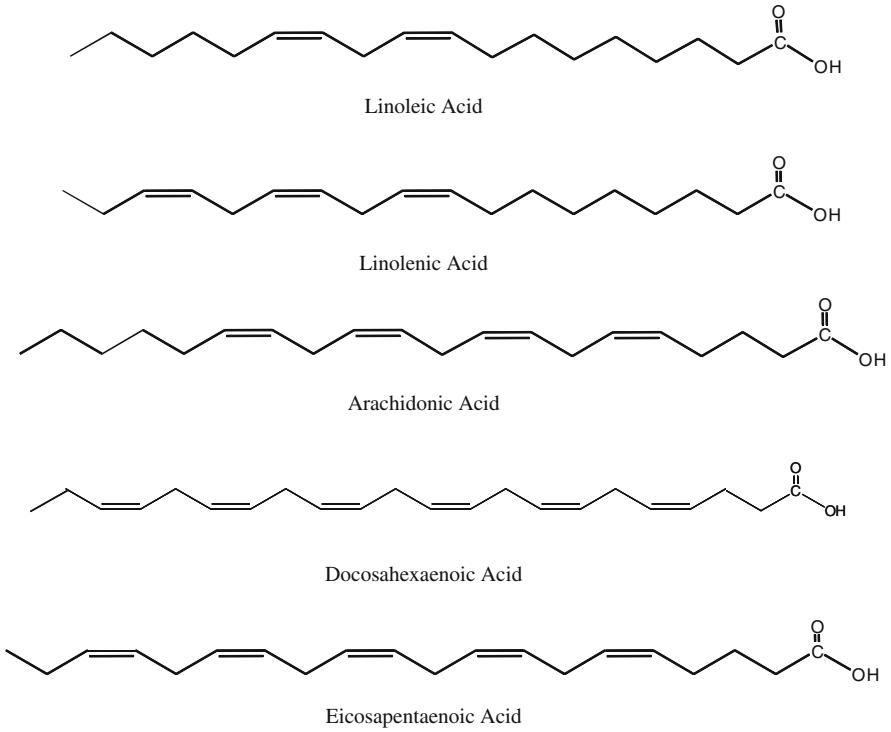

Fig. 1.1 Chemical structures of polyunsaturated fatty acids in brain linoleic acid (18:2*n*–6); α-linolenic acid (18:3*n*–3); arachidonic acid (20:4*n*−6); docosahexaenoic acid (22:6*n*−3); and eicosapentaenoic acid (20:5*n*−3)

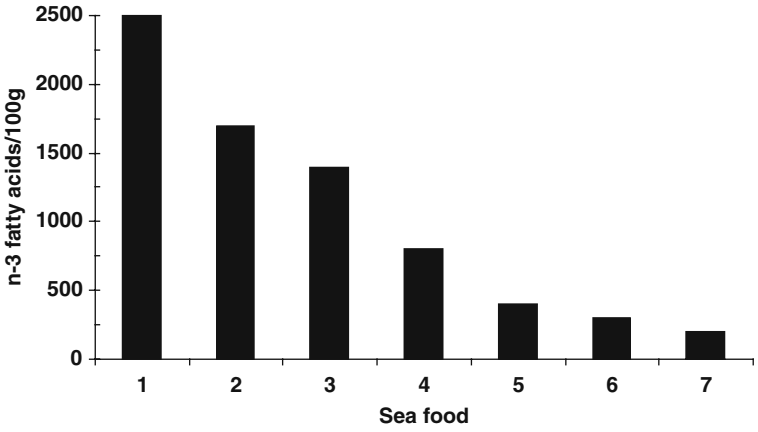

Fig. 1.2 *n*−3 Fatty acid content of various fishes. Mackerel (1); Anchovy (2); Coho salmon (3); Rainbow trout (4); Pacific halibut (5); Shrimp (6); Lobster (7). Modified from Logan (2003) and Psota et al. (2006)

vegetable oils from corn, sunflower seeds, cottonseed, rapeseed, and soybean are the richest sources of *n*–6 fatty acids.

1.2 *n*–3 Fatty Acids in Fish Oil Capsules and Krill Oil Capliques

Fish oil is obtained by extracting fish with food-grade ethanol and purified by molecular distillation to remove various contaminants (Fig. 1.3). Few studies have been performed on precise fatty acid composition of commercially available fish oil preparations that are used by the general population in Western countries (Hepburn et al., 1986; Chee et al., 1990). Gas liquid chromatography analysis of commonly available fish oil capsules indicates that these capsules contain 32% high content of saturated fatty acids (myristic and palmitic acids). EPA contents range from 18% to 30% while DHA contents range from 12% to 20% depending upon the brand name and manufacturing company. In addition, fish oil capsules also contain cholesterol (0.5–8.3 mg/g), vitamin A (66–10,000 IU/g) retinal, and vitamin E (0–0.6 mg/g) (Chee et al., 1990).

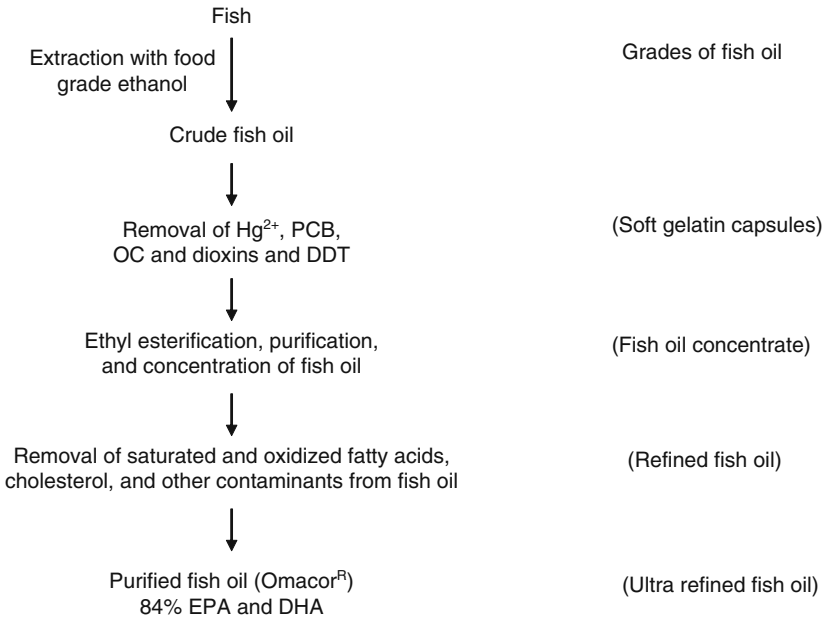

Fig. 1.3 Procedure for the purification of ultrapure fish oil from crude fish oil by molecular distillation. Best results are obtained using cold pressing and extraction with food grade ethanol. Soft gelatin capsules, fish oil concentrate, refined fish oil, and ultra-refined fish oil differ from each other in purity and concentration of DHA and EPA. More importantly, the long-term use of any fish oil is compromised by the impact of its ingradients on the digestive system, which ultimately determines the amounts consumed by the subject. Polychlorinated biphenyls (PCB); organochlorine (OC); and dichloro-diphenyl-trichloroethane (DDT)

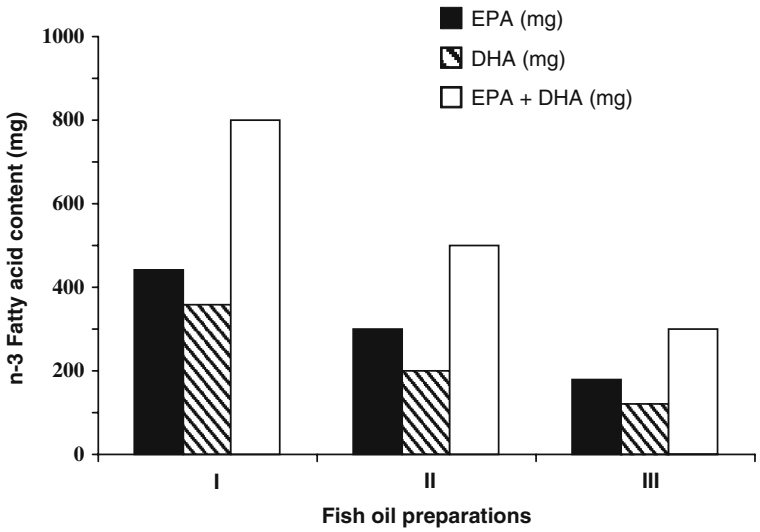

Fig. 1.4 *n*−3 Fatty acid content of fish oil supplements. Omacor (I); high-potency fish oil (II), and common fish oil capsules (III). Modified from Bays (2008)

High-potency fish oil supplements contain 300 mg EPA and 200 mg DHA (Bays, 2008) (Fig. 1.4**)**. Studies on the analysis of *n*–3 fatty acids in krill oil capliques have not been performed. So nothing is known about the composition of krill oil capliques. However, the stability of *n*–3 fatty acids and astaxanthin pigments in the aqueous suspension of krill oil/chitosan microcapsules has been studied. Physically stable and homogeneous microcapsules are obtained with chitosan of different molecular weight. This preparation retains over 95% of the EPA and DHA originally present in the oil. Although astaxanthin and its ethers are not as stable as the *n*–3 fatty acids, low molecular weight chitosan shows a better protective effect than high molecular weight chitosan (Butos et al., 2003). Collective evidence suggests that there is considerable variation in *n*–3 fatty acid contents of various brand names fish oil preparations. It is stated that capsules containing higher amounts of myristic and palmitic acid may have more adverse effects on plasma low-density lipoprotein cholesterol (LDL-C) than the capsules containing stearic acid and more monounsaturated fatty acids (Bonanome and Grundy, 1988; Ginsberg et al., 1990). Sometimes over-the-counter fish oil supplementation results in elevated LDL-C levels in human subjects (Malinowski and Metka, 2007). The significance of this increase on clinical outcome remains unknown.

1.2.1 Availability of Purified Fish Oil and Krill Oil Preparations

Fish oil is extracted from total fish body. The crude fish oil preparations contain 90% fatty acid triacylglycerols, 2–5% unsaponifiable matter containing sterols,

Table 1.1 Commercial names and manufacturers of *n*–3 fatty acid (DHA and EPA) preparations from fish and algal sources

Commercial name	Manufacturer
Purified fish oil	Carlson Laboratories Inc, Arlington Heights, IL, USA
Omegaven™	Kabi Bad Homburg, Germany
Lipoplus™ (LMF541)	B. Braun Melsungen, AG
Omacor™	Reliant pharmaceutical Inc Corner, NJ, USA
Zodin®	Solvary, Takeda, Kuhnil Pharmaceutical Co Ltd, Pierre Fabre Sante, France
DHASCO-S and T	Martek Biosciences Corporation, Columbia, MD, USA
Cardiozen™	Equazen Nutraceuticals, London, UK
DHActive TMCL	Nutrinova, Montpellier, France
Neuramin DHA	AllStar Health, Huntington, CA, USA
O-Mega-Zen^{3}™	Nu Tru Inc, Lincolnwood, IL, USA
Lovaza	GlaxoSmithKline, Philadelphia
Efalex™	Scotia Pharmaceuticals, Scotland, UK
Marinol D-40	Loders Croklaan Lipid Nutrition, Wormerveer, The Netherlands
Vegan omega-3	Deva Nutrition, Chelsea, AL, USA
Krill oil	Jedwards International inc, Quincy, MA, USA

fatty acid-esterified cholesterol, free fatty acids, minor quantities of vitamins A, D, and E, and some water-soluble amino acids, peptides, and minerals. These days fish oil is available in the form of a refined and purified liquid with different flavors (Table 1.1). The use of antioxidants for encapsulation/microencapsulation of fish oil prevents oxidation and extends the shelf life of fish oil. The use of monoacylglycerol citrate in purified fish oil as a sequestering agent prevents trace metal contamination. FDA-approved fish oil preparation "Omacor" is developed in the United States. Many clinical trials on humans are going on using Omacor (Davidson et al., 2007). One-gram capsule of Omacor (*n*–3 acid ethyl esters) has 800–840 mg of the ethyl esters of *n*–3 fatty acids. The *n*–3 fatty acids in Omacor include ethyl esters of EPA (440 mg) and DHA (360 mg) (Fig. 1.4). In Europe and Asia, Omacor is marketed as Zodin®. Omacor is used for treating hypertriglyceridemia (Brunton and Collin, 2007). Moderate hypertriglyceridemia is a very common condition, and elevated triacylglycerols are a risk factor for coronary heart disease. In patients with triacylglycerol levels above 500 mg/ml, approximately 4 g/day of EPA and DHA reduce triacylglycerol levels by 45% and very low-density lipoprotein cholesterol levels by more than 50%. Omacor is effective as monotherapy, but its effect is potentiated when used in conjunction with statins. There is little interaction with other drugs, although a small increase in bleeding time may be seen with anticoagulants. In hypertriglyceridemia, low-density lipoprotein cholesterol levels may increase following Omacor treatment depending on the baseline triacylglycerol level, but the net effect of EPA and DHA therapy is a reduction in non-high-density lipoprotein cholesterol level (McKenney and Sica, 2007). The most

common side effects observed in Omacor clinical trials were belching, infection, flu symptoms, upset stomach, rash, and change in sense of taste. Lovaza, a fish oil preparation by GlaxoSmithKline, has been recently approved by FDA. For vegetarians, *n*–3 fat preparation O-Mega-Zen^{3TM} is commercially available. O-Mega-Zen^{3TM} is derived from golden marine algae with little fishy odor or aftertaste. In addition, Martek Biosciences Corporation has introduced DHASCO-T and DHASCO-S from algal sources. Both DHASCO-T and DHASCO-S capsules deliver equivalent DHA levels in plasma glycerophospholipids and erythrocytes. DHA response is dose-dependent and linear over the dose range; plasma phospholipid DHA increased by 1.17, 2.28, and 3.03 g per 100 g fatty acid at 200, 600, and 1,000 mg dose, respectively (Artorburn et al., 2007). Omacor and O-Mega-Zen^{3TM}, DHASCO-T, and DHASCO-S preparations are murcurry- and DDT-free. These contaminants are commonly present in fishes from coastal water. In addition to lowering plasma triacylglycerol levels, these preparations upregulate intracellular degradation of apolipoprotein B-100-containing lipoproteins, depress LDL, increase bleeding time, downregulate gene expression for platelet-derived growth factor, lower blood pressure, decrease the number of endothelial adhesion molecules, improve lipoprotein size, and decrease the risk of cardiovascular and cerebrovascular diseases.

Both EPA and DHA are required for optimal human health. Studies on the comparison of pharmacological effects of commercially available preparations (DHA and EPA ethyl esters) and the naturally occurring DHA and EPA triacylglycerols present in fish indicate that purified commercially available preparations provide better health protection than naturally occurring ester found in fish not only because of contaminants (Hg^{2+} and DDT) but also because of rate of absorption through digestive tract. It is essential to build up stores of DHA and EPA in the body tissue for release of these fatty acids and their lipid mediators. This can be achieved through the sustained intake of commercially available fish oil preparation in the form of DHA and EPA ethyl esters (Rupp et al., 2004, 2006). In contrast to rapidly absorbed triacylglycerols from fish, ethyl esters of DHA and EPA are taken up more slowly within 24 h. From the administration of 1 g/day highly purified EPA + DHA ethyl esters (Omacor) to healthy volunteers, it is shown that EPA is increased from 0.6% to 1.4% within 10 days while DHA is increased from 2.9% to 4.3%. Baseline values can be obtained after the withdrawal of DHA and EPA within 10 days (Rupp et al., 2004, 2006).

Like fish oil, krill oil contains DHA and EPA, but chemical forms in which these fatty acids are present are different. In fish oil, DHA and EPA are found in the form of triacylglycerol form. In contrast, in krill oil, DHA and EPA are found in a double-chain phospholipid structure (Kidd, 2007). Thus in krill oil, DHA and EPA are mostly esterified in PtdCho. In esterified form, these fatty acids markedly outperform the effects of conventional fish oil DHA/EPA triglycerides in double-blind trials for premenstrual syndrome/dysmenorrhea and for normalizing blood lipid profiles (Table 1.2). Krill contains PtdCho

Table 1.2 Comparison of *n*–3 fats in fish oil capsules and krill oil capliques

Properties	Fish oil capsules	Krill oil capliques
Purity	Less	More
Intrinsic antioxidants	Present	Absent
Sensitivity to heat and moisture	More	Less
Allergenic ingredients	Present	Absent
Plasticizers	Present	Absent

(33–36%), PtdEtn (5–6%), triacylglycerol (33–40%), free fatty acids (FFA) (8–16%), and sterols (1.4–1.7%). Wax esters and sterol esters are present only in traces. More than 50 fatty acids can be identified using GLC/MS, the major ones being 14:0, 16:0, 16:1(n−7), 18:1(n−9), 18:1(n−7), 20:5(n−3), and 22:6(n−3) (Fricke et al., 2006). Short, medium-chain, and hydroxy fatty acids ($C \leq 10$) are not detectable. The sterol fraction contains very little cholesterol, desmosterol, and 22-dehydrocholesterol. In addition, it contains intrinsic antioxidants (vitamin E, vitamin A, vitamin D, and canthaxanthin) that prevent lipid peroxidation and make krill oil resistant to rancidity. Krill ω–3 phospholipids not only produce antiinflammatory effects but also lower C-reactive protein (CRP) levels in a double-blind trial (Kidd, 2007). The antioxidant potency of krill oil is, in terms of ORAC (oxygen radical absorptance capacity) values, 48 times more potent than fish oil. The astaxanthin found in krill oil provides also excellent protection against ultraviolet light and UV-induced skin damage. Krill oil is now supplied in the form of sealed capliques, which are non-allergenic and less sensitive to heat and moisture. Utilizing *n*–3 fats with attached phospholipids and antioxidants further increases the clinical ability of fish and krill oils for the treatment of cardiovascular, cerebrovascular, and neurological disorders (Kidd, 2007).

The studies on the impact of sources (from fish and fish oil) of *n*–3 fatty acids on their incorporation in serum, on blood lipid composition, and on cellular activation indicate that fish consumption is more effective for increasing serum EPA and DHA than supplementing the diet with fish oil and cod liver oil (Elvevoll et al., 2006; Visioli et al., 2003). These days, consumption of large amounts of fish is accompanied with several adverse side effects due to the potential presence of environmental toxins such as mercury, polychlorinated biphenyls, dioxins, and other contaminants (Bays, 2007). The risks of exposure to these toxins with fish oil consumption are substantially reduced through purification processes used for the preparation of concentrated fish oil supplements and prescription preparations. Availability of high-quality purified lemon and orange flavor fish oil has eliminated the problems of fishy smell and aftertaste (Farooqui, 2009). These commercially available preparations are stabilized with adequate amounts of vitamin E and are packaged in brown or opaque bottles to protect fish oil ingredients from light and oxygen. Emulsified fish oil preparations are much better absorbed than the neat oils in gelatin capsules.

1.2.2 Other n*–3-Enriched Manufactured Products*

Dutch group Loders Croklaan Lipid Nutrition, Wormerveer, The Netherland, has introduced Marinol D-40 and Marinol powder that contain 40% and 20% DHA, respectively. These products can be dry-blended with other ingredients, making them usable for bread, biscuits, and other bakery products. Canadian nutritional oil company, Bioriginal Food and Science of Saskatoon, Saskatchewan, is selling organic *n*–3-rich flax flour, BakOmega^{TM}. This flour is stable and tasty. Finally, NW Natural Products, WA, USA, and other companies are manufacturing ω–3 gummy fish tablets (chewable tablets) that contain micronutrients and ω–3 fatty acids. It is stated that this superfood reduces stress, boosts immune system, prevents mood swings, and boosts metabolism. I believe introduction of these supplements by the drug and food industry is a right step in protecting public health.

1.3 Effects of Fish Oil on Human Health

The *n*–3 fatty acids in fish oil markedly effect human health. To achieve a significant increase in human tissues in vivo, intake of high doses of fish oil is required because bioavailability of *n*–3 fatty acids from fish oil to neural cells involves not only digestion and transport but also metabolism (Kang et al., 2001). Furthermore, the efficacy of dietary intervention may also depend upon the health of the individual. For example, patients with gastrointestinal problems may not be able to absorb fish oil supplement. Daily use of *n*–3 fatty acids (3–5 g/day) by normal individuals may optimize the functions of human heart, brain, lungs, liver, spleen, and kidneys (Fig. 1.5). These fatty acids reduce cardiovascular disease, type 2 diabetes, hypertension, cancer, inflammatory and autoimmune diseases, and neurodegenerative diseases (Farooqui, 2009). In human nutritional studies, attempts have been made to increase the consumption of *n*–3 fatty acid enriched food because Western diet is deficient in *n*–3 fatty acids and has excessive levels of *n*–6 fatty acids compared with the diet on which human beings evolved and their genetic patterns are established (Simopoulos, 2006, 2008). Excessive levels of *n*–6 fatty acids and a very high *n*–6/ *n*–3 ratio (20 : 1), as are found in today's Western diets, promote the pathogenesis of inflammatory and autoimmune, and neurodegenerative diseases, whereas increased levels of *n*–3 fatty acids (a lower *n*–6/ *n*–3 ratio) exert suppressive effects and prevent cardiovascular disease, type 2 diabetes, hypertension, cancer, inflammatory and autoimmune diseases, and neurodegenerative diseases (Simopoulos, 2008). The American Heart Association recommends that everyone should eat oily fish twice per week and that those with coronary heart disease should consume 1 g/day of EPA plus DHA from oily fish or supplements.

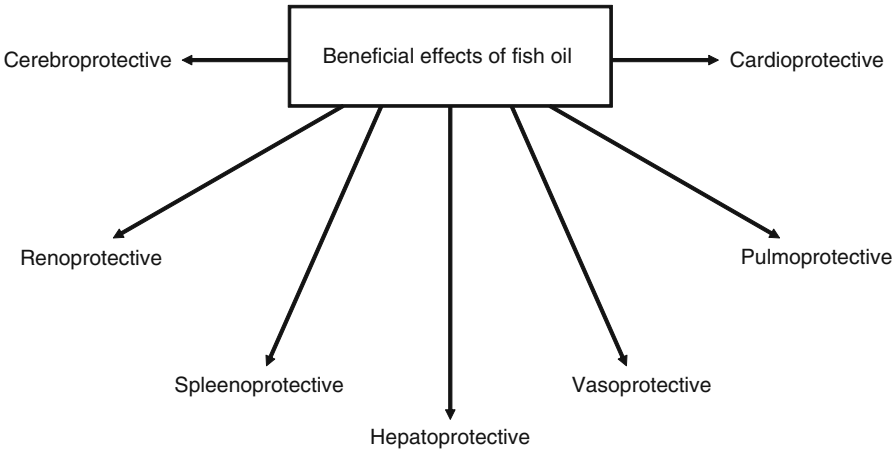

Fig. 1.5 Beneficial effects of fish oil on various organs of human body

1.4 Effects of Fish Oil on Heart

In heart, the incorporation of fish oil constituents, *n*–3 fatty acids, not only influences the physicochemical properties of membranes (fluidity, permeability) (Hashimoto et al., 1999) but also modulates the expression of many genes (De Caterina and Massaro, 2005; Deckelbaum et al., 2006). The *n*–3 fatty acids reduce proatherogenic cytokines, improve endothelial function, reduce vascular occlusion, and mitigate the course of coronary atherosclerosis. These fatty acids are also needed for vascular smooth muscle cell function (Hirafuji et al., 2003). The heart rate is reduced and heart rate variability is increased. An antiarrhythmic effect is also observed at the supraventricular and ventricular level (von Schacky, 2006). The *n*–3 fatty acids also modulate levels of plasma fibrinogen and coagulant factor VII levels (Schmidt, 1997). Collective evidence suggests that *n*–3 fatty acids reduce leukocyte reactivity, atherosclerosis, and thrombosis. In addition, fish oil exerts its effect through several different cellular mechanisms including the effects on lipoprotein metabolism, hemostatic function, platelet/vessel wall interactions, antiarrhythmic actions, and also through the inhibition of proliferation of smooth muscle cells and therefore growth of the atherosclerotic plaque (Abeywardena and Head, 2001). Fish oil intake also results in moderate reductions in blood pressure and modification of vascular neuroeffector mechanisms. The majority of cardiovascular benefits of fish oil are mediated in the vascular wall and at the vascular endothelium, which plays a central role in the regulation and maintenance of cardiovascular homeostasis and function (Abeywardena and Head, 2001) through the generation of nitric oxide and eicosanoids. In addition to direct effects on contractility, *n*–3 fatty acids in fish oil may affect atherogenesis via inhibition of vascular smooth muscle cell proliferation at the gene expression level and by modifying the expression of inflammatory cytokines and adhesion molecules (Abeywardena and Head, 2001; Verlengia et al., 2004).

1.4.1 Antiinflammatory and Antiatherosclerotic Effects of Fish Oil

Atherosclerosis, a pathological condition in which deposits of fatty substances, cholesterol, calcium, and other substances build up in the inner lining of an artery in the form of plaque, is an active process associated with vascular cell activation, inflammation, and thrombosis. Inflammatory processes in vascular wall destabilize atherosclerosis and produce lipid mediators and biomarkers that may provide insights into the molecular mechanism of pathogenesis of atherosclerosis and its relationship with inflammatory reactions. Although *n*–3 and *n*–6 fatty acids are incorporated in mammalian tissues, metabolism of these two families of fatty acids differs remarkably. The structural differences between *n*–3 and *n*–6 fatty acids modulate the generation of lipid mediators that produce opposing biochemical effects in vascular cells. Thus, *n*–6 fatty acid-derived lipid mediators (eicosanoids) are prothrombotic, proaggregatory, and proinflammatory. In addition, eicosanoids increase the expression of proinflammatory cytokines. In contrast, *n*–3 fatty acids generate lipid mediators (docosanoids) that are antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory. Docosanoids downregulate proinflammatory cytokines. Despite differences in biological properties of *n*–3 and *n*–6 fatty acid-derived lipid mediators, a balance between *n*–3 and *n*–6 fatty acids intake is important because these fatty acids compete for the same enzymes systems. Thus, ARA and EPA compete for cyclooxygenases and lipoxygenases. ARA produces 2- and 4-series eicosanoids with proinflammatory and prothrombic properties, whereas EPA generates 3- and 5-series eicosanoids that either oppose the above effects or have much lower levels of biological activity (De Caterina and Massaro, 2005). EPA-derived eicosanoids (thromboxanes A_3, B_3, and leukotriene B_5) are much less active than ARA-derived proinflammatory mediators such as prostaglandins, leukotrienes, and thromboxanes. EPA-derived resolvin E_1 (RvE_1) reduces inflammation by suppressing the activation of nuclear factor-κB (NF-κB) and consequently the expression and synthesis of cytokines and chemokines. At nanomolar levels, RvE_1 dramatically reduces dermal inflammation, peritonitis, dendritic cell migration, and interleukin IL-12 production. Its action is mediated by the ChemR23 receptor. Treatment of dendritic cells with the small interference RNA specific for ChemR23 sharply reduces RvE_1 regulation of IL-12 (Arita et al., 2005a). These results demonstrate novel counter-regulatory responses in inflammation initiated via RvE_1 receptor activation, which provide the first evidence for potent endogenous agonists of antiinflammation derived from EPA (Arita et al., 2005a,2005b). Another possible mechanism of RvE_1 may be that this metabolite prevents the binding of LTB_4 to its receptor and therefore blocks the propagation of a proinflammatory signal. The *n*–3 fatty acids also downregulate cyclooxygenase-2 gene and upregulate metalloproteinase gene in plaque angiogenesis and plaque rupture. The quenching of gene expression of proinflammatory proatherogenic genes by *n*–3 fatty acids has consequences on the extent of

leukocyte adhesion to vascular endothelium, early atherogenesis, and later stages of plaque development and plaque rupture, ultimately yielding a plausible comprehensive explanation for the vasculoprotective effects of *n*–3 fatty acids (De Caterina and Massaro, 2005). Ischemic heart disease is a multifactorial condition in which atherosclerosis is one important factor (De Caterina and Zampolli, 2001). Very little information is available on other factors that contribute to heart disease. Effect of fish oil on factors other than atherosclerosis remains unknown.

DHA is not a substrate for cyclooxygenase. Actions of a 15-lipoxygenase-like enzyme on DHA produce docosanoids (17S-resolvins, 10-,17S-docosatrienes, protectins, and neuroprotectins) (Hong et al., 2003; Marcheselli et al., 2003; Serhan et al., 2004; Phillis et al., 2006). Generation of docosanoids is cardioprotective and retards cellular injury and cellular death (Hong et al., 2003; Marcheselli et al., 2003; Serhan et al., 2004; Phillis et al., 2006).

DHA has more potent biochemical effects on cardiovascular diseases than EPA. Thus, DHA is more effective than EPA not only in suppressing ischemia-induced arrhythmia in rats and blocking thromboxane-mediated vasoconstrictor responses in aorta from spontaneously hypertensive rats, but also in retarding hypertension development in spontaneously hypertensive rats (Hirafuji et al., 2003). Collectively, these studies suggest that even EPA and DHA may produce different biological effects on membrane fluidity of mammalian tissue plasma membranes.

1.4.2 Antiarrythmic Effects of Fish Oil

Abnormality in heartbeat pattern or heart rhythms (faster or slower) is called as arrhythmia. The signs and symptoms of cardiac arrhythmias range from completely asymptomatic to loss of consciousness or sudden cardiac death. The *n*–3 fatty acids have antiarrhythmic effects on mammalian heart (Fig. 1.6). In vitro, these fatty acids stabilize the electrical activity of isolated cardiac myocytes by modulating sarcolemmal ion channels, needing a stronger electrical stimulus to elicit an action potential resulting in prolonged refractory period. The molecular mechanism associated with the antiarrhythmic action of *n*–3 fatty acids remains unknown. However, *n*–3 fatty acids activate protein kinase A (PKA) and induce their effects in the cardiac cell similar to β-adrenergic stimulation. Thus, in intact cardiac cells, EPA reduces the frequency of spontaneous waves of Ca^{2+} release while increasing sarcoplasmic reticulum Ca^{2+} content even when PKA activity is inhibited with H-89, a selective inhibitor of PKA. This observation suggests that the EPA-mediated inhibition of sarcoplasmic reticulum Ca^{2+} release is not dependent on the activation of PKA (Swan et al., 2003). Consistent with this, single-channel studies demonstrate that EPA, but not saturated fatty acids, reduces the open probability of the cardiac ryanodine receptor incorporated into phospholipid bilayers. EPA also blocks the binding

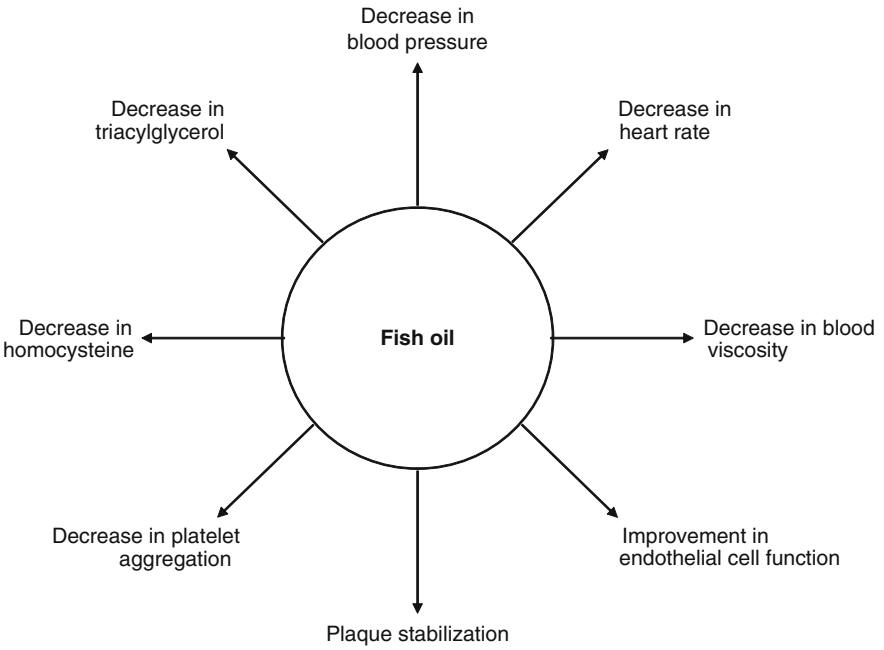

Fig. 1.6 Physiologic and pharmacologic effects of fish oil

of [^{3}H]ryanodine to isolated heavy sarcoplasmic reticulum. It is suggested that direct inhibition of ryanodine receptor channel gating by *n*–3 fatty acids plays an important role in the overall antiarrhythmic properties of these fatty acids (Swan et al., 2003; Honen et al., 2003). The *n*–3 fatty acids also modulate the generation of nitric oxide in vascular endothelial and smooth muscle cells. DHA and less potent EPA increase the nitric oxide generation mediated by IL-1β in a dose-dependent manner, and ARA has no significant effect on nitric oxide generation (Hirafuji et al., 2002). The stimulatory effect of DHA on the nitric oxide generation is more obvious at lower concentrations of IL-1β. DHA significantly potentiates IL-1β-mediated iNOS protein and mRNA expressions. Although EPA slightly increases the iNOS protein and mRNA expressions, ARA has no effect. IL-1β-mediated iNOS protein expression is significantly inhibited by PD 98059, a selective inhibitor of p44/42 MAPK kinase, both in the absence and in the presence of DHA. SB 203580, a selective inhibitor of p38 MAPK activity, has no significant effect. IL-1β upregulates the phosphorylation of p44/42 MAPK, but has no effect on the phosphorylation of p38 MAPK. DHA significantly potentiates the IL-1β-mediated phosphorylation of p44/42 MAPK, while having no effect on the phosphorylation of p38 MAPK. Collectively, these studies suggest that DHA increases nitric oxide production by potentiating IL-1β-mediated iNOS expression through the mechanism that involves p44/42 MAPK signaling cascade in rat vascular endothelial and

smooth muscle cells (Hirafuji et al., 2002). It is also proposed that like general anesthetics, *n*–3 fatty acids may also induce alterations in membrane physical properties, particularly membrane fluidity, which is increased in cardiac monocytes as a result of *n*–3 fatty acid treatment (Leifert et al., 2000).

DHA is the most potent and ALA the least potent with regard to effects on both membrane fluidity and sodium current block. The prevention of calcium overload in cardiac myocytes may also be closely associated with the antiarrhythmic mechanism of *n*–3 fatty acids (Leaf et al., 2003). In dogs, intravenous injection of fish oil prevents fatal ventricular fibrillation compared to soybean oil injections, supporting the view that fish oil has antiarrhythmic action on dog heart (Leaf et al., 1999). Similarly, injections of ALA, EPA, and DHA also have antiarrhythmic action in dog model of cardiac sudden death (Billman et al., 1999). Marmoset monkeys that consumed a diet containing *n*–3 fatty acids require higher threshold stimulation current than those consuming an *n*–6-enriched diet to induce ventricular fibrillation electrically under both control and ischemic conditions (McLennan et al., 1993; McLennan, 2001). Consumption of fish oil but not corn oil protects against fatal ventricular fibrillation induced by acute administration of a high dose of catecholamines in rats. In all the above models of cardiac sudden death, *n*–3 fatty acids may also provide cardioprotection by improving endothelial cell function, blood pressure, and vascular function by upregulating in nitric oxide production (Harris, 1997; Mori et al., 2000; Nestel et al., 2002). Modulation of cholesterol synthesis by *n*–3 fatty acids may also promote the antiarrhythmic effects. Thus in Reuber H35 hepatoma cells, EPA and DHA downregulate HMG-CoA reductase (Bastianetto and Quirion, 2004; Duncan et al., 2005), a rate-limiting intrinsic membrane protein (96 kDa), whose proteolysis releases an enzymically active soluble fragment (52–56 kDa) that regulates the synthesis of cholesterol. The downregulation of HMG-CoA reductase by *n*–3 fatty acids is not due to decreased protein synthesis, since similar levels of protein are found in the presence and absence of these fatty acids. In contrast, myristic acid upregulates HMG-CoA reductase. This is due to upregulation of the HMG-CoA reductase protein; therefore, protein synthesis is required for the increase in HMG-CoA reductase activity (Bastianetto and Quirion, 2004). Collectively, these studies suggest that incorporation of *n*–3 fatty acids in cardiac membranes modulates not only membrane composition but enzymic acitivities, which may modify the function of ion channels. These processes are closely associated with the antiarrhythmic effects of *n*–3 fatty acids (Lombardi and Terranova, 2007). In addition to antiarrhythmic effects, fish oil prolongs the ventricular effective refractory period, a measure of myocardial excitability (Albert, 2004).

1.4.3 Antithrombotic Effects of Fish Oil

Thrombosis is defined as the formation of a clot or thrombus inside a vessel, obstructing the flow of blood through the circulatory system. The *n*–3 fatty

acids also have antithrombotic effects on mammalian heart (Fig. 1.6). Administration of fish oil improves microvascular perfusion following ischemic/reperfusion injury. This may be due to inhibition of leukocyte adhesion and platelet reactivity (Kristensen et al., 2001). As stated above, ARA competes with EPA for the cyclooxygenase enzymes generating more potent proaggregatory thromboxane B_2 from ARA and less potent thromboxanes A_3, and B_3 from EPA. Consumption of fish and fish oil in diet blocks collagen-stimulated thromboxane B_2 generation without affecting prostacyclin synthesis. Based on these findings, it is proposed that *n*–3 fatty acids may have antithrombotic effects on mammalian heart (Mann et al., 1997). However, other studies indicate that fish oil has no effect on thrombosis (Archer et al., 1998). Large-scale clinical trials are needed to confirm the effects of *n*–3 fatty acids on thrombosis.

1.4.4 Effect of n–*3 Fatty Acids on Revascularization*

Revascularization is defined as the process of restoring the functionality of a diseased heart. Human heart vascularization procedures include coronary artery bypass grafting and percutaneous transluminal coronary angioplasty for coronary heart disease. In very ill patients who are not candidates for bypass surgery or angioplasty, transmyocardial revascularization (TMR) or transmyocardial laser revascularization (TMLR) procedures have been used. These procedures restore the flow of oxygen and nutrients to the heart. The *n*–3 fatty acids have positive clinical effects on heart after revascularization procedures. After coronary artery bypass grafting, supplementation of highly concentrated *n*–3 fatty acids (3.4 g/day) results in statistically significant reduction in angiographic vein graft occlusion. The reduction in occlusion rates is significantly related to the change in *n*–3 fatty acid concentration in serum glycerophospholipids (Eritsland et al., 1995; Arnesen, 2001). Incorporation of *n*–3 fatty acids in atrium glycerophospholipids also reduces incidences of postoperative atrial fibrillation, resulting in shorter hospital stay (Garg et al., 2006). Based on several studies, it is proposed that (a) *n*–3 fatty acid supplementation counteracts hypertension and normalizes coronary vasoreactivity to acetylcholine in heart transplantation patients, (b) counteracts occulusion of venous aortocoronary grafts 1 year after bypass operation, and (c) does not counteract restenosis 6 months after traditional percutaneous transluminal coronary angioplasty (Arnesen, 2001).

In addition, a pre-emptive *n*–3 infusion significantly reduces infarct size through the dual mechanisms of upregulation of heat shock protein 72, a key preconditioning protein, and a dramatic increase in the *n*–3 content of myocardial membranes, which appears to facilitate a shift in oxidant ischemia-reperfusion injury (McGuinness et al., 2006). Collective evidence suggests that *n*–3 fatty acids act on heart function in several ways. They prevent arrhythmias, generate docosanoids, inhibit synthesis of proinflammatory cytokines, and

retard plaque formation. In addition, these fatty acids stimulate endothelial cell nitric oxide synthase and generate NO, which relaxes vascular smooth muscle. This process promotes endothelial cell repair. The *n*–3 fatty acids also lower plasma triacylglycerol and very low-density lipoprotein levels (Table 1.3).

Table 1.3 Effects of *n*–3 fatty acids on cardiovascular systems

n–3 Fatty acid	Effect
DHA and EPA	Prevention of arrthymias
EPA	Synthesis of less inflammatory eicosanoids
DHA	Synthesis of antiinflammatory docosanoids
DHA and EPA	Inhibition of NF-κB and cytokine synthesis
DHA and EPA	Stimulation of nitric oxide production
DHA and EPA	Lowering of triacylglycerols and VLDLs
DHA and EPA	Prevention of plaques formation
DHA and EPA	Inhibition of thrombosis

1.4.5 **n–*3 or* ω *–3 Index and Heart Disease**

A reliable risk factor for sudden cardiac death for the general population remains to be defined. Ecological and prospective cohort studies as well as randomized controlled trials indicate that *n*–3 fatty acids are clinically relevant to coronary heart disease in general and sudden cardiac death in particular (Harris, 2004, 2007). The *n*–3 fatty acids operate via several mechanisms, starting from the incorporation of EPA and DHA into cardiac and vascular cell membranes to modulate membrane physicochemical properties and regulate activities of membrane-bound enzymes. Once released by intracellular calcium-independent phospholipase A_2, *n*–3 fatty acids interact with ion channels (Leaf et al., 2003). They are metabolized to a wide variety of bioactive lipid mediators such as thromboxanes A_3, B_3 and leukotriene B_5, resolvins, and protectin (Serhan et al., 2004). They also serve as ligands for several nuclear transcription factors, thereby altering gene expression (Harris, 2007). Thus, *n*–3 fatty acid levels in blood can be a strong reflection of their dietary intake. It is recently proposed that EPA plus DHA content of erythrocytes membrane expressed as a percent of total fatty acids may represent a new risk factor not only for death from coronary heart disease but also for sudden cardiac death. This biomarker is called as *n*–3 or ω–3 index (Harris, 2004; Geppert et al., 2005; Kelley et al., 2008). Thus, *n*–3 or ω–3 index represents the EPA plus DHA status of a given individual. It can be estimated by reproducible and standardized procedures. Many epidemiological studies support the view that *n*–3 or ω–3 index is an important risk factor or biomarker for sudden cardiac death. The *n*–3 or ω–3 index can assess the risk for sudden cardiac death and monitor therapy with EPA + DHA. Moreover, it compares very favorably with other risk factors for sudden cardiac death (Geppert et al., 2005; von Schacky and Harris, 2007; Harris, 2008). Recent intervention studies with EPA in patients at

high cardiovascular risk in Japan indicate that subjects rarely die by sudden cardiac death. Sudden cardiac death in Japan occurs 20 times less frequently than in the general population in Germany. In Japan, levels of EPA plus DHA are high (ω–3 index estimated around 11%), whereas in Germany levels of EPA plus DHA are low (ω–3 index around 4%) (von Schacky, 2008). These data strengthen the concept that a low ω–3 index is a risk factor for sudden cardiac death, as a tool to assess the status of a person in terms of EPA plus DHA and a means to monitor therapy with EPA plus DHA (von Schacky, 2008). An *n*–3 or ω–3 index of >8% provides the greatest cardioprotection, whereas an index of <4% indicates the highest risk. Collectively, these studies indicate that *n*–3 or ω–3 index fulfills several requirements for a risk factor, including consistent epidemiological studies, a plausible mechanism of action, a reproducible assay, independence from classic risk factors, modifiability, and, most important, the demonstration that raising levels will reduce risk for cardiac events (Harris, 2008; Harris et al., 2008).

1.5 Effects of Fish Oil on Brain

The *n*–3 fatty acids enter brain from circulation across blood–brain barrier and are incorporated at the *sn*-2 position of glycerol moiety of glycerophospholipids. They are released through the action of phospholipase A_2. The high levels of DHA in the retina and brain gray matter suggests that this fatty acid has important roles in retinal and neural function. Animal studies have shown that depletion of DHA from the retina and brain results in reduced visual function and learning deficits (Horrocks and Farooqui, 2004). Aging not only reduces levels of *n*–3 fatty acids in brain but also decreases neural membrane neuroplasticity. Decline in *n*–3 fatty acids with age is accompanied by loss of memory and learning. Reduction in *n*–3 fatty acid levels with age can be restored by DHA supplementation. Molecular mechanism associated with the loss of *n*–3 fatty acids from neural membrane is not fully understood. However, it is proposed that the loss of DHA with aging may be due to increased activity of plasmalogen-selective PLA_2 (André et al., 2006; Farooqui et al., 2008). This increase in plasmalogen-selective PLA_2 is also coupled with the loss of PlsEtn during aging. Dietary supplementation of DHA not only restores the levels of DHA but also increases cerebral choline and acetylcholine levels that improve passive avoidance performance in stroke-prone spontaneously hypertensive rats and also in rat hippocampus during aging (Barceló-Coblijn et al., 2003).

1.5.1 Effect of Fish Oil on Neural Membranes

Incorporation of EPA and DHA in neural membranes not only affects their physicochemical properties, such as membrane fluidity, permeability, and

viscosity, especially in neuronal synapses, but also modulates neurotransmission (Chalon et al., 1998; Zimmer et al., 2000; Chalon, 2006), gene expression (Puskás et al., 2003; De Caterina and Massaro, 2005; Deckelbaum et al., 2006), activities of enzymes and receptors, ion channels, and immunity (Yehuda et al., 2002). The *n*–3 fatty acids in brain protects against cognitive decline in healthy older people. It is proposed that this effect may be related to the role of *n*–3 fatty acids in maintaining the stability and structural integrity of synapse, which in turn modulates neurotransmission (Horrocks and Farooqui, 2004; Farooqui et al., 2007; Farooqui, 2009).

1.5.2 Effect of Fish Oil on Neuritogenesis

DHA promotes neuronal differentiation in rat embryonic hippocampal primary cultures. In vitro DHA deficiency can be produced by culturing hippocampal cells in chemically defined medium (Calderon and Kim, 2007). DHA supplementation restores DHA levels to the levels detected in freshly isolated hippocampus. DHA supplementation in hippocampal cultures induces morphological differentiation by increasing the population of neurons with more branches and longer neurites. In contrast, supplementation with ARA, oleic acid, or docosapentaenoic acid has no effect on hippocampal cultures, indicating specificity of DHA action on neurite growth. Hippocampal cultures prepared from *n*–3 fatty acid-deficient animals contain a lower DHA level and a neuronal population with shorter neurite length per neuron in comparison to those obtained from animals with adequate *n*–3 fatty acids. DHA supplementation to the deficient group recovered the neurite length to the level similar to *n*–3 fatty acid adequate cultures. Collective evidence suggests that DHA uniquely promotes neurite growth in hippocampal neurons. Inadequate neurite development due to DHA deficiency may contribute to the cognitive impairment associated with *n*–3 fatty acid deficiency (Calderon and Kim, 2007). The molecular mechanisms associated with neurite growth remain unknown. However, this process may partly involve soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) protein-mediated fusion of intracellular vesicles with plasma membrane followed by disassembly, membrane extension, and neurotransmitter release (Pongrac et al., 2007).

1.5.3 Effect of n–*3 Fatty Acids on Ion Channels*

Ion channels in neurons have channel proteins essentially homologous to those in the heart; therefore, it is possible that *n*–3 fatty acids may modulate the electrical activity in the brain in a manner similar to their effects in the heart (Leaf, 2001; Leaf et al., 2003). Among *n*–3 fatty acids, DHA inhibits voltage-stimulated Na^+ channels, whilst EPA has no effect on membrane excitability or Na^+ channels in hippocampal neurons (Xiao and Li, 1999). Similarly, DHA

modulates certain voltage-gated K^+ channels in Chinese hamster ovary cells, whereas EPA has no effect on K^+ channels. EPA modulates DHA synthesis in SH-SY5Y neuroblastoma cell cultures. EPA has antidepressant and antipsychotic activity whilst DHA does not. Quantitation of the mRNA levels of genes encoding for several key enzymes of both the endoplasmic reticulum and the peroxisomal steps of fatty acid metabolism indicates that EPA downregulates the enzymes involved in DHA synthesis and decreases DHA synthesis from its precursor ALA (Poumès-Ballihaut et al., 2001; Langelier et al., 2005). Collectively, these studies suggest that EPA and DHA differ on their effects on plasma lipid profiles, gene expression, and neural membrane structure.

1.5.4 Effect of n*–3 Fatty Acids on Receptors*

The dietary depletion of DHA stimulates caspases and downregulates NMDA receptors in Tg2576 mouse brain. Dietary supplementation of DHA partially protects the mice from NMDA receptor subunit loss (Calon et al., 2005). DHA also promotes neuronal survival not only by facilitating neural membrane translocation/activation of Akt but also through its ability to increase PtdSer levels in neural membranes (Akbar et al., 2005). In addition, DHA stimulates extracellular signal-regulated kinase in photoreceptors (German et al., 2006) and enhances the cerebral activities of catalase and glutathione peroxidase and increasing levels of glutathione in the cerebral cortex (Hossain et al., 1999). DHA also modulates physiological processes, such as long-term potentiation and memory processes, and inhibits ARA and its metabolite-mediated oxidative stress in brain tissue (Fujita et al., 2001; McGahon et al., 1999; Hashimoto et al., 2006). DHA consumption reduces reactive oxygen species in the hippocampus of Aβ-infused and aged rats (Hossain et al., 1998; Hossain et al., 1999).

Collective evidence suggests that *n*–3 fatty acids have many beneficial effects on brain tissue through their action on neural membrane structure and serving as precursors for potent lipid mediators. They are not only involved in cognitive development, memory-related learning, plasticity of nerve membranes, synaptogenesis, and synaptic transmission, but also in neuroprotection and neural cell survival. In a recent trial in 1,000 free living subjects, the intake of *n*–3 fatty acids is independently associated with lower levels of proinflammatory markers (IL-6, IL-1ra, TNF-α, C-reactive protein) and higher levels of antiinflammatory markers (soluble IL-6r, IL-10, TGF-β) (Ferrucci et al., 2006).

1.6 Effects of Fish Oil on Lungs

Lung diseases such as asthma and bronchitis are characterized by continuous inflammation. Thus, factors and drugs that downregulate inflammatory activity may be particularly beneficial for patients with asthma and bronchitis.

Treatment of asthmatic subjects with fish oil or *n*–3 fatty acid supplements results in improved ARA concentrations in neutrophils, reduced neutrophil chemotaxis, reduced leukotriene generation (Lee et al., 1985; Payon et al., 1986), and reduced airway late response to allergen exposure (Arm et al., 1988). However, relevant data are still sparse (Romieu and Trenga, 2001). Studies in the general adult population indicate that a high fish intake has a beneficial effect on lung function, but the relationship with respiratory symptoms and clinically manifest asthma and bronchitis is less evident (Smit et al., 1999; Schwartz, 2000).

1.7 Effects of Fish Oil on Kidneys

The *n*–3 fatty acid-enriched diet retards the progression of renal diseases in humans and experimental animal models (Donadio, 1993). Thus, administration of *n*–3 fatty acids reduces proteinuria and ameliorates renal injury in murine lupus nephritis, experimental focal segmental glomerulosclerosis, and other types of renal diseases (Chandrasekar and Fernandes, 1994; Clarke et al., 1991; Goldstein et al., 1995; Rahman et al., 1991; Donadio and Grande, 2004). Similarly, dietary supplementation of fish oil in dogs produces marked decrease in renal mass, followed by reduction in proteinuria, glomerulosclerosis, tubulointerstitial damage, and progressive loss of renal function (Brown et al., 1998, 2000). Furthermore, *n*–3 fatty acid supplementation reduces the progression of IgA nephropathy (Donadio et al., 1994, 1999). In contrast, dogs fed on *n*–6 fatty acid-enriched diet show progressive deterioration of kidney function associated with proteinuria, hypercholesterolemia, morphologic evidence glomerular and tubulointerstitial injury, and an increased prevalence of end-stage kidney failure. The molecular mechanism associated with *n*–6 fatty acid-mediated kidney damage and *n*–3 fatty acid-induced nephroprotection remains unknown. ARA-derived lipid mediators are vital for the proper control of renal hemodynamics. The lack of control of ARA-derived mediators can contribute to renal vascular injury and end-stage renal diseases (Imig, 2006). Three major enzymatic pathways, COX (cyclooxygenase), LOX (lipoxygenase), and EPOX (epoxygenase), are responsible for bioactive eicosanoids. These metabolites, through eicosanoid receptor-mediated mechanism, dilate or constrict the renal vasculature and maintain vascular resistance in the face of changing vasoactive hormones (Hao and Brever, 2007, 2008). Renal vascular generation of eicosanoids is altered in pathophysiological conditions such as hypertension, diabetes, metabolic syndrome, and acute renal failure. Collective evidence suggests that *n*–6-derived vasoactive eicosanoids modulate renal hemodynamics in rats, and early generation of vasoactive eicosanoids may induce glomerular hyperfunction, and subsequent overproduction of thromboxanes is closely associated with the pathogenesis of glomerular damage and proteinuria (De Rubertis and Craven, 1993; Hao and Brever, 2007, 2008). In contrast, *n*–3 fatty acids interfere

with the ARA cascade not only by competing for COX and LOX enzymes but also by generating eicosanoids that are less potent than ARA-derived eicosanoids. Studies on the effect of EPA on mesangial cells indicate that EPA modulates PDGF-stimulated proliferation by engendering changes in PDGF-stimulated TXA_2 synthesis (Schmitz et al., 2000). Fish oil also inhibits mesangial cell activation and proliferation in anti-Thy 1.1 ATS model of mesangial proliferative glomerulonephritis, reduces proteinuria, and decreases histologic evidence of glomerular damage. Potential molecular mechanism underlying the antiproliferative effect of DHA at low doses involves the inhibition of basal and epidermal growth factor (EGF)-stimulated ^{3}H-thymidine incorporation in mesangial cells. At higher doses EPA and DHA equally suppress basal and EGF-stimulated mesangial cells mitogenesis (Yusufi et al., 2003). Low levels of DHA decrease ERK activation by 30%. JNK activity is increased by low-dose DHA, but not by EPA. DHA and EPA have no significant effect on p38 activity. Low dose of DHA inhibits cyclin E activity. DHA upregulates the expression of the cell cycle inhibitor p21, but not p27. EPA has no effect on p21 or p27. It is proposed that the differential effect of low-dose DHA vs EPA in suppressing mesangial cells mitogenesis may be related to downregulation of ERK and cyclin E activity and to induction of p21. Collective evidence suggests that orally administered fish oil, or purified DHA, may have a suppressive effect in acute phases or relapses of glomerulopathies, by inhibiting the activation and proliferation of mesangial cells (Grande et al., 2000; Yusufi et al., 2003).

1.8 Effects of Fish Oil on Plasma Lipids

A predominance of small, dense, low-density lipoprotein (LDL) and increased plasma triacylglycerol levels are increased risk for the development of coronary heart disease (Griffin, 2001). Dietary long-chain *n*–3 fatty acids modulate the levels of triacylglycerols in several ways. They suppress the synthesis of triacylglycerols in the liver by inhibiting the diacyglycerol acyltransferase activity (Rustan et al., 1988). However, effects on very low-density lipoprotein (VLDL) synthesis and/or VLDL triacylglycerol removal rate via increased lipoprotein lipolysis have not been ruled out (Kasim-Karakas, 1995). Fish oil ingredients may limit the supply of lipid substrates for triacylglycerol synthesis. The *n*–3 fatty acids have been reported to increase the sensitivity of certain tissues to the action of insulin (Storlien et al., 1991). Thus, in rat liver, synthesis and secretion of triacylglycerol and cholesterol ester are reduced by 50–80% in the presence of EPA in comparison with oleic acid. Reduced synthesis of triacylglycerol and cholesterol ester is seen when EPA and oleic acid are given together. EPA causes higher incorporation of ^{3}H water into glycerophospholipids and lower incorporation into triacylglycerol and cholesterol ester as compared with oleic acid. Reduction in synthesis of triacylglycerol and cholesterol ester is also observed when EPA-CoA is given together with oleoyl-CoA,

whereas palmitoyl-CoA, stearoyl-CoA, linolenoyl-CoA, and arachidonoyl-CoA have no inhibitory effects (Rustan and Drevon, 1989). Collectively, these studies suggest that *n*–3 fatty acid-mediated triacylglycerol-lowering effect redistributes LDL subfractions toward larger and lighter particles, thus providing protection against the generation of atherogenic type of LDL (Griffin, 2001). This effect is more pronounced in carriers of the apoE4 polymorphism. Furthermore, EPA or EPA-CoA also reduces cellular cholesterol esterification by inhibiting the activity of acyl-CoA:cholesterol acyltransferase. The lowered cholesterol esterification induced by EPA also modulates the secretion of VLDL cholesterol ester. The molecular mechanism associated with hypotriglyceridemic effects of fish oil in humans may include the involvement of liver X receptor, hepatocyte nuclear factor-4α (HNF-4 α), farnesol X receptor, and peroxisome proliferator-activated receptors (PPARs). All these receptors are regulated by sterol receptor element binding protein-1c (SREBP-1c), the main genetic switch that modulates lipogenesis (Davidson et al., 2007). It is proposed that *n*–3 fatty acids elicit hypotriglyceridemic effects by coordinately suppressing hepatic lipogenesis through reducing levels of SREBP-1c, upregulating fatty oxidation in the liver and skeletal muscle through PPAR activation, and enhancing flux of glucose to glycogen through downregulation of HNF-4 α (Davidson et al., 2007). The net result of these processes is the repartitioning of metabolic fuel from triacylglycerol storage toward oxidation, thereby reducing the substrate available for VLDL synthesis. By simultaneously downregulating genes encoding the proteins that stimulate lipid synthesis and upregulating genes encoding the proteins that stimulate fatty acid oxidation, *n*–3 fatty acids are more potent hypotriglyceridemic agents than are *n*–6 fatty acids on a carbon-for-carbon basis (Davidson, 2006). Furthermore, as stated above peroxidation of *n*–3 fatty acids reduces VLDL secretion through stimulating apolipoprotein B degradation. The *n*–3 fatty acids may also act by enhancing postprandial chylomicron clearance through reduced VLDL secretion and by directly stimulating lipoprotein lipase activity. It is proposed that the use of fish oil is a valuable clinical tool for the treatment of hypertriglyceridemia (Davidson et al., 2007).

Studies on the effects of fish oil on hyperlipidemic apolipoprotein (APO) E*3-Leiden mice, which have impaired chylomicron and VLDL remnant metabolism, also indicate that fish oil-fed mice show a significant dose-dependent decrease in serum cholesterol (up to –43%) and triacylglycerol levels (up to –60%), mainly due to a reduction of VLDL (–80%). VLDL-apoB kinetic studies indicate that fish oil feeding produces a significant twofold increase in VLDL-apoB fractional catabolic rate (FCR), but hepatic VLDL-apoB synthesis is not affected by fish oil feeding. VLDL–triacylglycerol turnover studies show that fish oil significantly decreases the rate of synthesis of hepatic VLDL–triacylglycerol (–60%). A significant increase in VLDL–triacylglycerol FCR is observed (+70%), which is not related to elevation in lipolytic activity. It is suggested that APOE*3-Leiden mice are highly responsive to dietary fish oil and observed strong reduction in serum VLDL is primarily due to an effect

of fish oil to decrease hepatic VLDL–triacylglycerol production rate and to increase VLDL-apoB fractional catabolic rate (van Vlijmen et al., 1998). Collective evidence indicates that the consumption of fish oil is cardioprotective at doses >3 g/day. The cardioprotective effects are due to suppression of fatal arrhythmias rather than stabilization of atherosclerotic plaques. At doses >3 g/day, EPA plus DHA can improve cardiovascular disease risk factors, including decreasing plasma triacylglycerols, blood pressure, platelet aggregation, and inflammation, while improving vascular reactivity. Mainly on the basis of the results of randomized control trials, the American Heart Association recommends for everyone to eat oily fish twice per week and that those with coronary heart disease eat 1 g/day of EPA plus DHA from oily fish or supplements.

1.9 Effect of Fish Oil on Liver

Fish oil exerts hypolipidemic, antiobesity, and antiinflammatory effects on hepatic lipid metabolism. A slight increase in the serum activity of liver enzymes during *n*–3 fatty acid supplementation has been reported by several investigators (Schmidt et al., 1994). Treatment of obesity-prone C57BL/6 J mice with 8% fish oil + high-fat (30%) diet for 5 months results in the loss of body weight compared to mice fed a 30% triacylglycerol diet without fish oil (Mori et al., 2007). In addition to modulating mRNA levels in the liver, fish oil ingestion for 2 weeks upregulates the intestinal mRNA levels of lipid metabolism-related genes such as carnitine palmitoyltransferase 1a, cytochrome P450 4A10, and malic enzyme, compared with mice that are fed with the 30% triacylglycerol diet. Northern blot analysis demonstrate that the expression levels of most lipid metabolism-related genes in the small intestine of mice fed with 8% fish oil diet are comparable to those in the liver. Furthermore, fish oil ingestion also modulates lipid metabolism-related enzyme activity, fatty acid β-oxidation, ω-oxidation, and malic enzyme activities in the small intestine of mice fed the 8% fish oil diet compared to mice fed the 30% triacylglycerol-enriched diet. These findings suggest that an upregulation of intestinal lipid metabolism is associated with the antiobesity effect of fish oil (Mori et al., 2007). Microarray-based expression profiling of genes in rats fed with fish oil and ethanol diet indicates that a large number of genes show significant changes in female livers compared to males (Sharma et al., 2008). The upregulated genes in female liver are associated with proteosome endopeptidase activity, lipid metabolism, alcohol metabolism, mitochondrial and oxidoreductase activity. The downregulated genes are associated with oxidoreductase activity, chaperone activity, and electron transport activity in the female liver as shown by biological theme analysis. To identify specific regulatory networks of genes operative in promoting liver injury, ingenuity computational pathway analysis has been used. These studies identify a large cluster of genes associated with lipid metabolism, development, cellular growth and proliferation, apoptosis, carcinogenesis, and various signaling pathways (Sharma et al., 2008).

Diet enriched in fish and marine oils also improves serum lipid profiles by suppressing liver X receptor α (LXR α) activity in the liver. Studies on the effects of *trans* geometric isomers of eicosapentaenoic acid (TEPA) on triacylglycerol synthesis induced by a synthetic LXR α agonist (T0901317) on HepG2 cells indicate that TEPA significantly reduces the amount of cellular triacylglycerol and the expression of mRNAs encoding fatty acid synthase, stearoyl-CoA desaturase-1, and glycerol-3-phosphate acyltransferase induced by T0901317 compared with EPA (Zaima et al., 2006). However, there was no significant difference between the suppressive effect of TEPA or EPA on the expression of sterol-regulatory element binding protein-1c (SREBP-1c) induced by T0901317. It is shown that TEPA, but not EPA, downregulates the mRNA expression of peroxisome proliferator-activated receptor γ coactivator 1β (PGC-1 β), which is a coactivator of both LXR α and SREBP-1. It is proposed that the hypolipidemic effect of TEPA can be attributed to a decrease not only in SREBP-1 but also in PGC-1 β expression (Zaima et al., 2006). Collectively, these studies indicate that dietary fish oil modulates the fatty acid composition of hepatic membranes, plasma lipoprotein cholesterol profile, biliary lipids, and the expression of proteins involved in reverse cholesterol transport. Fish oil reduces plasma HDL cholesterol and increases biliary cholesterol without concomitant modifications in the expression of key genes and proteins involved in reverse cholesterol transport. These findings suggest that functional changes in the activity of these proteins as a consequence of the incorporation of *n*–3 fatty acids into hepatic membranes and plasma lipoproteins may underlie the effect of fish oil feeding on plasma and hepatic cholesterol metabolism in the rat (Morgado et al., 2005).

Chronic hyperglycemia is an additional risk factor for cardiovascular disease. Diabetes patients have two- to four fold increased risk of dying from complication of cardiovascular disease (Colwell, 1997). Considerable attempts using drugs (statins, fibric acid derivatives, and nicotinic acid) are made to control the dyslipidemia that severely deteriorates the diabetes in patients (Pyorala et al., 1997; Elkeles et al., 1998; Garg and Grundy, 1990). Studies on the effect of fish oil on glycemic control of streptozotocin (STZ; 90 mg/kg bodyweight)-treated type 1 diabetic rats, fish oil-treated or untreated non-diabetic rats have indicated that 3 weeks after fish oil treatment, plasma total cholesterol is reduced in both the non-diabetic and the diabetic rats by 35% and 10%, respectively (Mahmud et al., 2004). In non-diabetic and diabetic rats, levels of triacylglycerol are decreased by 69% and 20%, respectively, compared with control rats. Fish oil treatment decreases non-esterified fatty acids (NEFA) by 29% in diabetic rats, but the NEFA levels in non-diabetic rats are not affected. After fish oil treatment in non-diabetic and diabetic rats, platelet aggregation is decreased by 49% and 37%, respectively, and total antioxidant status is increased by 18% and 17%, respectively (Mahmud et al., 2004). Insulin levels are increased by 27% in the fish oil-fed non-diabetic rats, whereas insulin levels are markedly decreased in diabetic rats. Fish oil treatment does not alter glucose levels, but it reduces fructosamine levels by 29% only in fish oil-fed

diabetic rats (Mahmud et al., 2004). Collective evidence suggests that fish oil may ameliorate the atherogenic lipid profile, platelet hyperaggregation, and the antioxidative defence of STZ-diabetic rats, and amelioration is independent of the effects of fish oil on glycemic control. Similar results are obtained on non-insulin-dependent diabetic patients on supplementation of *n*–3 fatty acids-enriched diet. Based on these observations, it is proposed that fish oil treatment decreases vascular complications in diabetes (Kesavulu et al., 2002).

1.10 *n*–3 Fatty Acids and Bleeding Tendency

The bleeding tendencies of Greenland Eskimos, who consume *n*–3 fatty acid-enriched diet ($\geq$7–10 g/day), have been described by several investigators (Dyerberg and Bang, 1979; Bang and Dyerberg, 1980). Bleeding tendencies include prolonged cutaneous, nose, urinary tract, and obstetric bleedings (Dyerberg and Bang, 1979). Furthermore, Greenland Eskimos show reduced platelet in vitro aggregability, compared with Danish control subjects. This reduction in platelet function has been explained by a shift in eicosanoid metabolism when ARA (20:4n–6) is replaced by EPA (20:5n–3) in platelet membranes. This shift results in the synthesis of thromboxanes and prostacyclins, resulting in more vasodilatory and antiaggregatory hemostatic profiles (Dyerberg et al., 1978). There is no clinical evidence of an increased bleeding tendency when individuals consumed moderately enriched n–3 PUFA supplemention (2–5 g/day). Collective evidence suggests that daily ingestion of moderate amount of fish oil reduces chances of chronic heart disease, stroke, liver, and kidney diseases independent of the type of fat present in the daily diet.

1.11 Effect of *n*–3 Fatty Acids on Blood Pressure

DHA-enriched diet attenuates the development of high blood pressure in spontaneously hypertensive rats (Engler et al., 1999). Incorporation of dietary DHA produces changes in the fatty acid composition of the vasculature and organs (aorta, renal artery, plasma, liver, heart, kidney, and lung) involved in the maintenance of blood pressure regulation. It is proposed that alterations in fatty acid profiles of vasculature and organs affect systemic hemodynamics. The DHA-enriched diet markedly increases the levels of DHA in the aorta, renal artery, plasma, liver, heart, kidney, and lung by 5-, 15-, 7-, 6-, 3.8-, 3.5-, and 8.8-fold, respectively. The levels of EPA are also elevated, while there is a concomitant reduction in the levels of ARA, adrenic (AA), and docosapentaenoic acids (DPA). In addition, higher proportion of dihomo-γ-linolenic acid and a lower proportion of ARA indicate the impairment in Δ^5-desaturase activity. Treatment of DHA-treated stroke-prone spontaneously hypertensive rats (SHRSP) also causes a significant decrease in the levels of total cholesterol,

LDL, triglycerides, lipid peroxide, serum creatinine, and blood urea nitrogen as compared with those in non-treated SHRSP, suggesting that DHA-mediated antihypertensive action may be associated with the amelioration of both serum lipid alteration and renal dysfunction in non-treated SHRSP (Kimura, 2000). In addition, DHA-mediated alterations in biophysical properties of membrane and generation of docosanoids may also promote lowering of blood pressure in normal and hypertensive rats.

1.12 Recommendations for Intake of *n*−3 Fatty Acids

Commercial preparations of fish oil capsules are available over the counter in drug stores and supermarkets. An excessive use of *n*–3 fatty acids may produce harmful effects not only due to the effect on enzymic activities but also through their effect on membrane permeability. Recommendations for *n*–3 fatty acid intake have been made by several international scientific authorities. Thus, Health and Welfare Canada proposes the use of 1.0−1.8 g *n*–3 fatty acids/day. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends adequate intake of a minimum 0.22 g/day for *n*–3 fatty acids. British Nutrition Foundation (BNF) suggests a desirable dietary intake of 1.1 g and 1.4 g of *n*–3 fatty acids for females and males daily. FDA in the United States recommends the use of up to 3 g *n*–3 fatty acids/day.

1.13 Beneficial Effects of Olive Oil on Human Health

Interest in the health benefits of Mediterranean diet has increased tremendously due to its link with greater longevity and lower cardiovascular disease rate, cancer and age cognitive decline (Fig. 1.7) (Perez-Jimenez et al., 2005; Lopez-Miranda et al., 2007). Despite the high complexity of Mediterranean diet nutrients composition, olive oil (oleic acid) and polyphenolic antioxidants have emerged as its principal beneficial components. Oleic acid, a monounsaturated non-essential fatty acid, belongs to *n*–9 family of fatty acids and represents a large proportion of our dietary intake with low uptake in liver and neural cells. Olive oil not only provides the higher percent of energy but a lot of bioactive compounds that promote human health. The presence of polyphenolic antioxidants increases the shelf life of olive oil compared to other vegetable oil. These phenols have many beneficial effects on human neurovascular and cardiovascular systems (Fig. 1.8).

1.13.1 Effects of Olive Oil on Heart

Supplementation of olive oil in diet improves the major risk factors for cardiovascular disease, such as the lipoprotein profile, blood pressure, glucose

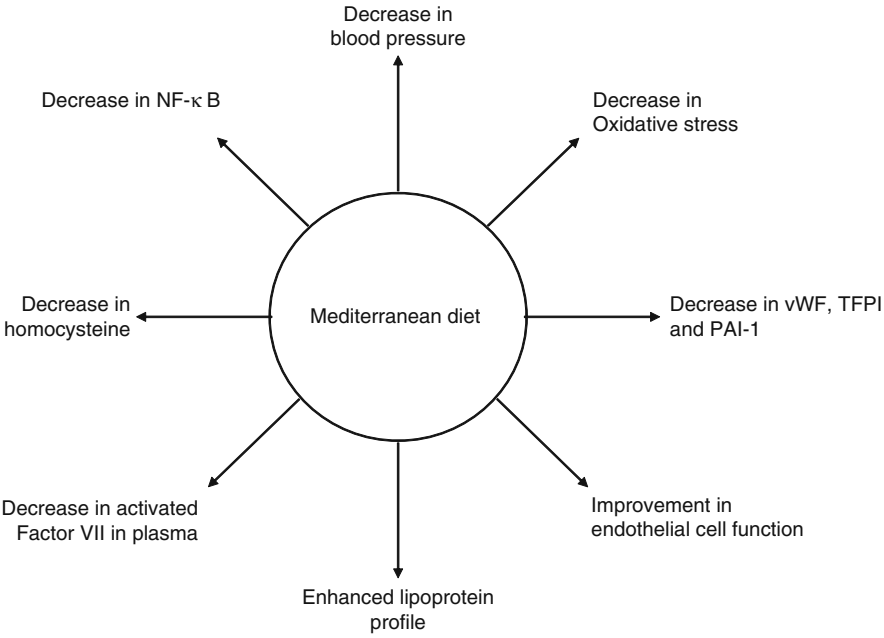

Fig. 1.7 Health benefits of olive oil-enriched Mediterranean-style diet in human. Nuclear factor κB (NF κB); von Willebrand factor 0 (vWF0); Tissue factor inhibitor (TFPI); Plasma activator inhibitor (PAI-1)

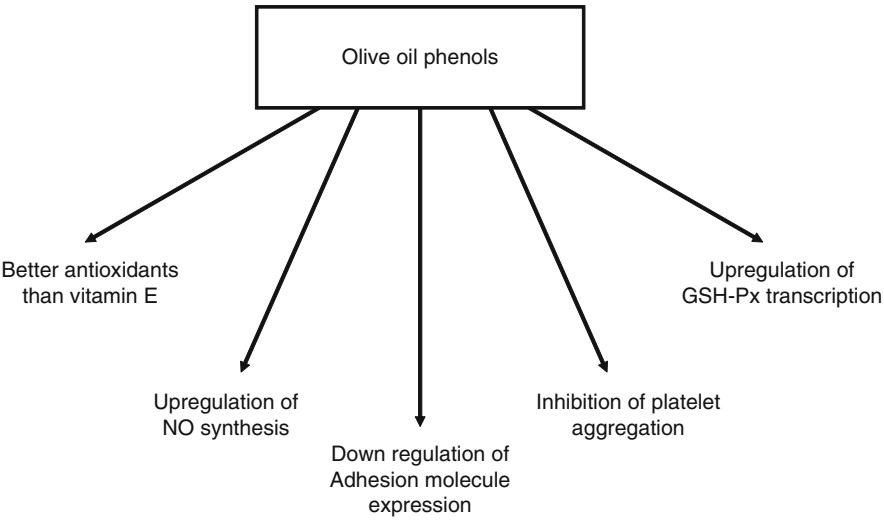

Fig. 1.8 Beneficial effects of olive oil phenols on cardiovascular system

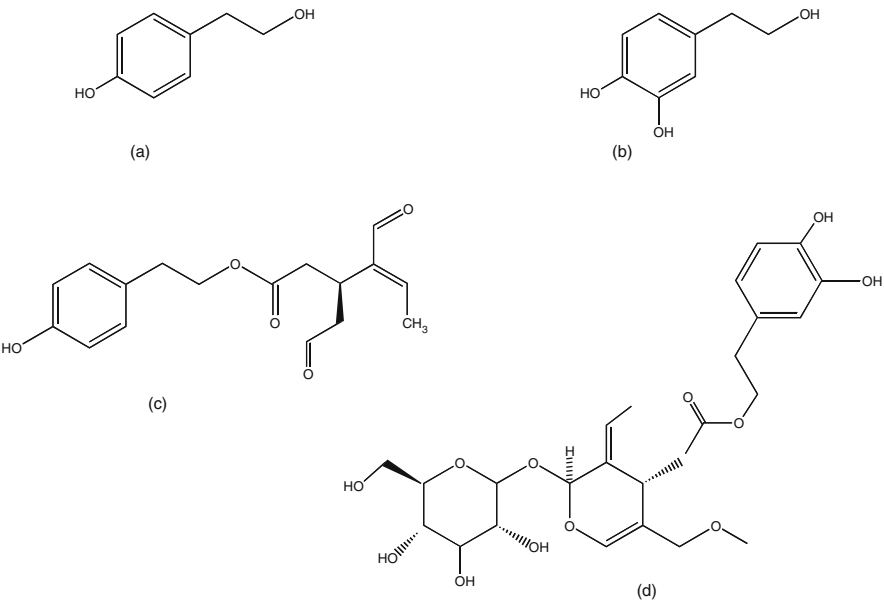

Fig. 1.9 Chemical structures of microconstituents in olive oil. Tyrosol [2-(4-hydroxyphenyl)-ethanol] (**a**), hydroxytyrosol (**b**), oleocanthal (**c**), and oleuropein (**d**)

metabolism, and antithrombotic profile (Perez-Jimenez et al., 2005). Olive oil is a good source of vitamins and polyphenolic antioxidants, such as tyrosol [2-(4-hydroxyphenyl)ethanol], hydroxytyrosol, oleuropein, and oleocanthal (Fig. 1.9), and has a balanced ratio of monounsaturated and polyunsaturated fatty acids (Ruano et al., 2005; Covas et al., 2006; Carluccio et al., 2007). In addition, olive oil contains many other components (Table 1.4) that have many effects on human health. Multiple mechanisms have been proposed to explain beneficial effects of Mediterranean diet. These mechanisms include decrease in LDL cholesterol and increase in HDL cholesterol, and also reduction of oxidative stress due to polyphenols, which are able to scavenge free radicals and protect LDL from oxidation. The Mediterranean diet diminishes NF-κB activation in mononuclear cells compared to Western diet (Perez-Martinez et al., 2007). Thus, in a dose-dependent manner, extra-virgin olive oil extract retards the translocation of p50 and p65 subunit of NF-κB in both unstimulated and phorbol-myristate acetate (PMA)-challenged monocytes and monocyte-derived macrophages. Inhibition is more effective for the p50 subunit than p65 (Brunelleschi et al., 2007). This effect occurs at concentrations found in human plasma after nutritional ingestion of virgin olive oil and is quantitatively similar to the effect exerted by ciglitazone, a PPAR-γ ligand. However, extra-virgin olive oil extract has no effect on PPAR-γ expression in monocytes. These results indicate the beneficial effects of extra-virgin olive oil are due to its ability to inhibit NF-κB activation in human monocyte/macrophages (Brunelleschi

Table 1.4 Non-fatty acid components of extra-virgin olive oil

Components	References
Tyrosol	Bendini et al. (2007)
Hydroxytyrosol	Bendini et al. (2007)
Oleuropein	Bendini et al. (2007)
Oleocanthal	Bendini et al. (2007); Buiarelli et al. (2004)
Apignin	Bendini et al. (2007); Buiarelli et al. (2004)
Luteolin	Bendini et al. (2007); Buiarelli et al. (2004)
Pinoresinol	Bendini et al. (2007); Buiarelli et al. (2004)
Gallic acid	Bendini et al. (2007); Buiarelli et al. (2004)
Protocatechnic acid	Bendini et al. (2007); Buiarelli et al. (2004)
p-Hydroxybenzoic acid	Bendini et al. (2007); Buiarelli et al. (2004)
Vanillic acid	Bendini et al. (2007); Carrasco Pancorbo et al. (2004); Carrasco Pancorbo et al. (2005)
Caffeic acid	Bendini et al. (2007); Carrasco Pancorbo et al. (2004); Carrasco Pancorbo et al. (2005)
Cinnamic acid	Bendini et al. (2007); Carrasco Pancorbo et al. (2004); Carrasco Pancorbo et al. (2005)
Syningic acid	Bendini et al. (2007); Carrasco Pancorbo et al. (2004); Carrasco Pancorbo et al. (2005)

et al., 2007). In RAW 264.7 macrophages, tyrosol inhibits exogenous ROS-mediated [^{3}H]ARA release. β-Sitosterol and tyrosol also reduce PMA-mediated nitric oxide generation, and this process correlates with the impairment of inducible nitric oxide synthase (iNOS) levels (Moreno, 2003). Polyphenolic compounds found in olive oil include oleuropein, an antioxidative and antiischemic compound that inhibits the adhesion of monocyte cells to the blood vessel lining, a process closely associated with the development of atherosclerosis. Another component of olive oil, (−)-oleocanthal (1), inhibits COX-1 and COX-2, the enzymes that convert ARA to eicosanoids. This property is similar to ibuprofen, a non-steroidal drug with antiinflammatory, analgestic, and antipyretic properties (Beauchamp et al., 2005; Smith et al., 2007). In addition, diet enriched in virgin olive oil reduces the sensitivity of platelets to aggregation by decreasing von Willebrand and thromboxane B2 plasma levels (Perez-Jemenez et al., 2006; Ruano et al., 2007). The capacity of Mediterranean diet to decrease fasting factor VII (proconvertin) plasma levels is due to components of extra-virgin olive oil. It results in modulation of postprandial activation, a process that is important in relation to its heart-protective effect (Lopez-Miranda et al., 2007). The chronic intake of virgin olive oil not only decreases the expression of tissue factor expression in mononuclear cells but also increases fibrinolytic activity by reducing plasma concentration of plasma activator inhibitor type-1 (PAI-1) (Perez-Jemenez et al., 2006). Virgin olive oil retards inflammation by inhibiting platelet activating factor, a lipid mediator that plays an important role not only in clotting process by mediating platelet aggregation but also in activating immune cells and their binding to the endothelial wall

(Karantonis et al., 2006). Collectively, these studies indicate that olive oil components interfere with the inflammatory response within atherosclerotic lesion by inhibiting the endothelial activation involved in monocyte recruitment during early atherogenesis and macrophage production of inflammatory cytokines and matrix-degrading enzymes, thus improving vascular stability (Carluccio et al., 2007). The intake of the polyphenol-enriched breakfast prepared from olive oil increases plasma concentrations of nitrates/nitrites and decreases levels of lipoperoxides and 8-epi prostaglandin-$F_{2\alpha}$ than the breakfast prepared from low polyphenol fat meal. Both these parameters are positively correlated to enhanced endothelial function (Ruano et al., 2005; Fuentez et al., 2008). In addition, olive oil components also inhibit platelet aggregation and the plasma homocysteine (rHcy) concentration. Collective evidence suggests that endothelial function, inflammation, and oxidative stress are also positively modulated by olive oil-enriched diet. However, despite the significant advances on health benefits of olive oil, the final proof of the specific mechanism(s) and contributing role of the different components of virgin olive oil to its beneficial effects requires further studies on health benefits of olive oil.

1.13.2 Effects of Olive Oil on Brain

Many studies in humans have shown that the intake of fat in olive oil may have protective effects against age-related cognitive decline and Alzheimer disease. The micronutrients found in virgin olive oil are readily bioavailable to humans and have antioxidant properties. These micronutrients also modulate hemostasis and antithrombotic properties in cardiovascular and cerebrovascular systems (Lopez-Miranda et al., 2007). Oleic acid inhibits gap junction permeability and increases glucose uptake in cultured rat astrocytes (Lavado et al., 1997). The molecular mechanism of oleic acid-mediated inhibition of gap junction remains unknown. However, it is proposed that oleic acid acts by stimulating protein kinase C (PKC) activity (Khan et al., 1992). Stimulation of PKC and oleic acid-mediated inhibition of gap junction can be prevented by inclusion of bovine serum albumin. The physiological significance of oleic acid-mediated gap junction inhibition is not fully understood. However, it is well known that oleic acid increases the rate of cell proliferation not only by enhancing glucose uptake but also by stimulating PKC. Both these factors are critical for neural cell proliferation. Metabolism of glucose through pentose phosphate shunt is essential for the supply of ribose phosphate needed for the synthesis of nucleic acids (Lavado et al., 1997), and activation of PKC is associated with neural cell proliferation. Neural cell proliferation requires the cessation of cell–cell communication through the inhibition of gap junction by oleic acid.

During brain development, neurons use oleic acid for the synthesis of glycerophospholipids and incorporate it into growth cones (Tabernero et al., 2001). Oleic acid promotes axonal growth, neuronal clustering, and expression of the

axonal growth-associated protein-43 (GAP-43). The effect of oleic acid on GAP-43 synthesis is mediated through the activation of PKC, since it can be blocked by inhibitors of this kinase, such as H-7, polymyxin, or sphingosine (Granda et al., 2003). The expression of GAP-43 is significantly increased in neurons co-cultured with astrocytes in the presence of albumin. Detailed investigations on this topic have indicated that astrocytes internalize albumin in vesicle-like structures by receptor-mediated endocytosis. Transcytosis-mediated albumin uptake is followed by passage through the endoplasmic reticulum, which is required for oleic acid synthesis (Tabernero et al., 2002). It is proposed that during brain development, oleic acid synthesis is the feedback mechanism regulated by the sterol regulatory element binding protein-1. This protein mediates the transcription of stearoyl-CoA 9-desaturase, the key rate-limiting enzyme for oleic acid synthesis. The presence of albumin activates the sterol regulatory element binding protein-1 and upregulates stearoyl-CoA 9-desaturase mRNA (Tabernero et al., 2002). Moreover, when the activity of sterol regulatory element binding protein-1 is blocked by the overexpression of a truncated form of this protein, albumin has no effect on stearoyl-CoA 9-desaturase mRNA, indicating that the effect of albumin is mediated by this transcription factor. The effect of albumin can be blocked either by retarding traffic to the endoplasmic reticulum or adding albumin–oleic acid complex. In addition, oleic acid also mediates the expression of microtubule-associated protein-2 (MAP-2), a marker of dendritic differentiation (Rodríguez-Rodríguez et al., 2004). The time course of MAP-2 expression during brain development coincides with that of stearoyl-CoA desaturase, the limiting enzyme of oleic acid synthesis, indicating that both phenomena coincide during development. The effect of oleic acid on MAP-2 expression is most probably independent of the autocrine factors synthesized by neurons (Rodríguez-Rodríguez et al., 2004), and exogenous or endogenous oleic acid by astrocytes exerts its neurotrophic effect through a PKC-dependent mechanism. This effect can be prevented by sphingosine or two myristoylated peptide inhibitors of PKC (Rodríguez-Rodríguez et al., 2004). Collectively, these studies indicate that during brain development, the presence of albumin plays an important role by triggering the synthesis and release of oleic acid by astrocytes, which induces neuronal differentiation through its interactions with transcription factor NeuroD2 (Tabernero et al., 2001, 2002; Rodríguez-Rodríguez et al., 2004).

In an animal model of stroke tyrosol, an antioxidant present in olive oil, shows a dose-dependent neuroprotective effect that peaked at 64.9% in rats treated with 30 mg/kg of tyrosol (Bu et al., 2007). In rotarod, beam balance, and foot fault tests, tyrosol exhibits protective effects against the sensory motor dysfunction. Collectively, these studies indicate that intake of virgin olive oil is compatible with a healthier aging, increased longevity, and neuroprotective effects (Perez-Jimenez et al., 2006, 2007; Bu et al., 2007).

Studies on supplementation of diet with *n*–3 fatty acids, *n*–9 fatty acids, and palm oil (control diet) for 3 weeks in mice indicate that *n*–3 fatty acid-enriched

diet suppresses the generation of both leukotrienes and prostaglandins, but the *n*–9 fatty acid-enriched diet only downregulates leukotrienes during acute inflammation (Doshi et al., 2004). Leukocyte accumulation during acute inflammation is not different in the *n*–3 or *n*–9 fatty acid-enriched diet as compared with the control group. The *n*–3 fatty acid-enriched diet also suppresses Freund's adjuvant-mediated granuloma formation. The *n*–9 PUFA-rich diet significantly attenuates galactosamine/lipopolysaccharide-mediated liver injury more effectively than the *n*–3 fatty acid-enriched diet. Collective evidence suggests that the differential modification of experimentally induced inflammation in mice by dietary *n*–3 fatty acid and *n*–9 fatty acid may be due to their differential effects on 5-lipoxygenease and cyclooxygenase metabolism of ARA during inflammatory processes (Doshi et al., 2004).

Studies on the in vivo effect of a diet rich in extra-virgin olive oil and a fish oil supplement on plasma and lipoprotein fatty acid composition and on LDL susceptibility to oxidative modification in free-living Spanish male patients with peripheral vascular disease have indicated that plasma triacylglycerols are decreased significantly after 3 months of dietary intervention (Ramirez-Tortose et al., 1999a,b). The LDL and plasma fatty acid pattern in the group consuming fish shows a significant increase in *n*–3 polyunsaturated fatty acids, mainly EPA and DHA. The slopes of LDL oxidative susceptibility are similar between baseline and endpoint values in extra-virgin olive oil and a fish oil-consuming groups (Ramirez-Tortose et al., 1999a,b). However, the uptake of oxidized LDL by macrophages is significantly reduced in fish oil-consuming patients in comparison with the control group. Collectively, these studies indicate that the daily consumption of extra-virgin olive oil in combination with a daily supplement of fish oil for 3 months in patients with peripheral vascular disease produces a plasma-lipid profile less atherogenic than in patients having refined olive oil as the main visible food fat (Ramirez-Tortose et al., 1999a,b). The simultaneous consumption of α-tocopherol and natural antioxidants provided by extra-virgin olive oil also has a protective effect on the LDL susceptibility to oxidative modifications in spite of a higher proportion of *n*–3 polyunsaturated fatty acids.

1.14 Harmful Effects of *Trans* Fatty Acids on Human Health

Trans fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration (Fig. 1.10). These fatty acids are produced when liquid oils are processed into solid fats like shortening and hard margarine through hydrogenation. Hydrogenation not only increases the shelf life but also elevates flavor and stability of foods having shortening or margarine. *Trans* fatty acids are also generated during heating and frying of oils at high temperatures. The major sources of *trans* fatty acids are partially hydrogenated fats commonly used for manufacturing processed food and food cooked in fast food resturants.

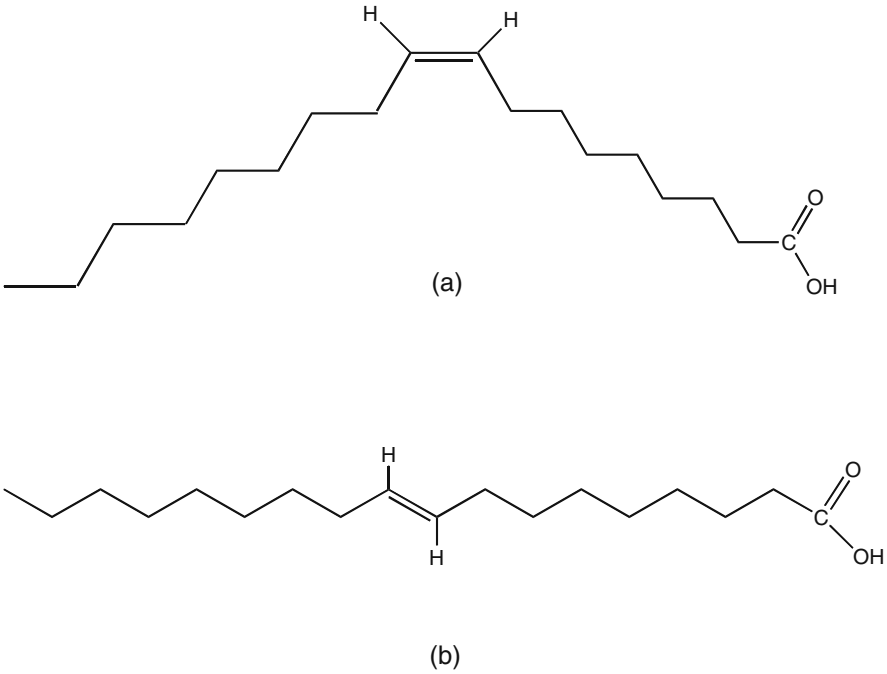

Fig. 1.10 Structural differences between *cis* and *trans* fatty acids. Oleic acid (**a**) and eiaidic acid (**b**)

Small amounts of *trans* fatty acids are also found naturally in dairy and beef meat as a result of bacterial transformation of unsaturated fatty acids in the rumen of ruminant animals (Hunter, 2006).

Trans fatty acids have many harmful effects and proposed to be associated with several pathological situations in humans. Many studies have indicated that *trans* fatty acids increase the level of the atherogenic LDL cholesterol and lower the level of HDL cholesterol compared to *cis* fatty acids (Katan, 1998; Hunter, 2006; Mozaffarian and Willett, 2007). This results in *trans* fatty acid diet-mediated increase in the ratio between total and HDL cholesterol. It is stated that this ratio is a more powerful predictor of coronary heart disease than total or LDL cholesterol alone. In addition, *trans* fatty acids also increase plasma triacylglycerol and VLDL levels. Although the molecular mechanism(s) associated with these processes are not fully understood, several possibilities for *trans* fatty acid-mediated pathologic effects have been proposed. They include (a) changes in cholesteryl ester transfer protein metabolism, which may play a prominent role in transfering cholesterol from HDL to LDL and VLDL, (b) modulation of endothelial cell function by *trans* fatty acids, and (c) *trans* fatty acid-mediated ligand-dependent effects on peroxisome proliferator-activated receptor (PPAR) or retinoid X receptor (RXR) pathways (Katan, 1998; Harvey

et al., 2008; Mozaffarian, 2006). It is hypothesized that the effects of *trans* fatty acids on insulin sensitivity may be mediated through alterations in gene expression.

In vitro studies demonstrate that the extent of *trans*-18 : 2 fatty acids incorporation is twofold greater in the glycerophospholipid fraction of human aortic endothelial cells than that of *cis*-18 : 2. Flow cytometric analysis indicates that *trans* fatty acid treatment of human aortic endothelial cells significantly upregulates the expression of endothelial adhesion molecules including intercellular adhesion molecule-1 (CD54) and vitronectin receptor (CD51/CD61) (Harvey et al., 2008). Incorporation of *trans* fatty acids into membrane glycerophospholipids increases human aortic endothelial cells adhesion to fibronectin- or vitronectin-coated plates by 1.5- to 2-fold, respectively. Neutrophil and monocyte adhesion to human aortic endothelial cells monolayers is also proportional to adhesion molecule expression. *Trans* fatty acid treatment also increases the release of monocyte chemoattractant protein-1 by nearly threefold in non-stimulated human aortic endothelial cells. Furthermore, chemotactic migration of *trans* fatty acid-treated human aortic endothelial cells toward sphingosine-1-phosphate (S1P) is significantly increased compared with controls. In contrast, capillary morphogenesis of *trans* fatty acid-treated endothelial cell is significantly prevented in response to S1P, suggesting that *trans* fatty acid incorporation suppresses endothelial cell differentiation. Collectively, these studies suggest that *trans* fatty acids play important roles not only in the induction of pro-inflammatory responses but also in endothelial cell dysfunction (Harvey et al., 2008). *Trans* fatty acids also increase the risk of coronary heart disease by adversely effecting plasma lipoprotein levels (Katan, 1998; Hunter, 2006; Mozaffarian and Willett, 2007) demonstrating strong positive relation among *trans* fatty acid consumption, alterations in cholesterol homeostasis, risk of myocardial infarction, coronary heart disease death, and sudden death (Katan, 1998; Hunter, 2006; Mozaffarian and Willett, 2007).

Putative differences between the effects of monounsaturated *trans* fatty acids from industrially produced and natural sources on cardiovascular disease risk markers have also been investigated, and it is reported that monounsaturated trans fatty acids in industrially produced food may have different effects on cardiovascular disease risk factors in women than the monounsaturated *trans* fatty acids from natural sources. It is proposed that HDL cholesterol-lowering property of monounsaturated *trans* fatty acids is specific to the monounsaturated *trans* fatty acids from industrial sources (Chardigny et al., 2008), and ruminant *trans* fatty acids intake is not associated with a higher risk of cardiovascular disease (Jakobsen et al., 2008). More studies are required to draw a meaningful conclusion from this study. Furthermore, many animal studies indicate that *trans* fatty acids (especially LA) retard the synthesis of ARA and DHA from their precursors. This inhibition may interfere with development of brain tissue in fetuses (Hunter, 2006).

In addition to their harmful effects on human heart, *trans* fatty acids have been linked to cancer, type 2 diabetes, maternal and child health, and macular degeneration, but the relationship of *trans* fatty acids with the above-mentioned conditions has been controversial and more studies are required on harmful effects of *trans* fatty acids on human health (Hunter, 2006). Collectively, these studies suggest that *trans* fatty acids may mediate the etiology of various metabolic and functional disorders, but the main concern about their pathological effects on human heart are mainly due to their structural similarity with saturated fatty acids, the lack of specific metabolic functions, and their competition with essential fatty acids (Valenzuela and Morgado, 1999; Hunter, 2006).

1.15 Conclusion

Fish oil contains *n*–3 fatty acids (DHA and EPA). These fatty acids have diverse physiological effects not only on normal health (neuronal development and heart, visual, immune, and renal functions) but also on chronic diseases, such as cerebrovascular and cardiovascular diseases. DHA and EPA also modulate plasma lipid levels and insulin action. Brain, heart, and kidney diseases are positively linked with the intake of saturated and *n*–6 enriched fatty acids and negatively linked with *n*–3 fatty acid-deficient diet. Thus, high intake of saturated fats and *n*–6 fatty acid-enriched diet increases the levels of eicosanoids, which induce oxidative stress and inflammation in brain, heart, and kidney and may promote the pathogenesis of cerebrovascular, cardiovascular, and renal diseases. Deficiency of DHA is associated with impairments in cognitive and behavioral performance effects, which are particularly important during brain development. DHA also modulates neurogenesis, neurotransmission, and protection against oxidative stress. The *n*–3 fatty acids in fish oil enhance hepatic fatty acid oxidation and inhibit fatty acid synthesis and VLDL secretion. This may be due to regulation of gene expression. The *n*–3 fatty acids regulate PPARα, SREBP-1, ChREBP, and MLX. These transcription factors control the levels of proteins involved in lipid and carbohydrate metabolism. Among *n*−3 fatty acids, DHA has recently been established as a key controller of hepatic lipid synthesis. This fatty acid controls the 26S proteasomal degradation of the nuclear form of SREBP-1. SREBP-1 is a major transcription factor that controls the expression of multiple genes involved in fatty acid synthesis and desaturation. DHA suppresses nuclear SREBP-1, which in turn suppresses lipogenesis. Molecular mechanism associated with these processes remains unknown. However, it is proposed that phosphorylation status of protein kinases may play an important role. In contrast, *n*–3 fatty acids in fish oil promote antioxidant, intiinflammatory, antiarryhthmic, and antinephropathic effects. It is proposed that an optimal balance between dietary *n*–6 and *n*–3 fatty acids is necessary for maintaining normal cerebrovascular, cardiovascular, renovascular, and hepatovascular functions.

Fish oil also improves vascular endothelial function and help lower blood pressure, platelet sensitivity, and the serum triglyceride level. It modestly increases the plasma levels of LDL cholesterol, increases HDL cholesterol levels, and has favorable effects on lipoprotein particle size and subclass distribution. Dietary fish oil also stabilizes the myocardium electrically, resulting in reduced susceptibility to ventricular arrhythmias, thereby reducing the risk of sudden death. It modulates coagulation factors, cell-mediated immunity, and markers of inflammation. Fish oil is well tolerated, with few side effects other than mild gastrointestinal symptoms. Neuroprotective and cardioprotective effects of fish oil may be due to antiinflammatory, antioxidant, and antiexcitotoxic properties of DHA and EPA.

Epidemiological and biochemical studies indicate that the Mediterranean diet, in which olive oil is the major source of fat, reduces the risk of coronary heart disease and cancer. It has been proposed that the beneficial effects of olive oil not only depend on oleic acid but are also associated with polyphenolic components of olive oil. A diet rich in oleic acid provides an adequate fluidity to the biological membranes, diminishing the hazard of lipid peroxidation, which affects polyunsaturated fatty acids. Moreover, the antioxidants present in olive oil are able to scavenge free radicals, and afford an adequate protection against lipid peroxidation. *Trans* fatty acids, which are generated by partial hydrogenation of vegetable oil, cause multiple adverse effects on human health. These effects include alterations in cholesterol homeostasis, inflammation, oxidative stress, endothelial health, body weight, insulin sensitivity, and cancer. It is not yet clear how specific *trans* fatty acids vary in their biological activity and mechanisms of action.

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Chapter 2
Transport, Synthesis, and Incorporation of *n*–3 and *n*–6 Fatty Acids in Brain Glycerophospholipids

2.1 Introduction

Neural membranes are composed of glycerophospholipids, sphingolipids, cholesterol, and proteins. Glycerophospholipids and sphingolipids contain nonpolar fatty acyl, alkenyl, and alkyl chains. The degree of unsaturation in polyunsaturated fatty acids (PUFA) determines many neural membrane properties including membrane order, packing pattern, and fluidity. Variations in the head group, length of the fatty acid acyl chains, and degree of saturation and unsaturation produce changes in surface charge and physicochemical characteristics of neural membranes. Collective evidence suggests that PUFA modulate many neural membrane properties and functions (Yehuda et al., 2002; Farooqui and Horrocks, 2007; Farooqui, 2009). Three families of PUFA are known to occur in neural membranes: the *n*–3 PUFA family is characterized by having their first double bond at carbon atom number 3; *n*–6 PUFA family members have their first double bond at carbon atom number 6, when counted from the methyl end of the carbon chain; and *n*–9 family member contains first double bond at carbon atom number 9 from fatty acid methyl end. Examples of *n*–3 fatty acids are α-linolenic acid (ALA; 18:3*n*–3), eicosapentaenoic acid (EPA; 20:5*n*–3), and docosahexaenoic acid (DHA; 22:6*n*–3). Examples of *n*–6 fatty acid family are linoleic acid (LA; 18:2*n*–6), arachidonic acid (ARA; 20:4*n*–6), and adrenic acid (AA; 22:4*n*–6), and example of *n*–9 family is oleic acid. Mammals cannot introduce double bond between carbon 1 and 6 due to the lack of desaturases but can introduce a double bond after the ninth carbon through the action of Δ^9-desaturase. In mammalian synaptosomal plasma membrane glycerophospholipids, *n*–6 and *n*–3 PUFA are mainly located at the *sn*-2 position of glycerol moiety. Majority of ARA and oleic acid is associated with phosphatidylcholine (PtdCho), whereas phosphatidylethanolamine (PtdEtn) and ethanolamine plasmalogens (PlsEtn) contain both ARA and DHA at the *sn*-2 position of glycerol moiety. Phosphatidylserine (PtdSer) is enriched in DHA (Glomset, 2006). Sphingomyelin and glycolipids in synaptosomal plasma membranes contain amide-linked stearic acid instead of a mixture of this acid with other amide-linked fatty acids. Although the physiological significance of this unique distribution of lipid head groups, esterified fatty acids, and amide-linked fatty acids is not fully understood, asymmetric distribution of glycerophospholipids and sphingolipids between the two leaflets of neural membrane may contribute to dynamic lipid substructures

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(Glomset, 2006). In neural membranes, DHA and ARA are substrates for lipid mediators. LA and ALA are precursors for the synthesis of ARA and DHA, respectively. The importance of ARA and DHA is related to their specific interactions with membrane proteins and their ability to serve as precursors for eicosanoids and docosanoids. Collective evidence suggests that the incorporation of ARA and DHA in neural membranes not only induces changes in physicochemical properties of membranes but also modulates membrane functions through the generation of lipid mediators (Horrocks and Farooqui, 2004).

2.2 Transport of Dietary ARA and DHA to Brain

Levels of fatty acids and other lipids in plasma are the net result of the balance between two opposite processes: the loading (the entry of new lipids through the ingestion) or endogenous synthesis and the unloading (energy utilization, incorporation into cell membranes, and storage). Even though mammals can synthesize very low amounts of many fatty acids, it is becoming increasingly evident that nearly all circulating fatty acids are derived from the diet (Galli and Rise, 2006; Visioli et al., 2006). ARA and DHA are present in diet as triacylglycerols, which are hydrolyzed by lipases in gastric and intestinal lumen. DHA is released more slowly than ARA. Its intestinal absorption is delayed but not decreased (Bezard et al., 1994). These fatty acids are incorporated in noticeable amounts in chylomicron glycerophospholipids. However, their uptake by various tissues is no more rapid than uptake of shorter-chain PUFAs. In tissues, LA and ALA, which constitute the major part of dietary essential fatty acids, are converted into ARA and DHA by alternate desaturation (Δ^6, Δ^5, and Δ^4)–elongation reactions. Animal tissues are more active in ARA and DHA biosynthesis than human tissues. Liver is one of the most active organs and its role is critical in providing long-chain PUFA secretion in VLDL (very low density lipoprotein) (Bezard et al., 1994). In liver, PUFA biosynthesis is regulated by nutritional, hormonal, and physiological factors. Dietary fatty acids modulate the biosynthesis of PUFA and are often inhibitory. Dietary ALA inhibits Δ^6 desaturation of LA. The desaturation products ARA, EPA, and DHA inhibit Δ^6 desaturation of LA and Δ^5 desaturation of DGLA (dihomo-gamma-linolenic acid). Insulin and thyroxin are necessary for Δ^6 and Δ^5 desaturation activities, whereas other hormones (glucagon, epinephrine, ACTH, glucocorticoids) downregulate desaturation. Age markedly affects activities of desaturases. Thus, in fetus, liver and brain are capable of converting LA and ALA into ARA and DHA, but these fatty acids are also delivered by the mother through placenta. After birth, the Δ^6 desaturase activity increases in liver but decreases in the brain (Bezard et al., 1994).

The concentration of free fatty acids in human serum of healthy subjects is about 7.5 nM with a standard deviation of 2.5 nM. Fasting elevates this value to

15nM±3.9. As stated earlier, liver plays a critical role in providing essential fatty acids to the brain, with secretion of long-chain PUFA in very low-density lipoprotein (VLDL). LA and ALA require the same desturases to form ARA and DHA, and therefore are in constant competition with each other for elongation and desaturation (Salem et al., 1999). Diet high in LA retards the synthesis of DHA in liver. In liver, ARA and DHA associate themselves either to albumin or to lipoproteins. From liver these fatty acids are transported in the blood either bound to albumin or in the form of triacylglycerol associated with lipoproteins. Total fatty acid concentration and fatty acid/albumin ratio regulate the levels of free fatty acids, but at physiological conditions (ratio 0.4–1.4) the effect is negligible. Understanding the mechanisms by which fatty acids cross the blood–brain barrier (BBB) and their utilization by neurons and glia is critical for understanding not only the normal brain development and function but also for the diagnosis and therapy of human neurological disorders. The rate of ARA and DHA crossing through BBB is higher from ARA and DHA–albumin complexes than from circulating ARA and DHA lipoproteins complexes (Hamilton and Brunaldi, 2007). The transport of ARA and DHA across BBB and other non-neural cellular membranes most likely occurs through passive diffusion. ARA and DHA transport is facilitated by a number of membrane-associated and cytoplasmic proteins. These include membrane proteins fatty acid translocase (FAT/CD36), plasma membrane fatty acid-binding protein (FABPpm) and fatty acid transport protein (FATP) (Utsunomiya et al., 1997) (Fig. 2.1). For net ARA or DHA influx, these fatty acids must be desorbed from the inner leaflet of the neural membrane and should bind with FATP/$FABP_{pm}$/acyl-CoA binding protein (ACBP) to prevent their repartitioning back into the membrane. If these events do not occur, fatty acids are repartitioned back into the outer leaflet and are desorbed back to the plasma to bind once again with serum albumin.

Although brain tissue abundantly expresses FABPpm and FATP, CD36 mRNA has not been detected in adult mouse and rat brain (Greenwalt et al., 1995; Utsunomiya et al., 1997). These transporters provide a high-affinity mechanism for recruitment of ARA and DHA off the albumin. These binding proteins may also function in the fine-tuning of cellular processes by modulating the metabolism of long-chain fatty acids implicated in the regulation of cell growth and various cellular functions (Dutta-Roy, 2000; Glatz et al., 2001). In brain tissue, ARA and DHA cross the luminal and transluminal leaflets of the endothelial cells and the plasma membrane of neural cells by reversible flip-flop (Stremmel et al., 2001; Hamilton and Brunaldi, 2007) (Fig. 2.2). From lipoprotein triacyglycerols, ARA and DHA are released in brain through the action of endothelial cell lipoprotein lipase (Vilaro et al., 1990). In an in vitro model of BBB, which is reconstituted by coculturing of brain-capillary endothelial cells and astrocytes, it is also reported that lysophosphatidylcholine (lyso-PtdCho)-containing DHA can cross the BBB more efficiently than free DHA or DHA in triacylglycerol (Lagarde et al., 2001). Although the physiological significance of this observation is not fully understood, based on labeling studies it is proposed

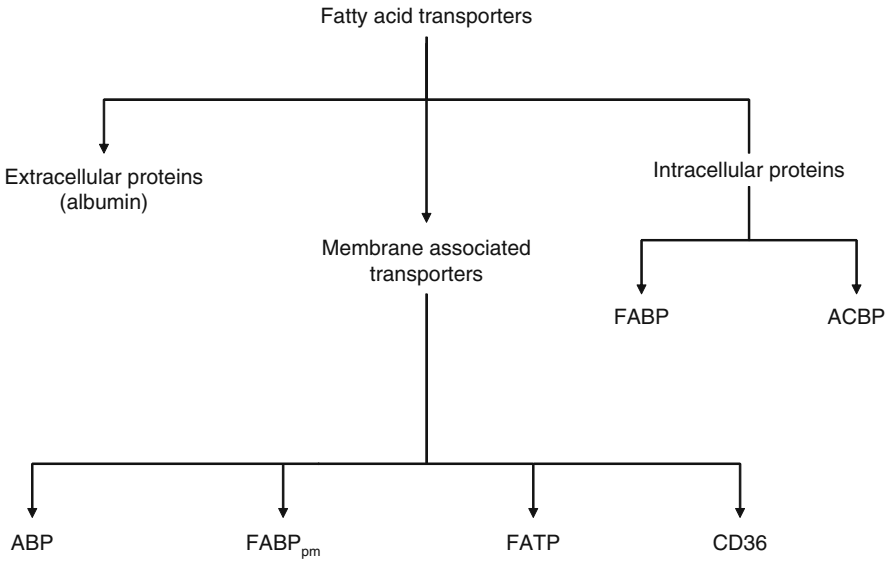

Fig. 2.1 Proteins associated with fatty acid transport. Albumin-binding protein (ABP); plasma membrane fatty acid-binding protein; fatty acid-transport protein (FATP); fatty acid translocase (CD36); cytoplasmic fatty acid-binding protein (FABP); and acyl-CoA-binding protein (ACBP)

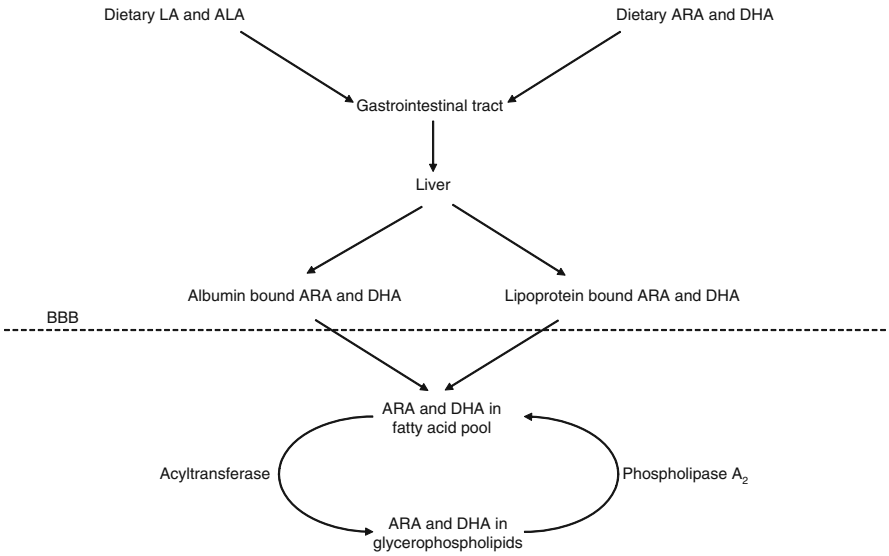

Fig. 2.2 Transport of ARA and DHA to brain

that DHA containing lyso-PtdCho may be a physiological carrier of DHA in the brain. In brain major proportion of ARA and DHA are activated and incorporated in glycerophospholipids through the action of Land cycle enzymes (acyl-CoA synthases and acyl-CoA:lysophospholipid acyltransferases) (Farooqui et al., 2000a), but small proportion of newly formed arachidonyl-CoA and docosahexaenoyl-CoA is β-oxidized. Positron emission tomography (PET) studies indicate that the adult human brain consumes ARA and DHA at rates of 17.8 and 4.6 mg/day, respectively. ARA and DHA consumption does not change significantly with age (Rapoport et al., 2007). In unanesthetized adult rats fed an *n*–3 fatty acid "adequate" diet containing 4.6% ALA as its only *n*–3 fatty acids, the rate of liver synthesis of DHA is more than sufficient to maintain brain DHA. In contrast, the rate of DHA synthesis in brain is very low. Reducing dietary ALA in the DHA-free diet produces upregulation of ALA to DHA conversion in liver but not brain due to increased expression of elongases and desaturases that catalyze the conversion of ALA into DHA (Rapoport et al., 2007). Concurrently, the loss of DHA in brain slows down due to downregulation of several of its DHA-metabolizing enzymes. Dietary ALA deficiency also facilitates the accumulation of brain docosapentaenoic acid (22:5*n*–6) and upregulates the expression of ARA-metabolizing enzymes such as cytosolic and secretory phospholipases A_2 ($cPLA_2$ and $sPLA_2$) and cyclooxygenase-2 (COX-2). It is stated that these changes along with reduced levels of brain derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB) in *n*–3 fatty acids diet may render these rats more vulnerable to neuropathological insults (Rapoport et al., 2007).

2.3 Importance of DHA in Neural Membranes

Fatty acid composition studies in various tissues indicate that DHA is highly enriched in retina and gray matter of cerebral cortex (Lauritzen et al., 2001). In adult rat brain, DHA accounts for more than 17% by weight of the total fatty acids. Similarly, in retina DHA accounts for more than 33% of the total fatty acids (Hamano et al., 1996). In human brain gray matter, DHA accounts for more than 36% and 24% of the total fatty acids in PtdSer and PtdEtn, respectively (Salem et al., 1986). However, the alkenyl groups from the PlsEtn are not included in these percentages. Because 40% of the ethanolamine glycerophospholipids in human gray matter are plasmalogens (Horrocks, 1972), ARA and DHA account for only 35–40% of the hydrocarbon chains in the gray matter ethanolamine glycerophospholipids (Farooqui and Horrocks, 2001a). The amount of DHA in the brain increases dramatically during the brain growth spurt (Lauritzen et al., 2001).

The turnover of DHA involves enzymes of deacylation/reacylation cycle (Farooqui et al., 2000a). These enzymes include plasmalogen-selective phospholipase A_2 (PlsEtn-PLA_2), docosahexaenoyl-CoA synthase, and acyl-CoA:lysolipid

acyltransferase. DHA is released from neural membrane plasmalogens by a PlsEtn-PLA_2. CoA-dependent or CoA-independent reacylation of lysoplasmalogens restores normal composition of neural membranes (Farooqui and Horrocks, 2001b; Strokin et al., 2003). Thus, DHA is used continuously for the biogenesis and maintenance of neuronal and retinal membranes throughout mammalian life (Farooqui et al., 2008). The incorporation of DHA in neural membranes affects many properties of neural membranes, including bilayer thickness, acyl chain packing free volume, and phase transition temperature (Mitchell et al., 1998; Wassall et al., 2004). DHA is specifically involved in inducing lateral phase separations into DHA-rich/cholesterol-poor and DHA-poor/cholesterol-rich lipid microdomains, and a reduced affinity between the DHA acyl chain and cholesterol has been proposed to promote phase separations (Wassall et al., 2004; Shaikh et al., 2003, 2004). This process may be involved in microdomain (raft) formation in neural membranes. Lipid microdomains are specialized regions within the plane of the plasma membrane. These regions play an important role in the compartmentalization and modulation of cell signaling. *n*–3 fatty acid-containing bilayers differentiate themselves from ARA-containing bilayers by having extremely high water permeability, minimal interaction with cholesterol, and loose acyl chain packing (Mitchell et al., 1998; Stillwell et al., 2005). *n*–3 fatty acids acyl chains provide neural membranes with lipid microdomain that serve as platform for compartmentalization, modulation, and integration of signaling (Ma et al., 2004). They also interact with proteins crucial for the assembly of membrane protein networks (Huster et al., 1998). For example, rhodopsin, a photo-receptor protein, is loosely bound to DHA-containing glycerophospholipids. This DHA-enriched environment may be involved in conformational changes in G protein-coupled photoreceptor inducing modification in activities of retinal enzymes (Mitchell et al., 1998; Rotstein et al., 2003). Thus, DHA modulates retinal cell signaling mechanisms involved in phototransduction (SanGiovanni and Chew, 2005). Studies on G protein-coupled receptor signaling in retinal rod outer segment membranes in DHA-deficient and DHA-adequate rat have indicated that second-generation DHA-deficient rats have 80% less DHA than DHA-adequate rats. In these rats, DHA is replaced by docosapentaenoic acid (DPA, 22:5*n*–6). This replacement correlates with desensitization of visual signaling in DHA-deficient retinal rod segment, and is related to reduced rhodopsin activation and cGMP phosphodiesterase activity. By analyzing the results of 26 independent 100-ns simulations of dark-adapted rhodopsin, it is suggested that DHA interacts tightly and specifically with rhodopsin at specific locations, which are different from non-specific interactions that occur with saturated acyl chains and cholesterol (Grossfield et al., 2006). Collectively, these studies suggest that DHA facilitates signaling cascades that not only modulate rhodopsin regeneration but promote the activation of other membrane-bound retinal proteins (Niu et al., 2004). Thus, DHA insufficiency is involved in retinal function alterations associated with visual defects.

In brain DHA-enriched ethanolamine and serine-containing glycerophospholipids are associated with specific proteins and are preferentially located in the intracellular leaflet in acetylcholine receptor-rich membranes. Similarly,

DHA-enriched PtdSer is also located in the inner lipid bilayer, and is an essential cofactor for the activation of protein kinase C and Raf-1 kinase. PtdSer modulates activities of diacylglycerol kinase, Na^+, K^+-ATPase, and nitric oxide synthase (Ikemoto et al., 2000). Thus, the presence of DHA in neural membranes provides them with an appropriate physical environment for the activity of integral membrane proteins. DHA also modulates ion channels and neurotransmitter receptors (Ferrier et al., 2002). As stated in Chapter 1, DHA stabilizes neuronal membrane by suppressing voltage-gated calcium currents and sodium channels (Young et al., 2000). It modulates T-cell activation via protein kinase C-α and C-ε and the NF-κB signaling pathway (Denys et al., 2005). DHA also alters the lipid composition of membrane microdomains and suppresses IL-2 receptor signaling that is involved in immunosuppressive effect of DHA (Li et al., 2005). The dietary deficiency of DHA in rhesus monkeys during their development produces diminished vision, abnormal electroretinographs, impaired visual evoked potential, and disturbed cognition. It is suggested that it is not only the amount of DHA and EPA but also the ratio of DHA and EPA to ARA that is important for the optimal brain development (Simopoulos, 2004, 2006; Farooqui, 2009).

2.4 ARA and Its Importance in Neural Membranes

PtdCho and PtdEtn are the major glycerophospholipids of neural membranes. In glycerophospholipid molecules, saturated fatty acids (palimatic or stearic acid) are located at the *sn*-1 position of glycerol moiety, whereas ARA and DHA are esterified with *sn*-2 position of glycerol moiety. ARA is evenly distributed in gray and white matter and among the different cell types in brain tissue. Rat brain contains 2.42, 0.58, 1.65, and 6.92 μmol ARA/g brain in PtdCho, PtdSer, PtdIns, and PtdEtn, respectively. Like ARA, the levels of DHA vary considerably in brain glycerophospholipids. Rat brain contains 1.69, 3.72, 0.14, and 11.08 DHA/g brain in PtdCho, PtdSer, PtdIns, and PtdEtn, respectively (Rapoport, 1999). Proportions of ARA and DHA are lower in myelin sheath than synaptic plasma membrane. The fatty acid composition of human brains glycerophospholipids is altered rapidly during development (Söderberg et al., 1991; Martínez and Mougan, 1998). In PtdEtn and PtdSer, DHA levels increase with age while ARA levels in PtdCho remain constant. During postnatal development, the levels of DHA increase less markedly than ARA, AA, and oleic acids in PlsEtn. In PtdSer, oleic acid levels increase dramatically throughout development, but the levels of ARA and DHA increase only until 6 months of age. After 6 months, DHA remains constant in PtdSer throughout life, but its percentage decreases due to the accretion of other PUFA (Martínez and Mougan, 1998; Akbar and Kim, 2002). Conversionof LA and ALA to ARA and DHA is important for brain because these fatty acids are not

only constituents of neural membranes but also precursors for eicosanoids and docosanoids, respectively. Both ARA and DHA play critical roles in cell signaling (Farooqui and Horrocks, 2006; Farooqui, 2009).

The turnover of ARA in brain glycerophospholipids is not uniform. It differs more than 10-fold among PtdIns, PtdCho, PtdSer, and ethanolamine glycerophospholipids (Corbin and Sun, 1978; Sun and Su, 1979; Washizaki et al., 1994; Rapoport, 1999), suggesting a separate role for each subclass of glycerophospholipids in the brain tissue. For example, ARA half-life in rat and mouse brain PtdIns is 1 h and is consistent with the role of polyphosphoinositides in the generation of receptor-mediated second messengers (Washizaki et al., 1994; Rapoport, 1999). In contrast, ARA half-life in PtdEtn is considerably longer, 24 h, suggesting a structural role of PtdEtn in neural membranes (Sun and Su, 1979; Washizaki et al., 1994).

2.5 Biosynthesis of *n*–3 and *n*–6 Fatty Acids in Liver

Intake of LA and ALA is important for the health of human brain and its development. The biosynthesis of ARA and DHA from LA and ALA requires the same enzyme systems and pathways involved in elongation and desaturation. Enrichment of excessive amounts of LA relative to ALA in diet not only favors oxidative modification of low-density lipoprotein cholesterol and increases platelet response to aggregation, but also elevates the levels of ARA-derived eicosanoids. These lipid mediators have prothrombotic and proinflammatory properties (Farooqui, 2009). In contrast, enrichment of ALA relative to LA in diet has inhibitory effects on the clotting activity of platelets in response to thrombin. It also promotes the production of less active eicosanoids from EPA and antiinflammatory lipid mediators (docosanoids) from DHA. Docosanoids not only downregulate proinflammatory cytokines production but also have antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory effects in cerebrovascular and cardiovascular systems (Farooqui, 2009). In past several decades, dietary shift toward the consumption of *n*–6 fatty acids at the expense of *n*–3 fatty acids is thought to be a primary cause of many diseases (Simopoulos, 2004; Farooqui et al., 2008; Farooqui, 2009). The balance between *n*–3 and *n*–6 fatty acids is essential for metabolism and maintenance of the functions of both classes of fatty acids. The availability of *n*–3 fatty acids plays a major role in regulating both fat accumulation and its elimination by the liver. Derangement of hepatic *n*–6:*n*–3 fatty acid ratio impacts on the pathogenesis of cerebrovascular and cardiovascular diseases through generation of lipid mediators that not only modulate microcirculation in various tissues but also modulate signal transduction processes associated with normal homeostasis.

2.5.1 *Biosynthesis of* n–*3 Fatty Acids in Liver*

As stated above, neurons lack enzymes necessary for de novo DHA synthesis. DHA is obtained either directly from the diet or synthesized from its main dietary *n*–3 precursor, ALA, in liver through a series of elongation and desaturation step (Scott and Bazan, 1989) (Fig. 2.3). Earlier steps of DHA synthesis in liver take place in the endoplasmic reticulum and consist of sequential alternating elongation and desaturation steps catalyzed by fatty acid elongase, Δ^6- and Δ^5-desaturase. Six elongases, namely elongase-1 to elongase-6, are known to occur in mammalian liver. Overnight starvation and enrichment of fish oil in diets represses hepatic elongase activity in livers of adult male rats (Wang et al., 2005). Diet-mediated changes in elongase activity correlate with elongase-5 and elongase-6 mRNA abundance. Desaturases are the key enzymes for the synthesis of DHA. They share common regulatory features, including dependence of expression on insulin and induction by peroxisome proliferators. A key regulator of desaturase gene expression is sterol-regulatory element binding protein-1c (SREBP-1c), which mediates transcriptional activation of Δ^6-desaturase gene by insulin (Nakamura and

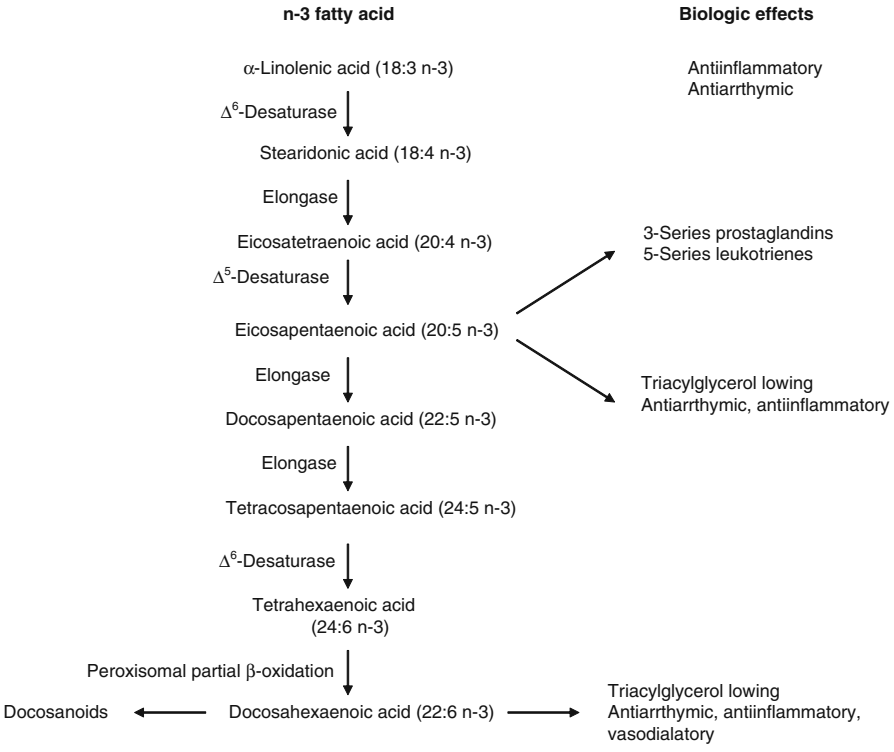

Fig. 2.3 Synthesis of DHA from ALA in liver and biologic effects of DHA

Nava, 2002). Feeding of the peroxisome proliferator-activated receptor α (PPARα) agonist, WY14,643, to adult rats increases not only hepatic elongase activity but also elongase-1, elongase-5, elongase-6, Δ^6-, Δ^5-, and Δ^9-desaturase mRNA abundance, and mead acid (20:3,*n*–9) content (Wang et al., 2005). Thus, PPARα agonists modulate both fatty acid elongation and desaturation pathways, resulting in alterations in hepatic lipid composition (Wang et al., 2005). Elongase-l activity is low in fetal liver but increases significantly after birth. Developmental changes in hepatic elongase activity parallel the postnatal induction of elongase-5 mRNA and mRNAs encoding the PPARα-regulated transcripts, Δ^6-, Δ^5-desaturase, and cytochrome P450 4A (Wang et al., 2005). In contrast, elongase-6, Δ^5-desaturase, and FAS mRNA abundance parallels alterations in hepatic sterol-regulatory element binding protein 1c (SREBP-1c) nuclear content. SREBP-1c is present in fetal liver nuclei, absent from nuclei immediately after birth, and reappears in nuclei at weaning, 21 days postpartum. Collective evidence suggests that changes in elongase-5 expression may account for much of the nutritional and developmental control of fatty acid elongation activity in the rat liver (Wang et al., 2005). Studies on comparison of DHA synthesis in rats fed with DHA-"deficient" (0.2% ALA, no DHA) diet and adequate diet (4.6% ALA of total fatty acid, no DHA) indicate that mRNA and activity levels of Δ^5- and Δ^6-desaturase and elongases 2 and 5 are upregulated in liver but not in brain. However, liver PPARα and SREBP-1 mRNA levels remain unchanged. These observations explain why the liver has a greater capacity to synthesize DHA from circulating ALA than does the brain in animals on an adequate *n*–3 PUFA diet and why liver synthesis capacity is increased by dietary deprivation (Igarashi et al., 2007a). It is proposed that Δ^6-desaturase step is the rate-limiting step in the biosynthesis of DHA (Sprecher et al., 1999). Another pathway for the synthesis of *n*–3 (DHA) involves a separate channeled carnitine-dependent mitochondrial pathway (Infante and Huszagh, 1997, 1998). In outer mitochondrial membrane, DHA synthesis starts with an 18:3*n*–3 carnitine complex. This complex is transported across the outer mitochondrial membrane, followed by binding of 18:3*n*–3 to a multifunctional enzyme complex. Multiple desaturation and elongation steps require elongases, Δ^6-, Δ^5-, and Δ^4-desaturases and two molecules of the α-tocopherol metabolites as an electron-withdrawing cofactor. Activities of desaturases and elongases are modulated by several factors. Δ^6- and Δ^5-desaturases require pyridoxine Zn^{2+} and Mg^{2+} for their activities, and saturated fats, cholesterol, and *trans* fatty acids inhibit their activities. Total fasting and glucose-rich diet decrease Δ^6-desaturase activities, whereas partial caloric restriction enhances enzymic activity. Activities of these enzymes are significantly decreased in chronic diseases such as diabetes, hypertension, hyperlipidemia, and metabolic syndrome (Das, 2007). These studies support the proposal that DHA is synthesized through endoplasmic reticulum, mitochondrial, and peroxisome pathways. Later pathway involves channeled carnitine and separate *n*–3-specific desaturases. The activity of Δ^6-desaturase is significantly lower in livers of rats fed with B-6-deficient diet than in the pair-fed control

group (approximately 64%). Acyl-CoA oxidase activity, an initial enzyme of the peroxisomal β-oxidation pathway, is reduced by approximately 80% in the B-6-deficient group. This suggests that B-6 deficiency impairs the metabolism of *n*–3 fatty acids from ALA to EPA and DHA with the most pronounced reduction occuring in the DHA synthesis (Tsuge et al., 2000). In most mammals, DHA synthesis occurs through the elongation and desaturation of ALA but, in humans, this conversion is very slow (less than 0.5%) or about 5–7 mg/day based on an ALA intake of 1,000–1,500 mg/day. Thus, in human, intake of preformed dietary DHA is necessary in order to preserve whole-body content of DHA in adults (Crawford, 2006). Metabolism of DHA is downregulated in response to intake of a small amount of *n*–3 fatty acids (Bourre et al., 1989). The continuous intake of DHA influences the rate of 22:6*n*–3 metabolism in the liver. The administration of 22:6*n*–3 to rats for 14 days induces an increase in β-oxidation and reduces Δ^5- and Δ^6-desaturations of *n*–6 fatty acid in hepatocytes, but has no affect on the synthesis of DHA from EPA (Gronn et al., 1992). In addition, hepatic peroxisomal β-oxidation is upregulated by the administration of a large amount of 22:6*n*–3 for 10 days in rats, while mitochondrial β-oxidation is not affected by DHA (Willumsen et al., 1993). Collectively, these studies suggest that essential DHA levels and metabolism are maintained when rats are fed with small amount of DHA.

As stated above, Δ^6- and Δ^5-desaturases have been purified, characterized, and cloned from several sources (Cho et al., 1999a,b; Nakamura and Nara, 2004). The open reading frame of the human Δ^5desaturase encodes a 444-amino acid peptide, which is identical in size to the Δ^6-desaturase. It also shares 61% identity with the human Δ^6-desaturase (Cho et al., 1999a,b). The Δ^5-desaturase contains two membrane-spanning domains, three histidine-rich regions, and a cytochrome b5 domain; all aligning perfectly with the same domains located in the Δ^6-desaturase. Expression of the open reading frame in Chinese hamster ovary cells results in the conversion of 20:3(*n*–6) to 20:4(*n*–6). Northern analysis indicate that many human tissues including skeletal muscle, lung, placenta, kidney, and pancreas express Δ^5-desaturase mRNA, but Δ^5-desaturase is abundantly distributed in the liver, brain, and heart. However, other tissues have low Δ^5-desaturase mRNA compared to Δ^6-desaturase. When diet is supplemented with 10% safflower oil or menhaden fish oil, the level of hepatic mRNA for Δ^6- and Δ^5-desaturase is only 25% of that found in the liver of rats fed a fat-free diet or a diet containing triolein. A BLAST and Genemap search of the human genome indicates that the Δ^6- and Δ^5desaturase genes reside in reverse orientation on chromosome 11 and that they are separated by $<$11,000 base pairs (Cho et al., 1999a,b). In mouse liver, during aging Δ^6-desaturase activity increases sevenfold between day 3 before birth and day 11 after birth, then decreases slightly up to weaning. The activity remains constant up to 9 months (Bourre and Piciotti, 1992).

Cerebral endothelium synthesizes DHA from dietary precursors via Δ^6-desaturation and retroconversion steps, whereas astrocytes are able to synthesize DHA either from 18-, 20-, and 22-carbon *n*–3 precursors (via elongation and

desaturation steps) or from 24-carbon precursors (Innis and Dyer, 2002). However, the synthesis of DHA in astrocytes is a minor process in quantitative terms as compared to DHA supplied to brain tissue from plasma. Brain development requires large amounts of PUFA for neural membrane synthesis, and DHA is specifically enriched in neuronal membranes for participation in neuronal signaling in response to various stimuli. Determination of Δ^6-desaturase activity in mouse brain during aging indicates that enzymic activity decreases dramatically (fourfold) during pre- and postnatal development up to weaning, and highest activity is detected in prenatal period (Bourre and Piciotti, 1992). Surprisingly, Δ^6-desaturase activity does not peak during intense period of myelinogenesis. It is estimated that from 2% to 8% of rat brain phospholipid DHA is replaced daily with DHA from the plasma unesterified fatty acid pool (Rapoport et al., 2001). Collective evidence suggests that synthesis of *n*–3 fatty acids from ALA requires movement of a 24-carbon intermediate from the endoplasmic reticulum to peroxisomes for retroconversion to DHA. The mechanism of this intra-organelle flux is unknown. It is suggested that fatty acid-binding proteins (L-FABP) facilitate this process. Studies on interactions of very long chain PUFAs to liver fatty acid-binding protein (L-FABP) indicate that fatty acids bind to L-FABP strongly with dissociation constant (K_d) value of approximately 10^{-8} to 10^{-7} M for 20-, 22-, and 24-carbon *n*–3 fatty acid (Norris and Spector, 2002).

2.5.2 Biosynthesis of n*–6 Fatty Acids in Liver*

The biosynthesis of ARA from LA also requires a series of desaturation and elongation steps catalyzed by same elongases and Δ^6- and Δ^5-desaturases involved in *n*–3 fatty acid synthesis (Fig. 2.4). Fatty acid Δ^6-desturation is a rate-limiting step in the conversion of LA to ARA. Although the synthesis of ARA in liver does not require intra-organelle transport from endoplasmic reticulum to peroxisomes, ARA binds to fatty acid-binding protein (L-FABP) with strong affinity and has K_d approximately 10^{-8} to 10^{-7} M. This observation raises the possibility that L-FABP may participate in the cytoplasmic processing of *n*–3 and *n*–6 fatty acids (Norris and Spector, 2002). Feeding of fresh Baobab seed oil containing cyclopropene fatty acids (malvalic acid and sterculic acid) or heated Baobab seed oil practically devoid of these fatty acids or control oil to rats downregulates both Δ^6- and Δ^5-desaturations in rat microsomes, but Δ^6- more than Δ^5-desaturation (Cao et al., 1993). The decreased capacity of microsomes to desaturate is reflected in the lower ARA content in microsomal glycerophospholipids from rats fed with fresh Baobab seed oil. Collective evidence suggests that cyclopropene fatty acids specifically inhibit incorporation of the Δ^5-desaturation product into glycerophospholipids. Other possibility is that they specifically inhibit desaturation of the substrate previously incorporated into a membrane glycerophospholipid (Cao et al., 1993). In

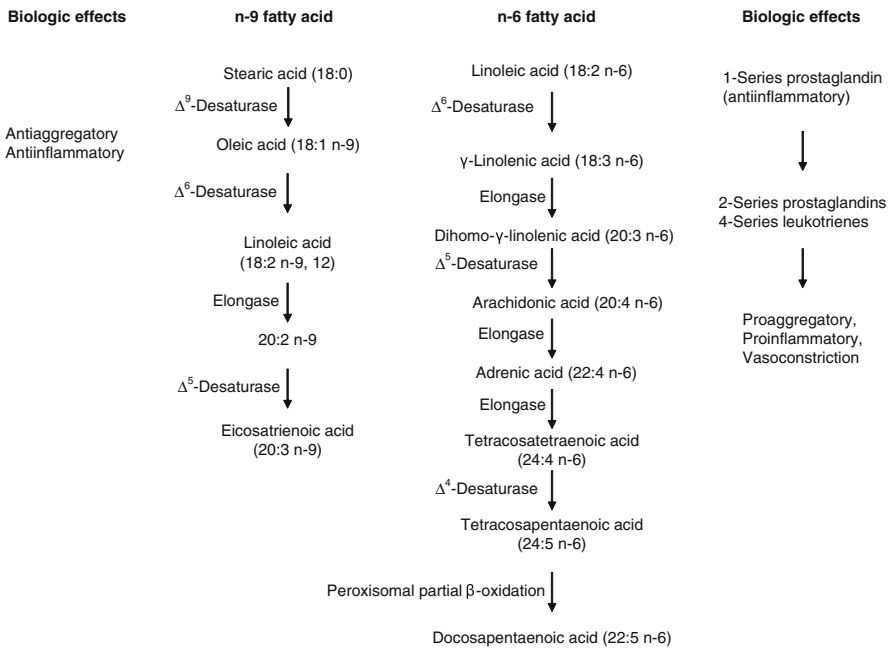

Fig. 2.4 Synthesis of ARA from LA and conversion of stearic acid into eicosatrienoic acid in liver along with their biologic effects

liver, many nutritional, hormonal, and physiological factors modulate ARA biosynthesis (Bezard et al., 1994). Dietary fatty acids exert a great influence and are often inhibitory. Thus, dietary ALA inhibits Δ^6-desaturation of LA. The desaturation products ARA, EPA, and DHA inhibit Δ^6-desaturation of LA and Δ^5-desaturation of DGLA (dihomo-gamma-linolenic acid). With regard to hormones, insulin and thyroxin are necessary for Δ^6- and Δ^5-desaturation activities, whereas other hormones (glucagon, epinephrine, ACTH, glucocorticoids) inhibit desaturation (Bezard et al., 1994). Δ^6-Desaturation is also modulated by vitamin B6. This vitamin is a cofactor for Δ^6-desaturase as well as *trans*-sulfuration pathway of homocysteine. It is proposed that low levels of this vitamin may contribute to heart disease by increasing homocysteine levels (Cabrini et al., 2005). The deletion of Δ^6-fatty acid desaturase gene expression in the mouse abolishes the initial step in the enzymic cascade of PUFA synthesis. The lack of PUFA and eicosanoids does not impair the normal viability and lifespan of male and female Δ^6-fatty acid desaturase deficient mice (fads2–/– mice) but causes sterility (Stoffel et al., 2008). The fads2–/– mouse is an auxotrophic mutant, which can be used to study glycerophospholipid membrane hemostasis, inflammation, and oxidative stress.

2.6 Incorporation of Fatty Acids in Glycerophospholipids

Turnover rates of ARA and DHA in brain membrane glycerophospholipids are rapid and energy consuming. The turnover rate of these fatty acids is regulated by acyl-CoA synthetases (ACSs) and isoforms of PLA_2 in Land cycle (Fig. 2.5) (Rapoport, 1999, 2008; Rapoport et al., 2001). The ARA turnover in brain glycerophospholipids is not only inhibited by $cPLA_2$ or ACSs inhibitors but also downregulated by inhibitors of cyclooxygenases (Farooqui and Horrocks, 2007). These inhibitors have no effect on DHA turnover and expression of DHA-selective calcium-independent $iPLA_2$. In addition, antimanic drugs (lithium and carbamazepine) downregulate transcription factor AP-2, which in turn modulates the expression and activity of $cPLA_2$ resulting in a selective downregulation of ARA turnover (Chen et al., 2008). Furthermore, targeting arachidonoyl-CoA formation via non-competitive inhibition of an ACS with valproate also selectively decreases brain ARA turnover. In contrast, NMDA and fluoxetine-mediated increase in $cPLA_2$ activity enhances brain ARA turnover in glycerophospholipids (Chen et al., 2008). Based on various studies, it is proposed that the brain ARA and DHA cascades are reciprocally modulated by dietary or genetic conditions. Thus, following 15 weeks of dietary *n*–3 fatty acid deprivation, DHA loss from rat brain is slowed because of low $iPLA_2$ and COX-1 expression, whereas expressions of $cPLA_2$, $sPLA_2$, and COX-2 are upregulated (Rapoport et al., 2001; Rapoport, 2007, 2008; Chen et al., 2008).

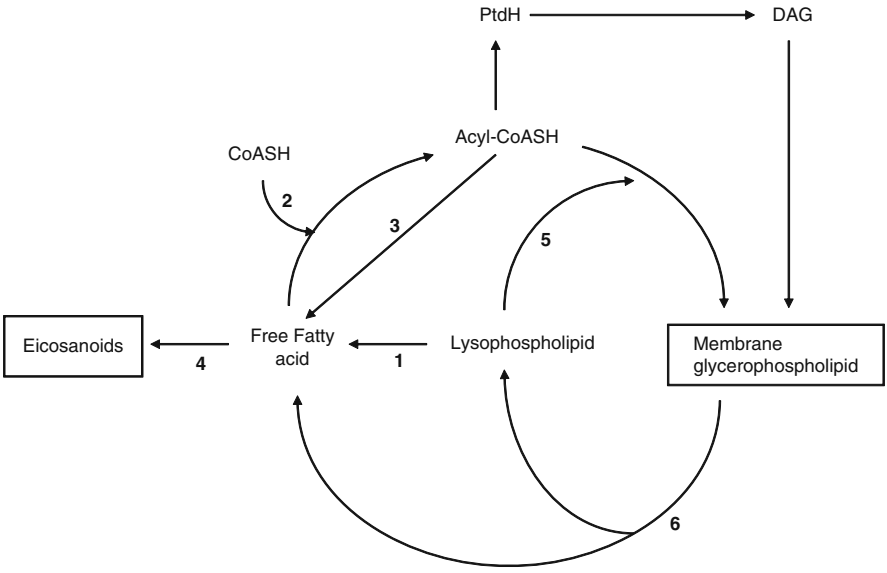

Fig. 2.5 Deacylation–reacylation of glycerophospholipids in brain. Lysophospholipase (1); acyl-CoA synthetase (2); acyl-CoA hydrolase (3); cyclooxygenase and lipoxygenase (4); acyl-CoA:lysophospholipid acyltransferase (5); and phospholipase A_2 (6)

2.6.1 Acyl-CoA Synthetases in Brain

ACSs are a group of enzymes that catalyze the thioesterification of fatty acids with coenzymeA to form activated acyl-CoA derivatives, which not only play a fundamental role in lipid metabolism but also modulate lipid homeostasis (Fig. 2.5). The products of the ACS enzyme reaction, acyl-CoAs, are required for complex lipid synthesis, energy production via β-oxidation, protein acylation, and fatty acid-dependent transcriptional regulation (Farooqui et al., 2000a). As stated earlier, free fatty acids can cross neural membranes either through passive diffusion, and the synthesis of acyl-CoA essentially locks them within the cell because acyl-CoA derivatives are impermeable to membranes (Pownall, 2001).

Activation of fatty acids is a two-step reaction catalyzed by ACSs. In the first step, an acyl-AMP intermediate is generated from ATP. AMP is then exchanged with CoA to produce the activated acyl-CoA. The generation of AMP in ACS-catalyzed reaction defines the superfamily of AMP-forming enzymes. The length of the carbon chain of the fatty acid species defines the substrate specificity for different ACSs. On this basis, several families of ACS have been characterized. They are encoded by separate genes and differ from each other in fatty acid preference, subcellular localization, and regulation (Cao et al., 2000; Lewin et al., 2001; Coleman et al., 2000). Although five genes, several isoforms, and variants have been identified, limited information is available on their biochemical properties and localization in brain (Nemazanyy et al., 2006; Soupene and Kuypers, 2008). Some isoforms are associated with the activation of short-chain fatty acids while others are involved in the activation of long-chain fatty acids. Long-chain ACSs are associated with the activation of ARA and DHA in mammalian brain. The incubation of [1-^{14}C]ARA with brain microsomes in the presence of ATP, CoA, and $MgCl_2$ results in the formation of [1-^{14}C]arachidonyl-CoA. The omission of ATP or CoA results in a 98% decrease in enzymic activity, indicating the absolute requirement of ATP and CoA for the ACS reaction (Reddy et al., 1984; Reddy and Bazan, 1984). The addition of unlabeled ARA and DHA results in the apparent inhibition of enzymic activity with K_i values of 31 μM for both fatty acids. Based on various kinetic data, a single ACS may be involved in fatty acyl-CoA formation. In contrast, other investigators have separated ACS activities of rat brain microsomes into three forms; (a) very long-chain acyl-CoA, (b) long-chain acyl-CoA, and (c) medium-chain acyl-CoA synthetases.

Several isoforms of long-chain acyl-CoA synthetase (ACSL) have been reported to occur in brain tissue. Some ACSL isoforms have been characterized and cloned from rat brain (Marszalek et al., 2005; Van Horn et al., 2005). ACSL3 and ACSL6 are the predominant ACSL isoforms in brain. These isoforms have been cloned from rat brain. ACSL3, ACSL4, and ACSL6 display similarities in kinetic parameters for CoA, palmitic acid, and ARA. The apparent K_m values of ACSL3 and ACSL6 for oleic acid are fourfold lower than for ACSL4. In a direct competition assay with palmitic acid, all the PUFA

competitively inhibit its activation. DHA is a preferred substrate for ACSL6. Two variants of ACSL6 (ACSL6 v1 and ACSL6 v2) are known to occur in brain tissue. The apparent K_m value for ATP of ACSL6 v1 is eightfold higher than that of ACSL6 v2. ACSL3 and ACSL6 v1 and ACSL6 v2 are more resistant to heat inactivation than ACSL4. Despite the high amino acid identity between ACSL3 and ACSL4, rosiglitazone, an antidiabetic drug, inhibits only ACSL4. Triacsin C, an inhibitor of ACSL1 and ACSL4, also inhibits ACSL3, but has no effect on ACSL6 variants (Van Horn et al., 2005). ACSL4 plays an important role in ARA and EPA metabolism. This form is highly expressed in brain, placenta, testis, spleen, and adrenal cortex (Cao et al., 2000). Acyl-CoA synthases compete with eicosanoid-synthesizing enzymes for ARA and DHA, thereby regulating the pool size of free ARA and DHA (Reddy et al., 1984; Reddy and Bazan, 1984; Farooqui et al., 2000a,b). Thus, upregulation of these enzymes and rapid synthesis of arachidonyl-CoA and docosahexaenoyl-CoA in brain may play an important role in retaining these essential fatty acids in neural membrane glycerophospholipids. In addition to being intermediates in fatty acid metabolism in brain, acyl-CoAs also modulate ion fluxes, vesicle trafficking, protein phosphorylation, and gene expression (Faergeman and Knudsen, 1997). Long-chain acyl-CoAs are involved not only in long-term potentiation in the hippocampus (Zhang et al., 2000) but also in synaptic vesicle formation (Schmidt et al., 1999). Different long-chain acyl-CoAs, existing in different cellular compartments, perform these functions (Fig. 2.6). In these compartments, the intracellular concentrations of acyl-CoAs are strictly controlled by the balance among acyl-CoA-synthesizing enzymes, acyl-CoA-utilizing enzymes (acyl-CoA:lysophospholipid acyltransferase, and acyl-CoA

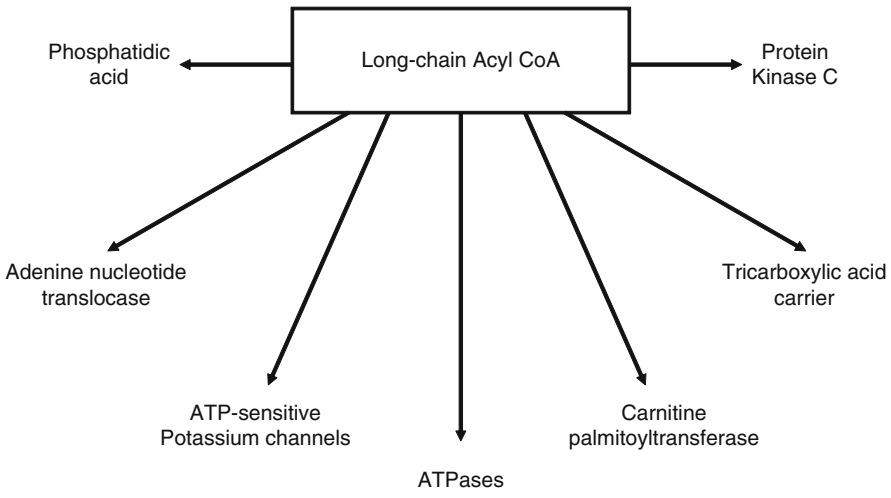

Fig. 2.6 Role of acyl-CoA in the modulation of various processes in brain

hydrolases), and fatty acid-metabolizing enzymes (Farooqui et al., 2000a; Corkey et al., 2000).

Another example of acyl-CoA functional variation is malonyl-CoA. This intermediate of fatty acid biosynthesis is involved in the inhibition of carnitine palmitoyl-transferase 1c (CPT1c), a mitochondrial outer membrane enzyme that initiates translocation of fatty acids into mitochondria for oxidation (Wolfgang et al., 2006, 2008). Brain contains three carnitine palmitoyl-transferases, which catalyze acyl transfer from various fatty acyl-CoAs to carnitine. Although CPT1a and CPT1b catalyze acyl transfer from various fatty acyl-CoAs to carnitine, CPT1c does not. These findings suggest that CPT1c has a unique function or activation mechanism. CPT1c has very high affinity for malonyl-CoA and binds with it rather tightly but does not facilitate fatty acid oxidation in vivo, in hypothalamic explants, or in heterologous cell culture systems (Wolfgang et al., 2008). CPT1c knockout (KO) mice have normal levels of metabolites and hypothalamic malonyl-CoA and fatty acyl-CoA in both fasted and refed states. The CPT1c KO mice show a decrease in food intake and lower body weight than wild-type littermates. CPT1c KO mice gain excessive body weight and body fat when given a high-fat diet while maintaining lower or equivalent food intake. Heterozygous mice display an intermediate phenotype. These observations support the view that CPT1c plays a role in maintaining energy homeostasis, but not through altered fatty acid oxidation (Wolfgang et al., 2006, 2008), and hypothalamic malonyl-CoA may be involved in the regulation of feeding behavior by altering the expression of key orexigenic (neuropeptide Y and agouti-related peptide) and anorexigenic (proopiomelanocortin and α-melanocyte-stimulating hormone) neuropeptides (Hu et al., 2005).

The intracellular levels of free unbound acyl-CoA esters are tightly regulated by feedback inhibition of the acyl-CoA synthetase and are buffered by specific acyl-CoA-binding proteins. In micromolar range, long-chain Acyl-CoAs regulate activities of many enzymes, receptors, and transporters. Long-chain-CoAs exclusively modulate activities of those proteins that contain adenine or guanine nucleotide-binding sites. This is because of similarities of their structures with Coenzyme A (Prentki and Corkey, 1996). In non-neural cells, long-chain-CoAs facilitate opening of the ATP-sensitive K^+-channels. This is contrast to free fatty acids that promote closing of ion channels. Long-chain-CoAs are not only essential for vesicular processing through the Golgi but also associated with complex needed for the insertion of VIP21-caveolin, a cholesterol-binding protein localized to both the plasma membrane caveolae and the *trans* Golgi network (Monier et al., 1996). Interactions between long-chain-CoA and VIP21-caveolin play important roles in vesicle or lipid trafficking (Monier et al., 1996). Excessive increase in acyl-CoA levels can be prevented by their conversion into acylcarnitines or by hydrolysis by acyl-CoA hydrolases (Lin et al., 1984; Corkey et al., 2000). Under normal physiological conditions, the free cytosolic concentrations of acyl-CoAs are in the low nanomolar range, and it is unlikely to exceed 200 nM under the most extreme conditions (Faergeman and Knudsen, 1997).

2.6.2 *Acyl-CoA:lysophospholipid Acyltransferase in Brain*

Acyl-CoA:lysophospholipid acyltransferase catalyzes the transfer of acyl group from acyl-CoA to lysophospholipid acceptor (Fig. 2.5). Subcellular distribution studies indicate that acyl-CoA:lysophospholipid acyltransferase is located in microsomal fraction and can be solubilized from bovine brain microsomes using Miranol (Baker and Chang, 1981; Deka et al., 1986). The solubilized enzyme is purified 3,000-fold from bovine brain microsomes using DEAE-cellulose, Blue-2-agarose, and Matrex green chromatographies, with an overall recovery of 6.0% (Deka et al., 1986). Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate revealed a single protein band with a molecular mass of 43 kDa (Table 2.1). The purified enzyme is specific for lyso-PtdCho. Enzyme shows less specificity toward the acyl-CoA derivatives. Among various acyl-CoAs, enzyme shows preference for arachidonyl-CoA. Short-chain acyl-CoA shows low activity. No activity is observed with palmitoyl-CoA or stearoyl-CoA (Deka et al., 1986). High concentrations of arachidonoyl-CoA inhibit the enzymic activity. Variation of lyso-PtdCho concentration at fixed concentration of arachidonyl-CoA and enzyme results in a sigmoidal shape substrate concentration curve. The specificity and kinetic properties of the enzyme can be altered by incorporation of enzyme into liposomes, which are composed of a mixture of glycerophospholipids. Decanoyl-CoA and myristoyl-CoA, which show significant activity with the soluble enzyme, do not serve as acyl donors for the liposome-bound acyltransferase. In contrast to the soluble form of the enzyme, the liposome-bound enzyme is active at concentrations of lyso-PtdCho below the critical micelle concentration. The liposome-bound enzyme is substantially less susceptible to thermal denaturation and proteolytic digestion than soluble enzyme (Deka et al., 1986). Differences in the rate of acylation of different lysophospholipid subclasses of glycerophospholipids by human brain acyl-CoA:lysophospholipid acyltransferase have also been reported (Ross and Kish, 1994). The rate with lysophosphatidylinositol (lyso-PtsIns) is highest, followed by lyso-PtdCho and Lyso-PtdSer. In contrast, acylation of lyso-PtdEtn is

Table 2.1 Physicochemical and kinetic properties of enzymes associated with remodeling pathway in the brain tissue

Properties	Acyl-CoA synthetase	AcylCoA:lysophospholipid acyltransferase	References
Localization	Endoplasmic reticulum	Endoplasmic reticulum	Reddy and Bazan (1983); Deka et al. (1986)
pH optimum	8.5	7.8	Reddy and Bazan (1983); Deka et al. (1986)
K_m value (μM)	36.0	2.0	Reddy and Bazan (1983); Deka et al. (1986)
V_{max} value (nmol/min/mg)	32.4	1300	Reddy and Bazan (1983); Deka et al. (1986)

Modify from Farooqui et al. (2000b).

barely detectable. Based on various kinetic and metabolic studies, lyso-PtdCho acyltransferase may compete with acyl-CoA hydrolase and lysophospholipase for acyl-CoA and lyso-PtdCho, respectively (Ross and Kish, 1994).

The occurrence of an acyl-CoA:lysophosphatidylcholine acyltransferase activity in rod outer segments has also been reported (Castagnet and Giusto, 1997). This enzyme shows maximum activity at pH 7.0. It transfers 60% of oleoyl group from [1-^{14}C]oleoyl-CoA to PtdCho in 5 min. Variation of oleoyl-CoA concentration at 46 μM lyso-PtdCho gives apparent K_m and V_{max} values of 100 μM and 2.5 pmol/min/mg protein, respectively. Variation of lyso-PtdCho concentration with 100 μM oleoyl-CoA gives apparent K_m and V_{max} values of 27 μM and 2.51 pmol/min/mg protein, respectively (Castagnet and Giusto, 1997). The enzymic activity is inhibited by $MgCl_2$. Substrate specificity experiments indicate that acyltransferase utilizes palmitoyl-CoA and arachidonoyl-CoA for the transfer of acyl group to rod outer segment glycerophospholipids and to acylate other lysophospholipids but less efficiently than lyso-PtdCho. Lyso-PtdCho is preferentially acylated with ARA, followed by oleic acid and less efficiently with palmitic acid. The high specific activity of acyl-CoA lysophosphatidylcholine acyltransferase that occurs in purified rod outer segments compared to the activity that is associated with other subcellular fractions of the bovine retina suggests that this enzymic activity is native to the rod outer segments (Castagnet and Giusto, 1997).

Identification and characterization of a murine gene that encodes an acyl-CoA:lysocardiolipin acyltransferase 1 (ALCAT1) has also been reported (Cao et al., 2004). Expression of the ALCAT1 cDNA in either insect or mammalian cells results in a significant increase in acyl-CoA:monolysocardiolipin acyltransferase and acyl-CoA:dilysocardiolipin acyltransferase activities. Substrate specificities studies indicate that recombinant ALCAT1 enzyme recognizes both monolysocardiolipin and dilysocardiolipin, and shows preference for linoleoyl-CoA and oleoyl-CoA as acyl donors (Cao et al., 2004). In contrast, no significant increases in acyltransferase activities by the recombinant ALCAT1 are detected either with glycerol-3-phosphate or with a variety of other lysophospholipids as substrates, including lyso-PtdCho, lyso-PtdEtn, and lyso-PtdSer (Cao et al., 2004). Immunocytohistochemical and Northern blotting studies indicate that liver and heart show highest expression of ALCAT1 enzyme, and this enzyme is localized in the endoplasmic reticulum. It is proposed that ALCAT1 plays an important role in cardiolipin-remodeling in endoplasmic reticulum (Cao et al., 2004).

Collective evidence suggests that several acyl-CoA:lysophospholipid acyltransferases occur in neural and non-neural tissues (Castagnet and Giusto, 1997; Zhao et al., 2008; Cao et al., 2004, 2008). At least three members of acyl-CoA:lysophospholipid acyltransferase family are found in mammalian tissues. Some isoforms have been purified, characterized, and cloned from mammalian RBC, liver, and brain tissues (Cao et al., 2008; Soupene et al., 2008; Zhao et al., 2008; Hishikawa et al., 2008), while others forms have not been characterized. There is evidence that multiple genes can encode one enzymic activity and that a given gene may encode multiple activities. A gene

encoding a mammalianacyl-CoA-dependent lysophospholipid acyltransferase 2 (LPEAT2, previously called as AYTL3 or AGPAT7) has been identified. LPEAT2 is predominantly expressed in brain along with an enrichment of PtdEtn in this tissue. Ectopic expression of LPEAT2 in mammalian HEK293T cells results in a dramatic increase (up to ninefold) in LPEAT activity compared to cells transfected with empty vector or an unrelated acyltransferase (Cao et al., 2008). LPEAT2 also shows significant acyl-CoA-dependent acyltransferase activity toward 1-*O*-alkenyl-lysophosphatidylethanolamine (1-alkenyl-Lyso-PtdEtn), lysophosphatidylglycerol (lyso-PtdGly), 1-*O*-alkyl-lysophosphatidylcholine (lyso-PAF), lysophosphatidylserine (lyso-PtdSer), and lyso-PtdCho, but displays no appreciable acylating activity toward glycerol 3-phosphate, lysophosphatidic acid (lyso-PtdH), lysophosphatidylinositol (lyso-PtdIns), and diacylglycerol (DAG), indicating that LPEAT2 is involved in multiple but selective functions in brain tissue. LPEAT2 can transfer acyl groups from medium- and long-chain fatty acyl-CoA, and its activity is not affected by Ca^{2+}. LPEAT2 can be overexpressed in mammalian cells where it is localized to the endoplasmic reticulum. In HEK293T cells, siRNA inhibits LPEAT2 activity against lyso-PtdEtn and 1-alkenyl-lysoPtdEtn, but has no affect on other lysophospholipid-acylating activities. Acyl-CoA:lysophospholipid acyltransferase-mediated remodeling of glycerophospholipids may be involved in maintaining membrane asymmetry and diversity, which modulates membrane fluidity and curvature. Presence of multiple forms of acyl-CoA:lysophospholipid acyltransferases and their genes encoding these activities explains the occurrence of more than 100 glycerophospholipids molecular species in brain and other mammalian tissues (Yamashita et al., 1997; Cao et al., 2008). Neural membrane diversity is produced by the concerted and overlapped reactions with multiple enzymes of remodeling pathway that recognizes both the polar head group of glycerophospholipids and various acyl-CoAs (Shindou et al., 2009). Signal transduction processes are facilitated by lipid mediators, which are generated by different acyl chains of glycerophospholipid molecular species. In the remodeling pathway, reacylation reaction catalyzed by specific acyltransferases controls glycerophospholipid composition and the availability of free ARA and DHA (Farooqui et al., 2000a,b). Lysophosphatidylcholine acyltransferases modulate inflammatory responses to lipopolysaccharide and other microbial stimuli (Jackson et al., 2008a). Specific inhibition of lysophosphatidylcholine acyltransferase down-regulates inflammatory cytokine production in monocytes and epithelial cells by preventing translocation of Toll-like receptor (TLR4) into membrane lipid raft domains. This observation indicates the existence of new regulatory mechanisms that not only facilitate the innate immune responses to microbial molecular patterns but also provide a basis for the antiinflammatory activity observed in many glycerophospholipid-derived metabolites (Jackson et al., 2008a; Farooqui, 2009). Lysophospholipid acyltransferases, phospholipipases A_2, C, and D, and cyclooxygenases and lipoxygenases are key enzymes that control cellular responses to a variety of stimuli through the generation of lipid

mediators that modulate oxidative stress and inflammation in brain (Farooqui et al., 2000a, 2000b; Farooqui, 2009). Regulation or manipulation of lysophospholipid acyltransferases, phospholipipases A_2, C, and D, and cyclooxygenases and lipoxygenases may thus provide important mechanisms for novel antioxidant and antiinflammatory therapies for neurotraumatic and neurodegenerative diseases (Jackson et al., 2008b; Farooqui et al., 2008; Farooqui, 2009).

Dietary deficiency of DHA reduces DHA recycling in brain glycerophospholipids, but has no significant effect on ARA recycling (Contreras et al., 2000, 2001), suggesting that Land cycle enzymes (Fig. 2.5) that regulate ARA recycling are different from those enzymes that regulate DHA recycling. Thus, rabbit platelet $cPLA_2$ can discriminate between DHA and ARA esterified at the *sn*-2 position of glycerophospholipids (Shikano et al., 1994). The incorporation of labeled DHA differs from that of ARA, as it incorporates into ethanolamine glycerophospholipids in greater amounts in comparison with its mass distribution. This indicates that substrate specificity of docosahexaenoyl-CoA:lysophospholipid acyltransferase is different from arachidonyl-CoA:lysophospholipid acyltransferase (Onuma et al., 1984; Laposata et al., 1985). Furthermore, chronic lithium feeding to rats downregulates ARA turnover in brain glycerophospholipids by 80% without affecting turnover of DHA or palmitate. These observations support the view that Land cycle utilizes different phospholipasesA_2, acyl-CoA synthetases, and acyltransferases to recycle DHA and ARA. Differences in substrate specificities of phospholipasesA_2, acyl-CoA synthetases, and acyltransferases may promote independent functions of DHA and ARA in neural membranes.

2.6.3 CoA-Independent Reacylation in Brain

Brain also contains the CoA-independent transacylation system in microsomes (Ojima et al., 1987; MacDonald and Sprecher, 1991; Yamashita et al., 1997). This system transfers fatty acids from diacyl glycerophospholipids to various glycerophospholipids in the absence of any cofactor. The acyl-substrate is a fatty acid esterified at the *sn*-2 position of a diacylglycerophospholipid. The only fatty acids transferred by this system are C_{20} and C_{22} PUFA. Diacylglycerophosphocholine is the most preferred substrate. Human brain homogenates possess the ability to transfer fatty acids from lysoPtdCho to lysoPtdEtn but not to lysoPtdSer or lysoPtdIns (Ross and Kish, 1994).

2.7 Incorporation of ALA and LA in Brain Lipids

Intracranial injections of either [1-^{14}C]ALA or [1-^{14}C]LA in19- to 20-day-old rat fetuses indicate a rapid disappearance of free LNA and LA, with apparent half-lives of 60 and 40 min, respectively (Green and Yavin, 1993). One hour

after [1-^{14}C]ALA injection, 32.3% and 14.3% of the total brain radioactivity is associated with neutral acylglycerol and glycerophospholipid fractions, respectively. After 20 h, 75% of radioactive fatty acid ends up in glycerophospholipids. PtdCho, diacylglycerol (DAG), and triacylglycerol (TAG) account 40, 23, and 9% of the total brain label at 1 h, and 35, 10 and 14% at 20 h, respectively (Green and Yavin, 1993). Ethanolamine-containing glycerophospholipids (including plasmalogen) contain about 10% radioactivity after 6 h, and radioactivity is increased nearly threefold at 20 h primarily due to an increase in the amount of labeled docosapentaenoic acid (DPA) and DHA, the elongation–-desaturation products of ALA. The pattern of incorporation of LA into neutral and glycerophospholipid fraction is similar to ALA. Thus, after 1 h, PtdCho, DAG, and TAG show 23, 10, and 23% of the total brain radioactivity, whereas after 20 h these lipids contain 44, 6, and 10% radioactivity, respectively. Although radioactivity in the ethanolamine glycerophospholipids also increases substantially from 4% at 1 h to 29% at 20 h, the main radioactivity is in LA. Labeled ARA constitutes 42.7% of the total radioactivity in PtdIns at 20 h (Green and Yavin, 1993). Comparison of the total amounts of LA and ALA and their corresponding labeled ARA and DHA metabolites in brain and liver after 3 and 6 h indicates that the contribution of liver metabolism to the elongation–desaturation under these conditions is negligible. One hour after intracerebral injection of [^{3}H]DHA or [^{3}H]ARA, 29.2% and 12% of total radioactivity, respectively, is associated with the ethanolamine glycerophospholipids while 20% and 40% incorporates in PtdCho, respectively. PI labeling by [^{3}H]ARA is six- to eightfold higher than that observed in the presence of DHA. A high percent of radioactivity (26.9% and 18.2%) is found in DAG and TAG species (Green and Yavin, 1993). These studies suggest that there is a high degree of selectivity in esterification of ALA and LA in brain tissue.

2.8 Incorporation of Docosahexaenoic Acid in Neural Membranes in Glycerophospholipids

Studies on the fate of intravenously co-injected ^{14}C-DHA and ^{3}H-oleic acid (OA) into mice indicate that 5 min after injection, more than 40% of the ^{14}C-DHA but less than 20% of the ^{3}H-OA, labels are associated with the liver. The uptake of ^{14}C-DHA in mice heart is higher than ^{3}H-OA. The incorporation of ^{14}C-DHA in brain is very slow. The incorporation for ^{14}C-DHA and ^{3}H-OA reaches 0.7% at 24 h and 1–1.5% level at 48 h, respectively (Polozova and Salem, 2007). Total ^{14}C activity in plasma reaches 2% of the injected dose at 20 min and levels off at 0.5% after 1.5 h. Fifteen percent of ^{14}C-DHA plasma activity at 30 min remains associated with non-esterified fatty acids, whereas about 85% goes to triacylglycerol in very low-density lipoprotein (VLDL) and LDL fractions. Only 30% of ^{3}H-OA remains associated with the VLDL fraction at 30 min. These studies indicate that liver plays an important

role in loading DHA lipoproteins and then delivering it to brain, heart, and other target tissues (Polozova et al., 2006; Polozova and Salem, 2007).

Studies on the incorporation of DHA in glycerophospholipids have been performed in rat retina (Stinson et al., 1991) and brain (DeMar et al., 2004; Igarashi et al., 2007a, b) of DHA-deprived and DHA-adequate rats. The half-life of DHA in retinal glycerophospholipids is 19 days (Stinson et al., 1991). Half-lives of DHA in individual glycerophospholipids of DHA-adequate rat brain ranges from 23 to 56 days. The docosahexaenoyl-CoA that enters the brain and passes through the docosahexaenoyl-CoA is esterified into brain glycerophospholipids at a rate of 13–15 pmol/g brain/s. This DHA pool is predominantly esterified to ethanolamine-containing glycerophospholipids (6–7 pmol/g brain/s) and choline-containing glycerophospholipids (4–5 pmol/g brain /s) (Lee et al., 2005; Green et al., 2008). Studies on fatty acid composition of brain microsomes from offspring of rats artificially reared on an *n*–3-deficient diet indicate a dramatic reduction of DHA content when compared with control animals. This decrease is accompanied by an increase in DPA content, which replaces the DHA-containing glycerophospholipids with DPA molecular species (Garcia et al., 1998). The *n*–3 deficiency has no effect on the total PUFA in brain microsomal glycerophospholipids. However, *n*–3-deficient diet results in a decrease in the total polyunsaturated PtdSer content and with an increase in levels of 1-stearoyl-2-docosapentanoyl (18:0/22:5*n*–6) species, particularly in PtdCho. Incorporation of [^{3}H]serine into PtdSer in rat brain microsomes from *n*–3-deficient animals is significantly lower than that of the control animals (Garcia et al., 1998), indicating that neuronal and glial PtdSer synthesis is sensitive to changes in the DHA levels.

Subcellular distribution studies on the distribution of [^{3}H]DHA in rat brain after intravenous infusion show that 60% and 30% radioactivity incorporates into synaptosomal and microsomal membrane fraction, respectively. The administration of arecoline, a cholinergic agonist, results in a marked increase in the incorporation of [^{3}H]DHA in the synaptosomal and microsomal fractions. In contrast, arecoline treatment has no effect on the incorporation of [^{3}H]palmitic acid. The identification of glycerophospholipids subclasses in various subcellular fraction indicates that DHA mainly incorporates at the *sn*-2 position of ethanolamine glycerophospholipids whereas [^{3}H]palmitic acid is esterified to the *sn*-1 position of PtdCho (Jones et al., 1997; Rapoport, 1999). The differential incorporation of DHA and palmitic acid in various glycerophospholipids may be responsible for better penetration, binding, and packing of neural membrane proteins into the lipid bilayer (Mitchell et al., 1998).

Uptake and incorporation studies in [^{3}H]DHA in PC12 indicate that DHA preferentially incorporates into cell bodies and nerve growth cones (Martin, 1998), and esterified into intermediates of de novo lipid synthesis and acyl chain turnover. During this process, [^{3}H]DHA labeling of neutral lipids decreases with time whereas glycerophospholipids labeling increases. This suggests that in PC12 cells DHA metabolism may be compartmentalized (Martin et al., 2000). Among PC12 cell glycerophospholipids, [^{3}H]DHA incorporates into

PlsEtn. Collectively, these studies suggest that [^{3}H]DHA-labeled lipids are exclusively synthesized in the cell body and then trafficked to nerve growth cones (Martin, 1998; Martin et al., 2000). This trafficking of DHA from cell bodies to growth cones may be associated with synaptogenesis.

2.9 Incorporation of Arachidonic Acid in Neural Membranes

As stated above, ARA is evenly distributed in gray and white matter and among the different cell types in brain. Incubation of [^{3}H]ARA with neural membranes in vitro indicates that the bulk of radioactivity is incorporated in PtdCho and PtdIns. After 10 min incubation, the incorporation in PtdIns was 8- to10-fold higher than in PtdCho. After 35 min, there is no further increase of PtdIns labeling, but that of PtdCho increased significantly by twofold. The esterification of ARA into PtdIns quickly reaches a steady-state, whereas such esterification into PtdCho is not attained so rapidly (Fonlupt et al., 1994). The total incorporation of ARA in glycerophospholipids is fivefold higher than DHA (DeGeorge et al., 1989). Acyl-CoA synthetase converts ARA into fatty acyl-CoA esters (Cao et al., 2000). Microsomal acyl-CoA:lysophospholipid acyltransferase transfers ARA from arachidonyl-CoA to lysoPtdCho (Sun and MacQuarrie, 1989).

The turnover of ARA in brain glycerophospholipids is not uniform. It differs more than 10-fold among PtdIns, PtdCho, PtdSer, and ethanolamine glycerophospholipids (Sun and Su, 1979; Washizaki et al., 1994; Rapoport, 1999), suggesting a separate role for each subclass of glycerophospholipids in the brain tissue. For example, the ARA half-life in rat and mouse brain PtdIns is 1 h and is consistent with the role of polyphosphoinositides in the generation of receptor-mediated second messengers (Washizaki et al., 1994; Rapoport, 1999). In contrast, the half-life of ARA in these brains in PtdEtn is considerably longer, 24 h, suggesting a structural role in neural membranes (Sun and Su, 1979; Washizaki et al., 1994). ARA is the precursor of circulating vasoactive eicosanoids, namely prostacyclin and thromboxanes A_2. The ratio of thromboxane A_2 to prostacyclin is an index of the relative activity of the opposing stimuli that not only effect vascular tone but also modulate platelet activation (Dutta-Roy, 2000). These factors play an important role in the pathogenesis of cardiovascular disease.

2.10 Conclusion

DHA and ARA are fundamental components of neural membranes. They are obtained either directly from the diet or synthesized from their dietary precursors ALA and LA in liver through a series of elongation and desaturation steps, which take place in the endoplasmic reticulum. Liver is the major site for the

synthesis of DHA and ARA. It synthesizes DHA and ARA not only for its own membrane glycerophospholipids but also for export and uptake by brain tissue. DHA and ARA syntheses consist of sequential alternating elongation and desaturation steps catalyzed by fatty acid elongase, Δ^6- and Δ^5-desaturase. These desaturases are the key enzymes for the synthesis of DHA and ARA. A key regulator of desaturase gene expression is sterol-regulatory element binding protein-1c (SREBP-1c), which mediates transcriptional activation of Δ^6-desaturase gene. The identification of SREBP-1c as a key regulator of Δ^6-desaturase suggests that the major physiological function of SREBP-1c in liver may be the regulation of phospholipid synthesis. Incorporation of DHA and ARA involves thioesterification of fatty acids with coenzymeA to form acyl-CoA. This reaction is catalyzed by acyl-CoA synthetase. The transfer of acyl group from acyl-CoA to a lysophospholipid acceptor results in the synthesis of glycerophospholipids. This reaction is catalyzed by acyl-CoA:lysophospholipid acyltransferase. Several acyl-CoA:lysophospholipid acyltransferases have been shown to occur in the brain tissue. They play an important role in attaining the appropriate molecular species of glycerophospholipids in neural membranes. DHA and ARA compete for incorporation into the glycerophospholipids of neuronal membranes; increased levels of DHA/EPA in neurons result in a decrease in the level of ARA in glycerophospholipids. This reduces the synthesis of ARA-derived eicosanoids. The incorporation of labeled DHA differs from that of ARA, as it incorporates into ethanolamine glycerophospholipids in greater amounts in comparison with its mass distribution. Further division of ethanolamine glycerophospholipids into diacyl, alkylacyl, and alkenylacyl types indicates that the high incorporation of radioactive DHA in ethanolamine glycerophospholipids is predominantly due to its rapid incorporation into diacyl and alkylacylglycerophospholipids. It is tempting to speculate that the quality of dietary fat may influence the activity of enzymes involved in the desaturation of fatty acids and their acylation in the brain tissue. A number of genes encoding these activities have been identified. It is proposed that in mammalian tissues, membrane diversity is produced by the concerted and overlapped reactions with multiple forms of acyl-CoA synthetases and acyl-CoA:lysophospholipid acyltransferases that recognize both the polar head group of glycerophospholipids and various acyl-CoAs. Collectively, these processes facilitate and maintain fluidity, diversity, and asymmetry in neural membranes.

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Chapter 3
Release of *n*–3 and *n*–6 Fatty Acids from Glycerophospholipids in Brain

3.1 Introduction

Fatty acids are key constituents of glycerophospholipids, which form the backbone of neural membranes. Neural membranes are complex, heterogeneous, highly interactive, and cooperative system of lipids and proteins that perform many important functions (Farooqui and Horrocks, 2007; Farooqui, 2009). Fatty acids are classified into essential and non-essential fatty acids. Essential fatty acids cannot be synthesized in human body and therefore have to be supplied by diet. Examples of essential fatty acids are *n*–6 (LA and ARA) and *n*–3 (EPA and DHA) fatty acids. In contrast, non-essential fatty acids can be synthesized by the human body. Example of non-essential fatty acid is *n*–9 (oleic acid) acid. The *n*–3 and *n*–6 fatty acids are of particular nutritional importance for humans. The *n*–3 fatty acids are enriched in fish, fish oil, and krill oil. These fatty acids are also found in green vegetables, sea algae, and shellfish. In contrast, *n*–6 fatty acids are mainly found in vegetable oils, processed food, and corn-fed animal meat.

In brain, major proportions of DHA are found in ethanolamine- and serine-containing glycerophospholipids. DHA-enriched ethanolamine and serine glycerophospholipids are preferentially located in the inner leaflet of lipid bilayer. PtdSer is synthesized by serine base-exchange reaction from pre-existing membrane PtdCho or PtdEtn. This reaction is catalyzed by PtdSer synthase-1 or PS synthase-2, respectively (Vance and Vance, 2004; Suzuki and Kanfer, 1985). The serine base-exchange reaction is energy-independent and occurs in a Ca^{2+}-dependent manner in the endoplasmic reticulum and mitochondrial membranes. Considerable serine base-exchange activity has been detected in the plasma membranes of neuronal cell bodies (Goracci et al., 1973) and synaptosomes (Holdbrook and Wurtman, 1988). The serine base-exchange activity of neuronal microsomal and plasma membrane preparations is higher than the corresponding preparations from glial cells. Although PtdSer can be converted to PtdCho or PtdEtn by base-exchange reactions, most of PtdSer is transferred to mitochondria where it is decarboxylated to PtdEtn by PtdSer-decarboxylase (Voelker, 1984). The mitochondrial decarboxylation of PtdSer is the primary means of metabolizing PtdSer into PtdEtn. Therefore, the substrate preference

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in PtdSer synthesis or decarboxylation is an important determinant for the accumulation of specific PtdSer molecular species in neural membranes. In neural cells, PtdSer pool is modulated by DHA and is responsible for maintaining membrane asymmetry (Kim, 2007).

Other DHA-containing glycerophospholipids include phosphatidylethanolamine and ethanolamine and choline plasmalogens. The biosynthesis of ethanolamine and choline plasmalogens is initiated in peroxisomes and completed in the endoplasmic reticulum. Dihydroxyacetone phosphate is the precursor for plasmalogen. First three enzymes of plasmalogen biosynthesis, dihydroxyacetone phosphate acyltransferase, alkyl dihydroxyacetone phosphate synthase, and acyl/alkyl dihydroxyacetone reductase, are associated with peroxisomes. The endoplasmic reticulum contains the other enzymes, namely 1-alkyl-*sn*-Gro*P* acyltransferase, 1-alkyl-2-acyl-*sn*-Gro*P* phosphohydrolase, and 1-alkyl-2-acyl-*sn*-Gro:CDP-choline (CDP-ethanolamine) choline (ethanolamine) phosphotransferase (Farooqui et al., 2008a). Ethanolamine and choline plasmalogens are located in synapse. They are closely associated not only with the stability of synapse and functioning of acetylcholine receptor but also with membrane fluidity and permeability (Farooqui et al., 2008a), whereas DHA-enriched PtdSer is an essential cofactor for the activation of several proteins including protein kinase C (PKC) and Raf-1 kinase. PtdSer is synthesized from PtdEtn or PtdCho through a base-exchange reaction, which catalyzes the replacement of ethanolamine or choline by serine (Garcia et al., 1998). PtdSer also modulates the activities of diacylglycerol kinase and nitric oxide synthase (Ikemoto et al., 2000). In addition, PtdSer modulates the activity of Na^+, K^+-ATPase, an integral membrane protein found at high densities at the nodes of Ranvier in neuron. This ATPase generates and maintains Na^+ and K^+ gradient needed for maintaining resting membrane potential. Collective evidence suggests that the presence of DHA-containing glycerophospholipids in neural membranes provides them with an appropriate physical environment for the activity of integral membrane proteins (Farooqui et al., 2008a).

In neural membranes, ARA is enriched in PtdCho, PtdIns, and PtdH. These glycerophospholipids play important roles in maintaining function and integrity of membranes at cellular and subcellular levels (Farooqui and Horrocks, 2007). Thus, ARA-enriched glycerophospholipids are associated with neural membrane stability, transport, and generation of lipid mediators that regulate growth and development.

3.2 Release of DHA from Ethanolamine or Choline Plasmalogen

The release of DHA from plasmalogen is a receptor-mediated process. It is catalyzed by plasmalogen-selective phospholipase A_2 (PlsEtn-PLA_2). This enzyme has been purified and characterized from bovine brain, rabbit heart,

and kidney (Hirashima et al., 1992; Farooqui et al., 1995; Hazen and Gross, 1993; Portilla and Dai, 1996).

PlsEtn-PLA_2 occurs in the cytosolic fraction of brains from various animal species (Farooqui et al., 2008a). Determination of isoforms of PLA_2 activity in brain has indicated that PlsEtn-PLA_2 accounts for the greater proportion of total PLA_2 activity in mammalian brains (Yang et al., 1997; Farooqui et al., 2008a). Brain PlsEtn-PLA_2 can be separated from $cPLA_2$ and $sPLA_2$ by gel filtration on Sephadex G-75 column (Hirashima et al., 1992).

3.2.1 Plasmalogen-Selective-Phospholipase A_2 in Brain

Bovine brain PlsEtn-PLA_2 can be separated from cytosolic PLA_2 by Sephadex G-75 column chromatography. It has a molecular mass of 39 kDa and does not require calcium for its activity (Hirashima et al., 1992; Farooqui et al., 1995). It is markedly inhibited by ATP and ADP above 2 mM, and AMP and cAMP have no effect on enzymic activity (Table 3.1). Bovine brain PlsEtn-PLA_2 is stimulated by Triton X-100, octylglucoside, and Tween-20 (Figs. 3.1 and 3.2). Anionic detergents, such as sodium deoxycholate and sodium taurocholate, inhibit the PlsEtn-PLA_2 in a dose-dependent manner (Fig. 3.3). PlsEtn-PLA_2 is inhibited by iodoacetate (Fig. 3.4), and this inhibition is reversed by mercaptoethanol (Fig. 3.5). Other SH-group-blocking agents such as dithio-*bis*-2-nitrobenzoic acid and *N*-ethylmaleimide also inhibit PlsEtn-PLA_2 activity. The inhibitory effect of SH-group-blocking agents can be reversed by dithiothreitol, suggesting that reduced sulfhydryl groups are required for the enzymic activity. Polyvalent anions (citrate > sulfate > phosphate) also inhibit PlsEtn-PLA_2 activity (Farooqui et al., 1995). The purified PlsEtn-PLA_2 is also inhibited by metal ions such as $Ag^+ + Hg^{2+} > Fe^{3+}$ (Table 3.1). Quinacrine and nordihydroguariaretic acid, the non-specific inhibitors of PLA_2, also inhibit the PlsEtn-PLA_2 in a dose-dependent manner (Yang et al., 1997). Purified enzyme

Table 3.1 Physicochemical and kinetic properties of bovine brain PlsEtn-PLA_2 and $cPLA_2$

Properties	PlsEtn-PLA_2	$cPLA_2$	References
pH optimum	7.4	8.0	Hirashima et al. (1992)
K_m value (μM)	29.0	70.0	Hirashima et al. (1992)
V_{max} (nmol/min/mg)	76	214	Hirashima et al. (1992)
Thermal stability	More sensitive	Less sensitive	Yang et al. (1994a)
Effect of iodoacetate	More sensitive	Less sensitive	Farooqui et al. (1995)
Effect of GM3 ganglioside	Strong inhibition	No effect	Yang et al. (1994b)
Effect of NANA	Strong inhibition	Inhibition	Yang et al. (1994b)
Effect of mucin	Inhibited	No effect	Yang et al. (1994b)
Effect of glycosaminoglycans	Inhibited	No effect	Yang et al. (1994a)
Effect of ATP	Inhibition	No effect	Farooqui et al. (2008a)
Molecular mass (kDa)	39	110	Hirashima et al. (1992)

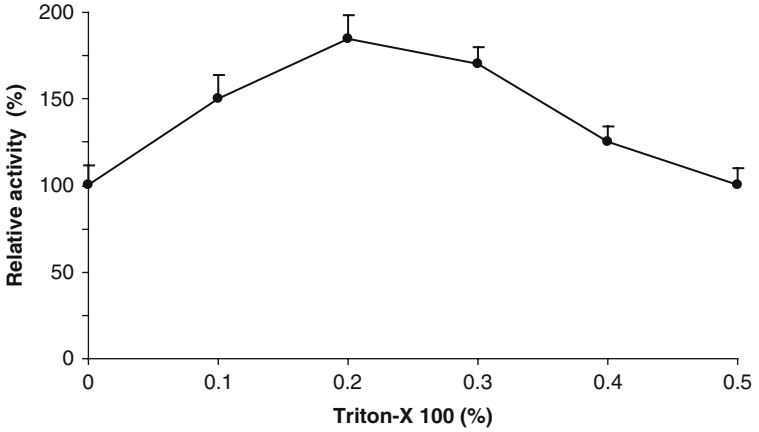

Fig. 3.1 Effect of Triton X-100 on bovine PlsEtn-PLA_2 activity. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). Purified enzyme had specific activity of 76 pmol/min/mg protein. Reaction mixture without the addition of detergent is used as control, and expressed as 100%. The data represent values are mean ± S.E.M. Each data point represents five determinations

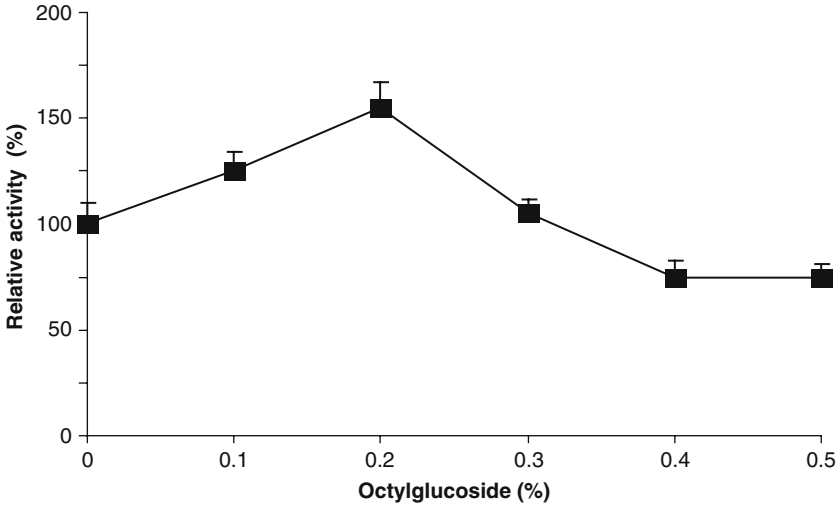

Fig. 3.2 Effect of octylglucoside on bovine PlsEtn-PLA_2 activity. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). Purified enzyme had specific activity of 76 pmol/min/mg protein. Reaction mixture without the addition of detergent is used as control, and expressed as 100%. The data represent values are mean ± S.E.M. Each data point represents five determinations

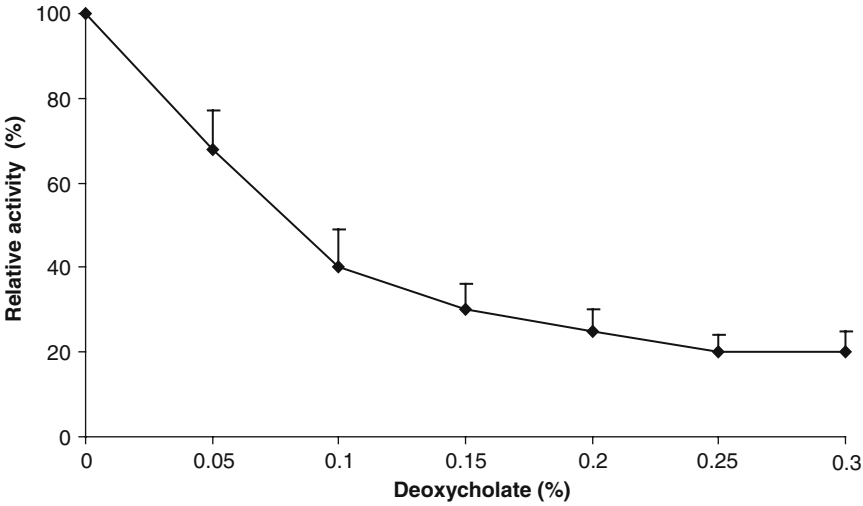

Fig. 3.3 Effect of sodium deoxycholate on bovine PlsEtn-PLA_2 activity. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). Purified enzyme had specific activity of 76 pmol/min/mg protein. Reaction mixture without the addition of detergent is used as control, and expressed as 100%. The data represent values are mean ± S.E.M. Each data point represents five determinations

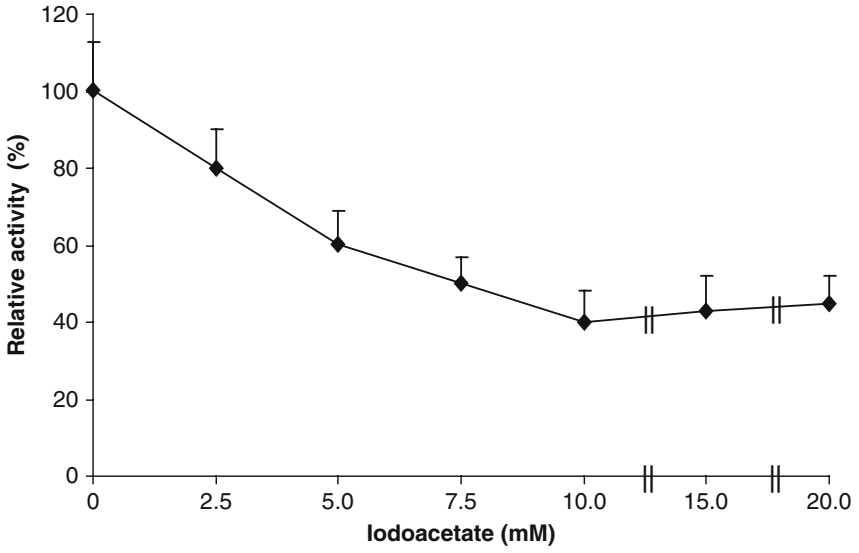

Fig. 3.4 Effect of iodoacetate on bovine PlsEtn-PLA_2 activity. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). Purified enzyme had specific activity of 76 pmol/min/mg protein. Reaction mixture without the addition of detergent is used as control, and expressed as 100%. The data represent values are mean ± S.E.M. Each data point represents five determinations

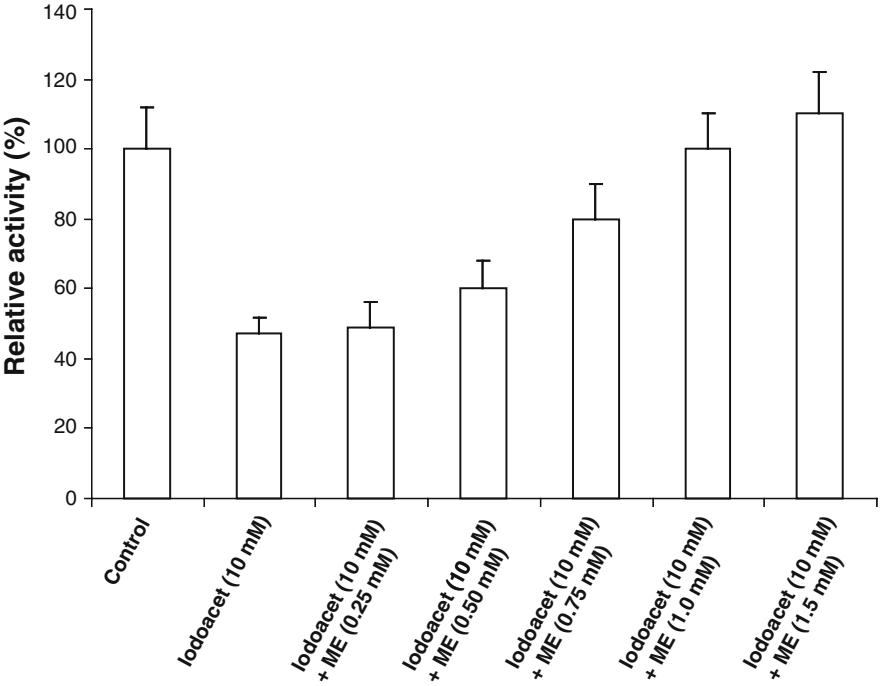

Fig. 3.5 Inhibitory effect of iodoacetate on bovine PlsEtn-PLA_2 activity and its reversal by β-mercaptoethanol. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). Purified enzyme has specific activity of 76 pmol/min/mg protein. Reaction mixture without the addition of detergent is used as control, and expressed as 100%. The data represent values are mean ± S.E.M. Each data point represents five determinations. Iodoacetetate (iodoacet) and β-mercaptoethanol (ME)

effectively utilizes ether-linked glycerophospholipid substrates and displays preference for those phospholipids that contain DHA at the *sn*-2 position.

Glycosaminoglycans also inhibit purified bovine brain PlsEtn-PLA_2. Heparan sulfate is the most potent inhibitor, followed by hyaluronic acid, chondroitin sulfate, and heparin (Yang et al., 1994a). The inhibition by glycosaminoglycans can be reversed by the addition of protamine sulfate. *N*-Acetylneuraminic acid, gangliosides, and sialoglycoproteins also inhibit the plasmalogen-selective PLA_2 in a dose-dependent manner (Figs. 3.6, 3.7 and 3.8). However, colominic acid (poly 2, 8-*N*-acetylneuraminic acid) has no effect on its enzymic activity (Yang et al., 1994b). The significance of *N*-acetylneuraminic acid-, ganglioside-, and mucin-mediated inhibition of PlsEtn-PLA_2 is not fully understood. However, interactions of plasmalogen-selective PLA_2 with glycosaminoglycans and sialoglycoconjugates may be involved in the anchoring of this enzyme to plasma membranes, where its substrate is located.

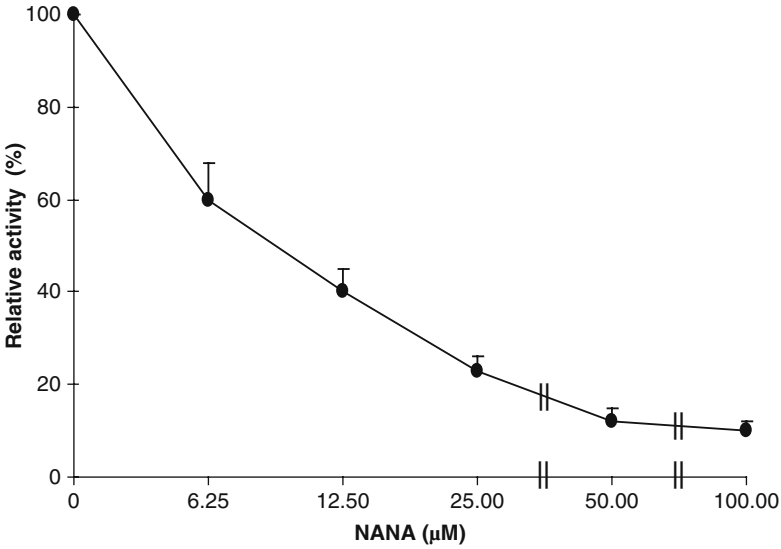

Fig. 3.6 Effect of N-acetylneuraminic acid (NANA) on bovine PlsEtn-PLA_2 activity. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). Purified enzyme had specific activity of 76 pmol/min/mg protein. Reaction mixture without the addition of detergent is used as control, and expressed as 100%. The data represent values are mean ± S.E.M. Each data point represents five determinations

Immunocytochemical localization of plasmalogen-selective PLA_2 has been performed in neuronal and astrocytic cultures (Yang et al., 1997; Farooqui et al., 2008a). Colocalization of plasmalogen-selective PLA_2 with glial fibrillary acidic protein (GFAP) suggests that this PLA_2 is predominantly associated with astrocytes (Farooqui and Horrocks, 2001b). In contrast, the 85-kDa cytosolic PLA_2 is localized in neurons as well as astrocytes (Farooqui et al., 2000b).

Treatment of neuronal cultures with glutamate analog, kainate (KA), enhances PlsEtn-PLA_2 activity in a dose- and time-dependent manner (Fig. 3.9). The stimulation of PlsEtn-PLA_2 by KA can be prevented by 6-cyano-7-nitroquinoxaline 2,3-dione (CNQX), an AMPA receptor antagonist (Fig. 3.10) (Farooqui et al., 2003). This observation suggests that the release of DHA from neural membrane glycerophospholipids is a receptor-mediated process that may involve kainate receptor. The stimulation of PlsEtn-PLA_2 by KA in neuronal cultures is also blocked by bromoenol lactone (BEL) in a dose-dependent manner (Fig. 3.11).

Free fatty acids inhibit PlsEtn-PLA_2 in a dose-dependent manner (Farooqui et al., 2006). Unsaturated fatty acids such as DHA and EPA are more potent inhibitors than palmitate. Unsaturated fatty acid-mediated inhibition can be reversed by bovine serum albumin.

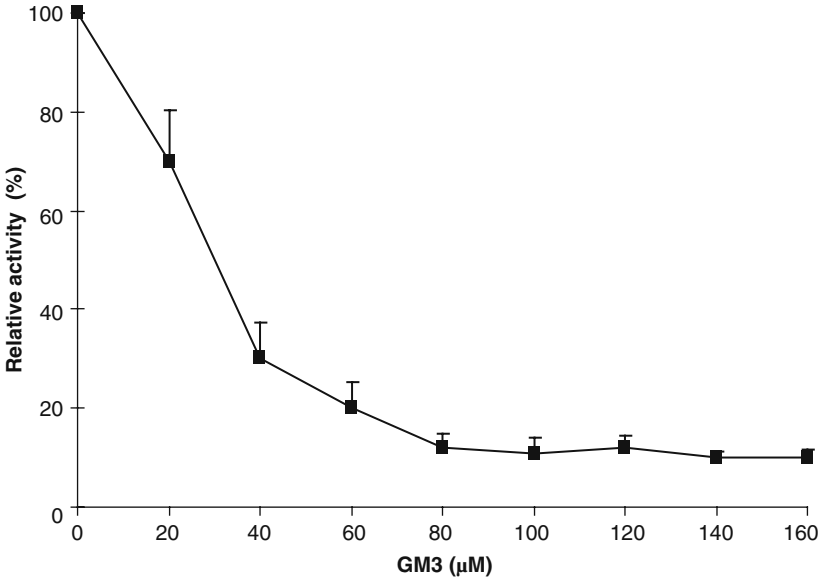

Fig. 3.7 Effect of GM_3 on bovine PlsEtn-PLA_2 activity. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). Purified enzyme had specific activity of 76 pmol/min/mg protein. Reaction mixture without the addition of detergent is used as control, and expressed as 100%. The data represent values are mean ± S.E.M. Each data point represents five determinations

Human LA-N-1 cell neuroblastoma nuclei contain PlsEtn-PLA_2 activity that is stimulated by retinoic acid in a dose- and time-dependent manner (Antony et al., 2001). A pan retinoic acid receptor antagonist, BMS943 (Farooqui et al., 2004a), blocks the stimulation of PlsEtn-PLA_2. Thus, the stimulation of PlsEtn-PLA_2 is a receptor-mediated process. The activation of a nuclear transcription factor, cAMP response element binding protein (CREB), also depends upon the activity of PlsEtn-PLA_2. Collectively, these studies indicate that metabolism of plasmalogens and release of DHA may also be involved in signal transduction processes in the nucleus.

Based on the treatment of hippocampal cultures with PLA_2 inhibitor, it is suggested that the release of ARA and DHA in astrocytes is controlled by different isoforms of PLA_2, i.e., $cPLA_2$ and PlsEtn-PLA_2, respectively (Sergeeva et al., 2005). The enrichment of hippocampal tissue with DHA results in neuroprotective effect. This indicates that the incorporation of DHA in plasmalogens is required for the neuroprotective effect on hippocampal and astrocytic cultures. The protective effect is substantially higher in dentate gyrus than in CA1 and CA3 areas. Moreover, in astrocytic cultures, the release of arachidonic and docosahexaenoic acids is differently regulated through Ca^{2+}- and cAMP-dependent signal transduction pathways (Strokin et al., 2003), supporting the view that release of arachidonic and docosahexaenoic acids is

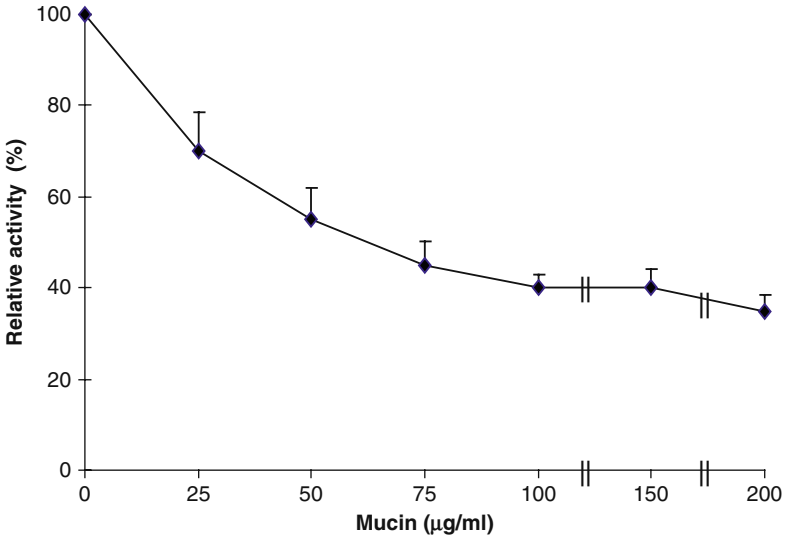

Fig. 3.8 Effect of mucin on bovine PlsEtn-PLA_2 activity. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). Purified enzyme had specific activity of 76 pmol/min/mg protein. Reaction mixture without the addition of detergent is used as control, and expressed as 100%. The data represent values are mean ± S.E.M. Each data point represents five determinations

differentially modulated under normal and pathological situations (Strokin et al., 2006; Farooqui et al., 2006). Detailed investigations on the release of DHA in astrocytes indicate that treatment of astrocytic cultures with siRNA VIB Ca^{2+}-independent phospholipase A_2 (PlsEtn-PLA_2) strongly suppresses the DHA in astrocytes (Strokin et al., 2007). Unlike bovine brain PlsEtn-PLA_2, astrocytic enzyme is stimulated with ATP. In astrocytic cultures, DHA release proceeds simultaneously with ARA release and prostaglandin generation, and prostaglandin synthesis is negatively controlled by endogenous DHA (Strokin et al., 2007). Exogenous addition of DHA also blocks prostaglandin synthesis, suggesting that the negative control of prostaglandin production observed in astrocytic cultures is likely due to competitive inhibition of cyclooxygenase-1 and cyclooxygenase-2 by free DHA. Furthermore, treatment of astrocytes with DHA also results in downregulation of cyclooxygenase-1, an enzyme that generates prostaglandin in lipopolysaccharide-stimulated astrocytes. Collective evidence suggests that PlsEtn-PLA_2-mediated release of DHA negatively modulates prostaglandin synthesis in astrocytic cultures (Strokin et al., 2007).

Lactate dehydrogenase and propidium iodide (PI) release by α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) are markedly reduced by DHA in hippocampal slices (Ménard et al., 2008). It is shown that neuroprotection by DHA is restricted to the hippocampal CA1 region and cannot be reproduced by EPA or ARA. Moreover, the protective effect of DHA is specific

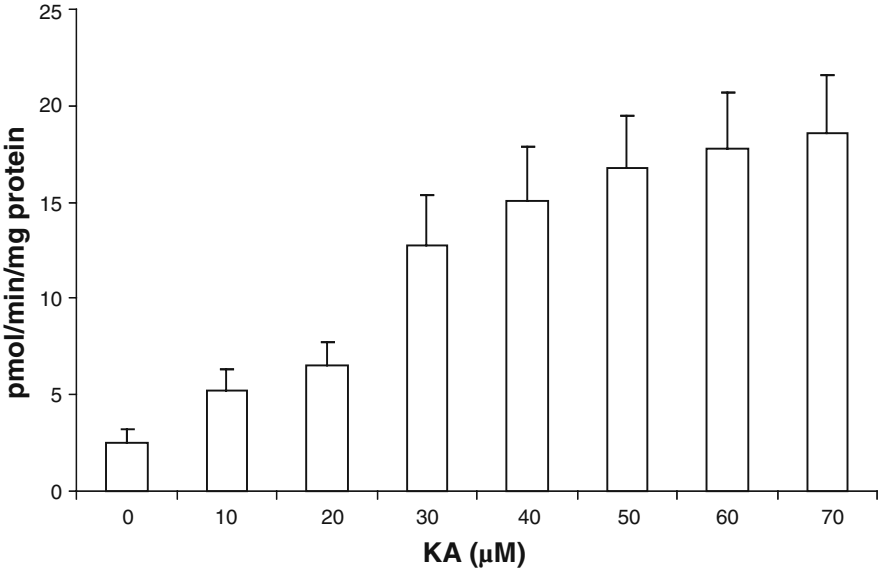

Fig. 3.9 Effect of kainic acid treatment on PlsEtn-PLA_2 activity of neuron-enriched cultures from rat cerebral cortex. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). The data represent values are mean ± S.E.M. Each data point represents five determinations

to AMPA receptor stimulation and *N*-methyl-d-aspartate (NMDA), or kainate receptor-mediated toxicity is not diminished upon pre-incubation with DHA. Biotinylation experiments clearly show that the neuroprotective actions of DHA are due to the downregulation of AMPA receptors in hippocampal membranes (Ménard et al., 2008).

3.2.2 Canine Myocardium PlsCho-PLA_2

Canine myocardium PlsCho-PLA_2 is purified by multiple column chromatographic procedure, which includes anionic exchange, chromatofocusing, ATP agarose affinity chromatography, mono Q chromatography, and hydroxylapatite chromatography. Canine myocardium PlsCho-PLA_2 exists as a complex with phosphofructokinase. This complex has a molecular mass of 400 kDa. Interactions between phosphofructokinase and myocardial PlsCho-PLA_2 are highly specific, and it is proposed that these interactions may be involved in coordinated regulation of phospholipolysis and glycolysis in heart tissue (Hazen and Gross, 1993). Myocardial PlsCho-PLA_2 also binds with calmodulin, a calcium-binding protein, and these interactions may also have implications in regulation of PlsCho-PLA_2 activity in heart muscle (Wolf and Gross, 1996).

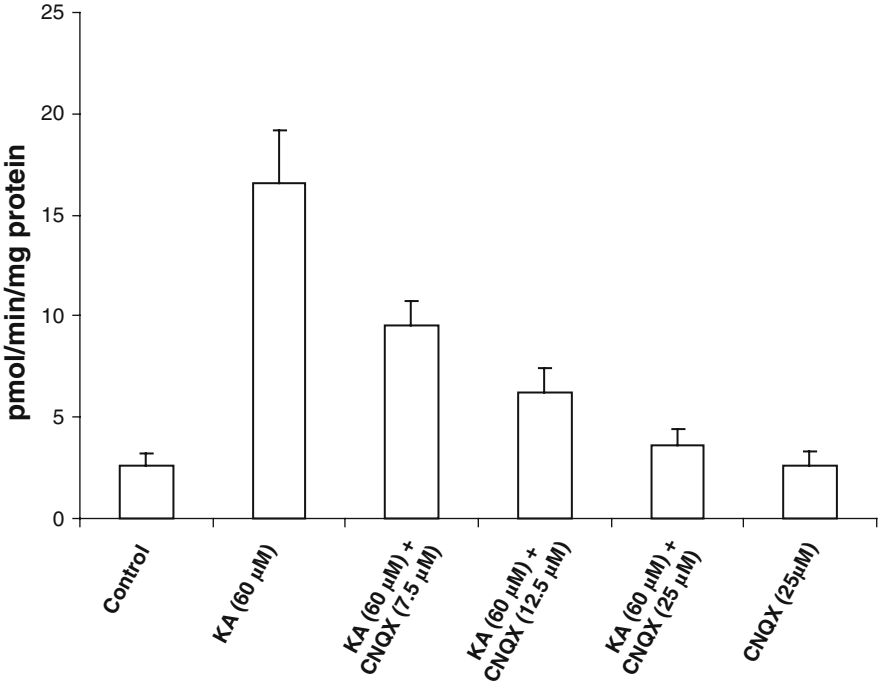

Fig. 3.10 Effect of various concentrations of 6-cyano-7-nitroquinoxaline 2,3-dione (CNQX) on kainic acid-mediated stimulation of PlsEtn-PLA_2 activity of neuron-enriched cultures from rat cerebral cortex. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). The data represent values are mean ± S.E.M. Each data point represents five determinations

Purified brain PlsEtn-PLA_2 does not interact with calmodulin. Myocardial PlsCho-PLA_2 differs from bovine brain PlsEtn-PLA_2 in ATP-mediated stimulation of enzymic activity and inhibition by Triton X-100 (Hazen et al., 1991).

3.2.3 PlsEtn-PLA_2 from Rabbit Kidney

A plasmalogen-selective PLA_2 has also been purified to homogeneity from rabbit kidney cortex through multiple column chromatographic procedure, which involves anion exchange, hydrophobic interaction, mono Q, hydroxylapatite, phenyl-Sepharose, and chromatofocusing fast protein liquid chromatography (Portilla and Dai, 1996). The purified enzyme shows optimal activity at neutral pH, and has specific activity of 1.2 μmol/min/mg protein. It has a molecular mass of 28 kDa and hydrolyzes PlsCho > PtdCho (Table 3.2). It selectively cleaves phospholipids containing arachidonic acid at the *sn*-2 position in comparison to oleic acid. Antibodies against the purified protein

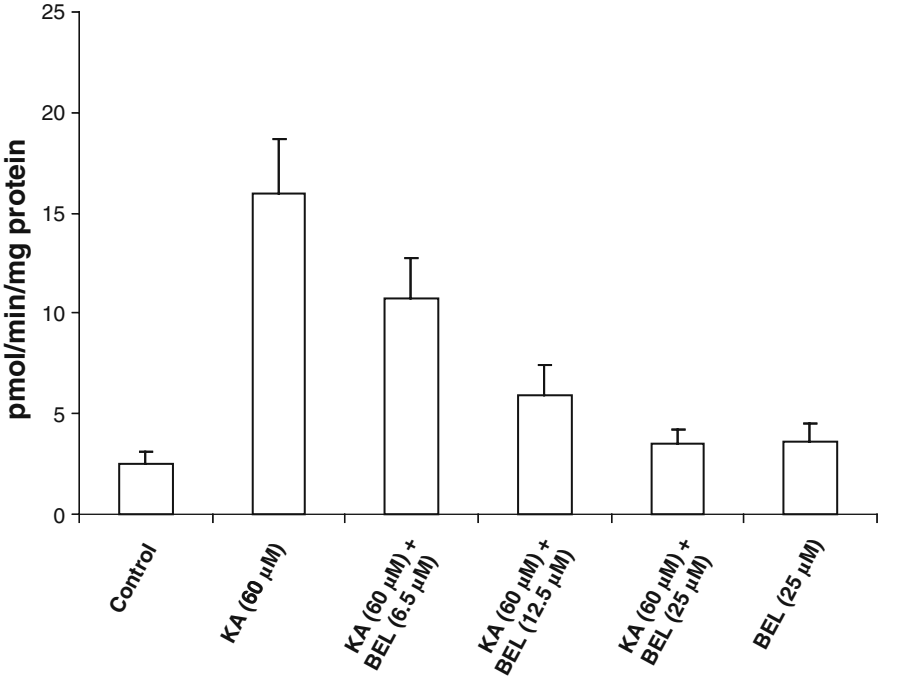

Fig. 3.11 Effect of various concentrations of bromoenol lactone (BEL) on kainic acid-mediated stimulation of PlsEtn-PLA_2 activity of neuron-enriched cultures from rat cerebral cortex. Enzymic activity is determined by an earlier described procedure (Hirashima et al., 1992). The data represent values are mean ± S.E.M. Each data point represents five determinations

Table 3.2 Coupling of PLA_2 isoforms with various receptors in brain tissue

Receptor type	PLA_2 isoform	References
Glutamate receptor	cPLA$_2$, PlsEtn-PLA$_2$,	Lazarewicz et al. (1990); Kolko et al. (1996); Farooqui et al. (2003)
Dopamine receptor	cPLA$_2$	Ross (2003)
Serotonin receptor	cPLA$_2$	Qu et al. (2003a); Kurrasch-Orbaugh et al. (2003); Qu et al. (2003b)
P_2-purinergic receptor	cPLA$_2$	Xing et al. (1994)
TNF-α receptor	cPLA$_2$	Atsumi et al. (1998); Jupp et al. (2003)
IL-receptor	cPLA$_2$	Xu et al. (2003)
Interferon receptor	cPLA$_2$	Xu et al. (2003)
Growth factor receptor	cPLA$_2$	Jupp et al. (2003); Akiyama et al. (2004)
Endothelin receptor	cPLA$_2$ PlsEtn-PLA$_2$	Trevisi et al. (2002)
Muscarinic receptor	cPLA$_2$	Bayón et al. (1997)

precipitate all of the soluble calcium-independent PLA_2 activity from rabbit kidney cortex. Cloning of a full-length rat cDNA encoding PlsCho-PLA_2 has been performed using sequence derived from the purified kidney cortex enzyme. These studies indicate that a cDNA from rabbit kidney that encodes the rabbit homolog of the PlsCho-PLA_2, a closely related isoform of PlsCho-PLA_2, can also be isolated. The rat cDNA encodes a 24-kDa protein and contains the sequence G-F-S-Q-G, which fits the active site consensus sequence G-X-S-X-G of carboxylesterase (Portilla et al., 1998). Collective evidence suggests that PlsCho-PLA_2 enzymes are encoded by a multigene family in rats, mice, rabbits, and human. Northern blot analyses of various rat tissues indicate that PlsCho-PLA_2 gene is ubiquitously expressed with highest mRNA in kidney and small intestine. The expression of rat PlsCho-PLA_2 in a baculovirus expression system results in the expression of PlsCho-PLA_2 and lysophospholipase activities (Portilla et al., 1998).

3.3 Release of DHA from PtdSer

Very little information is available on the release of DHA from PtdSer (Garcia and Kim, 1997). The treatment of [^{3}H]DHA-labeled C6 glioma with (+/−)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI), a 5-HT_{2A} receptor agonist, promotes the release of [^{3}H]DHA. [^{3}H]DHA release is blocked by PLA_2 inhibitor, indicating the presence of PtdSer-hydrolyzing PLA_2 activity in C6 glioma cells. It is proposed that release of DHA in response to serotonin may represent a mechanism through which astroglia provide DHA to neurons (Garcia and Kim, 1997). My attempts to detect the presence of PtdSer-selective PLA_2 in mammalian brain have been unsuccessful.

3.4 Receptor-Mediated Degradation of Plasmalogens

PlsEtn-PLA_2 may be coupled to glutamatergic (Farooqui et al., 2003) and 5-HT_{2A} receptors. It is estimated that isoforms of PLA_2 release DHA at a rate 102–131 pmol/g brain/s. A portion (about 10%) of the released DHA is utilized by 15-lipoxygenases-like enzyme for docosanoids synthesis, and the remaining DHA (90%) is reesterified at the *sn*-2 position of glycerophospholipids (probably PlsEtn) (Lee et al., 2005; Bazinet et al., 2005; Green et al., 2008). Based on plasmalogen turnover studies in the brain, it is proposed that interaction of agonist with receptors at the neural cell surface results in the stimulation of PlsEtn-PLA_2 and generation of lysoplasmalogen and DHA. In neural membranes, lysoplasmalogen is usually reacylated or hydrolyzed by lysoplasmalogenase, and DHA is metabolized to docosanoids (Farooqui and Horrocks, 2001a). During receptor stimulation, the release of docosanoids may constitute the first wave of second-messenger generation during the initial phase of signal transduction (Turini and Holub, 1994).

The generation of lysoplasmalogen in neural membrane may induce changes in membrane fluidity and permeability, and allow the influx of external Ca^{2+} via plasma membrane channels. This influx of Ca^{2+} results in the translocation and stimulation of Ca^{2+}-dependent $cPLA_2$, a PLA_2, which is specific for the release of arachidonic acid from the *sn*-2 position of PtdCho. The degradation of this glycerophospholipid produces the subsequent wave of second-messenger generation in the late phase of signal transduction. Collectively, these studies suggest DHA-containing plasmalogens are consumed first in second-messenger generation during the initial phase of the signal transduction process. In contrast, ARA-containing PtdCho is utilized at the later stages.

3.5 Release of *n*–6 Fatty Acids from Neural Membrane Glycerophospholipids

Release of *n*–6 fatty acids from glycerophospholipids in brain is catalyzed by cytosolic phospholipase A_2 ($cPLA_2$). Although $cPLA_2$ activity occurs in the brain, it has never been purified to homogeneity. Partially purified preparations have been used to study kinetic properties of this enzyme. Rat brain cytoplasm contains two PLA_2 activities, PLA_2-H and PLA_2-L. PLA_2-H has an apparent molecular mass of 200–500 kDa. Its activity is partially inhibited by Ca^{2+}. In contrast, PLA_2-L has a molecular mass of 100 kDa and requires Ca^{2+} for binding to glycerophospholipid substrate (Yoshihara and Watanabe, 1990). Based on several enzymic properties, such as Ca^{2+}-sensitivity, molecular mass, and Ca^{2+}-mediated translocation, PLA_2-L appears to be identical to $cPLA_2$ (Yoshihara et al., 1992).

3.5.1 $cPLA_2$ in Brain

$cPLA_2$ has been partially purified from bovine brain using multiple column chromatographic procedures (Hirashima et al., 1992). The purified enzyme shows optimal activity at pH 7.4 and displays a distinct preference for glycerphospholipids having esterified ARA at the *sn*-2 position of glycerol moiety (Clark et al., 1995; Farooqui et al., 2000a). Owing to the presence of a Ca^{2+}-dependent phospholipid-binding domain at the N-terminal region, $cPLA_2$ is translocated in a Ca^{2+}-dependent manner from cytosol to the nuclear or other cellular membranes (Clark et al., 1995; Hirabayashi et al., 2004), where other downstream enzymes, including the cyclooxygenases and lipoxygenases, responsible for the metabolism of ARA to eicosanoids are located. This gives $cPLA_2$ access to its membrane-associated phospholipid substrate. The C-terminal region of $cPLA_2$ contains the phosphorylation site and catalytic site. These sites may be involved in regulation of the enzymic activity. The activation of $cPLA_2$ can be through serine residues, notably Ser 505 and Ser 727, by mitogen-activated protein kinase (MAPK) and PKC (Hirabayashi and Shimizu, 2000; Hirabayashi et al., 2004). In

neural membranes, $cPLA_2$ activity and arachidonic acid release are linked to dopamine, glutamate, serotonin, P2-purinergic, muscarinic, cytokine, and growth factor receptors through different coupling mechanisms (Table 3.2). Some receptors involve G-proteins and others do not. The ligand-mediated stimulation of the above receptors modulates the release of arachidonic acid and levels of other second messengers in the brain tissue (Farooqui et al., 2000b).

Unlike PlsEtn-PLA_2, $cPLA_2$ is not inhibited by glycosaminoglycans (Yang et al., 1994a). GM1 and GM3 gangliosides have no effect on bovine brain $cPLA_2$ activity. Although NANA and mucin inhibit PlsEtn-PLA_2 and $cPLA_2$, the degree of inhibition for PlsEtn-PLA_2 is greater than $cPLA_2$. Thus at 10 μM NANA produces 50% inhibition of PlsEtn-PLA_2. In contrast, 10 μM NANA causes only 15% inhibition of $cPLA_2$ activity. Bovine PlsEtn-PLA_2 is more thermolabile than $cPLA_2$. Bovine brain PlsEtn-PLA_2 is inhibited by Ag^{+}, Hg^{2+}, and Fe^{3+} (Table 3.3) but these ions have very little effect on $cPLA_2$. There is considerable similarity in metal ion inhibition patterns of bovine brain $cPLA_2$ and recombinant human $cPLA_2$ (Reynolds et al., 1993).

Table 3.3 Effect of metal ion on partially purified bovine PlsEtn-PLA_2 and $cPLA_2$

Metal ion (μM)	PlsEtn-PLA_2 relative activity (%)	$cPLA_2$ relative activity (%)
AgCl		
Control	100±15	100±8
20	75±10	100±5
40	52±10	93±7
60	33±7	95±6
80	22±8	97±5
100	20±4	90±4
$HgCl_2$		
Control	100±15	100±7
20	80±15	75±9
40	59±15	45±7
60	38±8	25±7
80	29±6	15±7
100	25±5	4±2
$FeCl_3$		
Control	100±13	100±13
20	87±10	93±5
40	65±10	77±6
60	52±8	63±8
80	35±7	50±8
100	25±4	37±8
150	20±5	30±5
200	20±5	32±7

Values are means ± S.E.M., $n = 5$.

The treatment of cortical, striatal, hippocampal, and hypothalamic neurons and cerebellar granule cells with glutamate, NMDA, and kainate results in a dose-dependent increase in PLA_2 activity and arachidonic acid release (Dumuis et al., 1988; Lazarewicz et al., 1990; Farooqui et al., 2003). An NMDA antagonist, 2-amino-5-phosphonovalerate (APV), an AMPA receptor antagonist, 6-cyano-7-nitroquinoxaline (CNQX), quinacrine (a non-specific $cPLA_2$ inhibitor) (Sanfeliu et al., 1990; Kim et al., 1995; Farooqui et al., 2003), and also arachidonoyl trifluoromethylketone (a potent inhibitor of $cPLA_2$) block this release in a dose-dependent manner. These studies strongly suggest that the stimulation of $cPLA_2$ is a glutamate receptor-mediated process in neuronal cultures.

Treatment of hippocampal slices with kainic acid alone or kainic acid plus quinacrine results in significant differences in $cPLA_2$ immunoreactivity (Lu et al., 2001). After 1 week of treatment with kainic acid in the presence of quinacrine, significantly less immunoreactivity is observed in slices treated with quinacrine. The decrease in $cPLA_2$ immunoreactivity by quinacrine may be due to inhibition of $cPLA_2$mRNA expression (Ong et al., 2002). In addition, quinacrine and other PLA_2 inhibitors may also act through the downregulation of cytokine, nuclear factor-κB, AP1, and intracellular adhesion molecule expression (Farooqui et al., 2001).

The chronic administration of NMDA (25 mg/kg i.p.) to rats also upregulates brain $cPLA_2$ and AP-2 expression, and antimanic drugs (lithium and carbamazepine) downregulate excessive NMDA signaling (Rao et al., 2007). Daily administration of subconvulsive dose of NMDA to rats for 21 days not only downregulates frontal cortex NMDA receptor (NR)-1 and NR-3A subunits but also upregulate $cPLA_2$ activity, phosphorylation, protein, and mRNA levels. The activity and protein levels of $sPLA_2$ or $iPLA_2$ are not affected. Chronic NMDA also upregulates the DNA-binding activity of AP-2 and the protein levels of its a and b subunits. These changes in $cPLA_2$ and AP-2 are observed following acute NMDA administration (Rao et al., 2007). Collective evidence suggests that $cPLA_2$ activity is linked to glutamate receptors, and glutamate and its analogs modulate $cPLA_2$ activity (Farooqui et al., 2008b).

Treatment of astroglial cultures with ATP also evokes ARA release in a dose- and time-dependent manner. Thus, PLA_2 activity may be linked to purinergic receptors in astrocytes. This suggestion is supported by studies on the occurrence of various isoforms of PLA_2 in immortalized astrocytes and the astrocytoma cell line 1321N1 and on ARA release by various agonists (Sun et al., 2005; Hernández et al., 2000). Astrocytes may be particularly sensitive to the ATP/UTP ratio. These nucleotides act on the $P2Y_2$ receptor, and stimulate signaling pathways leading to AA release (Sun et al., 2005). PLA_2 inhibitors can prevent this ARA release (Sun et al., 2005).

Four paralogs of $cPLA_2$ occur in brain and other non-neural tissues (Diaz-Arrastia and Scott, 1999; Farooqui et al., 2000b; Hirabayashi et al., 2004). They are $cPLA_2$-α (mol. mass 85 kDa), $cPLA_2$-β (mol. mass 114 kDa), $cPLA_2$-γ (mol. mass 61 kDa), and cPLA2-δ (mol. mass 93 kDa). $cPLA_2$-β is found mainly in

the cerebellum and shares more similarities with cPLA$_2$-α than with cPLA$_2$-γ. cPLA$_2$-γ lacks the C2 domain, but contains a prenyl-group-binding motif that behaves as a lipid anchor and allows binding of the enzyme to the membrane. cPLA$_2$-α activity is uniformly distributed in various regions of rat brain (Farooqui et al., 2000a). The basal expression of cPLA$_2$-α mRNA under normal conditions is very low in neuronal and glial cells of brain tissue (Farooqui et al., 2000a; Pardue et al., 2003). Forebrain and midbrain are very lightly stained with cPLA$_2$-α antibody, except for the arcuate nucleus and mammillary nuclei. In contrast, hindbrain contains many densely labeled nuclei. Most of the cPLA$_2$-α is present in dendrites postsynaptic to unlabeled axon terminals and in a small number of myelinated axons (Sandhya et al., 1998; Kishimoto et al., 1999; Strokin et al., 2003; Shirai and Ito, 2004). This paralog is involved in the production of lipid mediators, such as ARA, eicosanoids, and platelet-activating factor under physiological and pathophysiological conditions.

cPLA$_2$-β is found mainly in the cerebellar granule cells (Shirai and Ito, 7004). It shares more similarities with cPLA$_2$-α than with cPLA$_2$-γ. cPLA$_2$-β contains an N-terminal C2 domain that confers Ca^{2+} sensitivity and an additional N-terminal extension containing a JmjC domain with an unknown function (Pickard et al., 1999). cPLA$_2$-β lacks the regulatory phosphorylation sites that are present in cPLA$_2$-α. Besides the C2 domain, cPLA$_2$-γ also lacks regulatory phosphorylation sites that are present in cPLA$_2$-α. (Diaz-Arrastia and Scott, 1999; Farooqui et al., 2000a; Hirabayashi et al., 2004). However, it contains multiple putative PKC phosphorylation sites. cPLA$_2$-γ is constitutively associated with cellular membranes, and also has farnesylated, palmitoylated, and oleoylated sites (Tucker et al., 2005). Unlike cPLA$_2$-α, cPLA$_2$-γ acts on other fatty acid residues at the *sn*-2 and *sn*-1 positions of glycerophospholipids. cPLA$_2$-α hydrolyzes fatty acids at the *sn*-2 position; cPLA$_2$-β prefers to cleave fatty acids at the *sn*-1 position; and cPLA$_2$-γ efficiently hydrolyzes fatty acids at *sn*-1 as well as *sn*-2 positions of the glycerol moiety (Song et al., 1999). cPLA$_2$-γ is constitutively expressed in the mitochondrial, endoplasmic reticulum, and Golgi apparatus membranes, where it is involved in remodeling and maintaining membrane phospholipid composition under oxidative stress. Recombinantly expressed cPLA$_2$-γ liberates ARA from phosphatidylcholine. Unlike cPLA$_2$-α, cPLA$_2$-γ also acts on other fatty acid residues at the *sn*-2 and *sn*-1 positions of glycerophospholipids. cPLA$_2$-α hydrolyzes fatty acids at the *sn*-2 position; cPLA$_2$-β prefers to cleave fatty acids at the *sn*-1 position; and cPLA$_2$-γ efficiently hydrolyzes fatty acids at *sn*-1 as well as *sn*-2 positions of the glycerol moiety (Song et al., 1999). cPLA$_2$-γ is constitutively expressed in the endoplasmic reticulum, where it is involved in remodeling and maintaining membrane phospholipid composition under oxidative stress. The genes for human cPLA$_2$-α, β, and γ map to chromosomes 1, 15, and 19, respectively. cPLA$_2$-δ is mainly found in skin. In contrast to other cPLA$_2$ paralogs, it has a preference for linoleic acid instead of arachidonic acid release (Chiba et al., 2004).

3.5.2 Other Phospholipases A_2

Rat brain contains a novel PLA_2 that hydrolyzes fatty acids at the *sn*-1 and *sn*-2 positions of the glycerol moiety (Yoshida et al., 1998). This enzyme has been purified using multiple column chromatographic procedures. The purified enzyme shows optimal activity under alkaline conditions. It is independent of Ca^{2+} concentration. This PLA_2 is not affected by ATP and has a molecular mass of 300 kDa, and hydrolyzes oleic, linoleic, and arachidonic acid from the *sn*-2 position phosphatidylcholine. This enzyme also releases palmitic and stearic acids from the *sn*-1 position. PtdCho, PtdEtn, PtdIns, and PtdH are hydrolyzed with almost similar rates (Yoshida et al., 1998).

3.6 Regulation of PLA_2 Activity in Brain

Many stimuli regulate multiple forms of PLA_2, and modulate the generation of PLA_2-derived lipid mediators. They include neurotransmitters, growth factors, cytokines, glycosaminoglycans, ganglioside, and endogenous inhibitors like annexins. Regulation of multiple forms of PLA_2 depends not only on endogenous and exogenous stimuli but also on the type of neural cells involved (neurons versus glia or neural cells versus non-neural cells). In addition to signaling role of PLA_2, regulatory processes also maintain neural cell glycerophospholipid mass and therefore glycerophospholipid homeostasis by modulating the recycling of fatty acid moieties.

3.6.1 Regulation of PlsEtn-PLA_2

Bovine brain PlsEtn-PLA_2 is inhibited by glycosaminoglycans and sialoglycoconjugates. The interactions among PlsEtn-PLA_2, glycosaminoglycans, and sialoglycoconjugates may be involved in anchoring this enzyme to neural membranes where its plasmalogens and phosphatidylserine are localized. Sphingomyelinase, an enzyme that generates ceramide, decreases the levels of plasmalogens in rat brain slices (Latorre et al., 1999). This effect can be mimicked by C2-ceramide (Latorre et al., 2003). The decrease in plasmalogens by sphingomyelinase or C2-ceramide is inhibited by quinacrine, ganglioside, and bromoenol lactone, which are non-specific inhibitors of PlsEtn-PLA_2 activity (Farooqui and Horrocks, 2001b). It is interesting to note that addition of the caspase-3 inhibitor, acetyl-l-aspartyl-l-glutamyl-l-valyl-l-aspartyl-cholomethylketone (Ac-DEVD-CMK), partially blocks the ceramide-induced stimulation of plasmalogen-selective PLA_2 without altering sphingomyelinase-elicited ceramide accumulation (Latorre et al., 1999). This suggests the involvement at the nuclear level of plasmalogen hydrolysis in signal transduction related to apoptotic cell death (Farooqui et al., 2004b). Arachidonic acid

is known to stimulate sphingomyelinase, and ceramide activates plasmalogen-selective PLA_2 activity (Farooqui et al., 2001), plasmalogen-selective PLA_2 activity (Farooqui et al., 2001) indicating a close interaction (cross-talk) between glycerophospholipid and sphingolipid metabolism (Farooqui et al., 2004b; Farooqui et al., 2007). Ceramide and ceramide-1-phosphate stimulate PKC while sphingosine and sphingosine-1-phosphate inhibit PKC, Ca^{2+}/calmodulin-dependent kinase, Na^+/K^+-ATPase, CTP:phosphocholine cytidylyltransferase, and PLC (Farooqui et al., 2007). Both ceramide-1-phosphate and sphingosine-1-phosphate promote the induction of COX-2 (Pettus et al., 2004a,b). Ceramide-1-phosphate induces translocation of PLA_2 to the nuclear membrane. Collective evidence suggests that sphingolipid-derived metabolites may act in concert to regulate the production of eicosanoids from arachidonic acid. Collectively, these studies suggest that interactions between plasmalogen and sphingolipid-derived lipid mediators regulate cellular functions such as proliferation, migration, cytoskeletal organization, inflammation, and differentiation (Pyne, 2004; Pettus et al., 2004b; Farooqui et al., 2007). The regulation of PlsEtn-PLA_2 may be a complex process. In brain tissue, it may vary from one cell type to another. Regulation of PlsEtn-PLA_2 may have superimposed mechanisms. Thus, it is important to understand not only the molecular mechanism of receptor-mediated plasmalogen degradation but also the interactions of plasmalogen-derived lipid mediators with other neural membrane constituents such as glycosaminoglycans and sialoglycoconjugates.

3.6.2 Regulation of $cPLA_2$

In neural membranes, $cPLA_2$ activity and arachidonic acid release are linked to dopamine, glutamate, serotonin, P2-purinergic, cytokine, growth factor receptors, and RA receptors through different coupling mechanisms (Table 3.2). Some receptors involve G-proteins and others do not (Farooqui et al., 2006). $cPLA_2$ activity is regulated by translocation in the presence of Ca^{2+} and by phosphorylation. Calcium mediates binding of the enzyme to phospholipid substrate without being involved in the catalytic mechanism itself. An increase in the intracellular calcium produces translocation of $cPLA_2$ to nuclear and other cellular membranes through a calcium-dependent lipid-binding motif (Clark et al., 1995). The translocation of $cPLA_2$ not only allows the interaction between enzyme protein and its phospholipid substrate but it brings $cPLA_2$ into close proximity with other downstream enzymes responsible for the conversion of arachidonic acid into eicosanoids. $cPLA_2$ also contains a consensus sequence for MAPK (Pro-Leu-Ser-505-Pro). In astrocytes, Ser-505 is phosphorylated by both p38-MAP kinase and *c*-Jun N-terminal kinase (Hernández et al., 1999, 2000).

In neural and non-neural tissues, $cPLA_2$ activity is modulated through a cooperative binding mechanism with glycerophospholipids containing

ARA(Burke et al., 1995) or through binding of anionic phospholipids, such as phosphatidylinositol 4,5-bisphosphate (PIP_2), phosphatidylinositol 3,4,5-trisphosphate (PIP_3), and ceramide-1-phosphate (Hirabayashi et al., 2004; Pettus et al., 2004a), to a pleckstrin homology domain (Mosior et al., 1998). Role of glycosphingolipids as regulators of signal transduction processes has been recently appreciated through the identification of sphingolipid-derived lipid mediators such as ceramide, ceramide-1-phosphate, sphingosine, and sphingosine-1-phosphate (Pettus et al., 2004a; Pyne, 2004). These sphingolipid-derived metabolites modulate activities of various isoforms of PLA_2 and modulate the release of ARA and DHA.

$cPLA_2$ activity is also regulated by inhibitory proteins called annexins (Gerke and Moss, 1997; Kaetzel and Dedman, 1995; Clemen et al., 2001). Studies on the regulation of $cPLA_2$ activity by annexins in non-neural cells are complicated not only by the presence of several annexins (at least five) but also by the occurrence of several paralogs of $cPLA_2$ activity. Annexin 1(ANXA1) specifically targets $cPLA_2$ through direct enzyme inhibition and suppression of cytokine-mediated activation of the enzyme. ANXA1 also downregulates the activity of enzymes such as inducible nitric oxide synthase (iNOS) in macrophages and inducible cyclooxygenase-2 (COX-2) in activated microglia. The inhibition of iNOS expression may be due to ANXA1-mediated IL-10 release in macrophages (Parente and Solito, 2004).

The occurrence of PLA_2-activating proteins has also been reported in non-neural cells such as monocytes and endothelial cells (Clark et al., 1987). $cPLA_2$-activating proteins occur in Aplysia neurons and rat cerebral cortex (Calignano et al., 1991). The partially purified $cPLA_2$ stimulatory protein is phosphorylated by PKC. It has been proposed that phosphorylation of this stimulatory protein by PKC regulates $cPLA_2$ activity in neurons (Calignano et al., 1991).

3.7 Conclusion

In brain the release of ARA and DHA from neural membrane glycerophospholipids is catalyzed by $cPLA_2$ and PlsEtn-PLA_2, respectively. Bovine brain PlsEtn-PLA_2 differs from rabbit heart and kidney PlsEtn-PLA_2 in molecular mass, inhibitory effect of ATP, stimulatory effect of non-ionic detergents (Triton-X-100 and glucoside), and inhibitory effect of anionic detergents (sodium deoxycholate). Addition of iodoacetate results in a dose-dependent inhibition of PlsEtn-PLA_2 activity, and this inhibition is reversed by β-mercaptoethanol. Bovine brain PlsEtn-PLA_2 is inhibited by NANA, GM_3 ganglioside, and mucin in a dose-dependent manner, with an inhibitory pattern of NANA > GM3 > mucin. Treatment of neuronal cultures with kainic acid results in a dose-dependent stimulation of PlsEtn-PLA_2 activity, and this stimulation is blocked by CNQX, a kainic and AMPA receptor antagonist. ARA release from glycerophospholipids is

catalyzed by $cPLA_2$. Several paralogs of $cPLA_2$ occur in brain tissue. They differ from each other in molecular mass, presence or absence of C2 domain, and response to inhibitors. $cPLA_2$ differs from PlsEtn-PLA_2 in molecular mass, response to inhibitory effects of ATP, NANA, GM_3 ganglioside, and mucin. These enzymes also differ from each other in thermo-stabilities at pH 7.4. Under normal conditions, these enzymes control neural membrane integrity by altering levels of essential glycerophospholipids and modulating membrane fluidity, permeability, and ion homeostasis transiently. The reacylation of lysophospholipids through a series of energy-dependent reactions restores normal neural membrane integrity and function.

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Chapter 4
Oxidation of Arachidonic and Docosahexaenoic Acids and Neurochemical Effects of Their Metabolites on Brain

4.1 Introduction

In brain tissue, ARA and DHA are released by the action of $cPLA_2$ and PlsEtn-PLA_2, respectively (Horrocks and Farooqui, 2004). As stated in Chapter 3, free ARA and DHA are either reincorporated in neural membrane phospholipids by reacylation reactions or oxidized by several enzymic and non-enzymic mechanisms to various oxygenated metabolites with important neurochemical functions (Rapoport, 1999, 2003; Lee et al., 2004a; Farooqui et al., 2000a,b; Green et al., 2008). Thus, COX, LOX, and EPOX metabolize ARA to prostaglandins, thromboxanes, leukotrienes, lipoxins, and epoxyeicosatrienoic acids (Table 4.1). Prostaglandins, thromboxanes, leukotrienes, and lipoxins are collectively called as eicosanoids (Fig. 4.1). Eicosanoids have been detected in neural cells (neurons, astrocytes, oligodendrocytes, and microglia), cerebral vascular endothelial cells, and cerebrospinal fluid (Wolfe and Horrocks, 1994; O'Banion, 1999). Eicosanoids not only regulate signal transduction and gene transcription processes but also induce acute inflammatory responses, induce oxidative stress, and mediate fever and pain (Wolfe and Horrocks, 1994). Brain tissue expresses several different isoforms of $cPLA_2$, PlsEtn-PLA_2, COX, LOX, and EPOX both under normal and stimulated situations (Horrocks and Farooqui, 2004; Kis et al., 2003, 2004; Snipes et al., 2005; Shaftel et al., 2003). Cross talk among isoforms of $cPLA_2$, PlsEtn-PLA_2, and COX, LOX, and EPOX enzymes modulate the synthesis of eicosanoids among neural cells, macrophages, platelets, and endothelial cells (Shinohara et al., 1999; Farooqui and Horrocks, 2007; Farooqui et al., 2007). Eicosanoids synthesis during receptor stimulation and pathological conditions involves different ARA substrate pools that may be coupled to distinct PLA_2, COX, LOX, and EPOX isoforms at different cellular and subcellular levels (Ueno et al., 2001; Farooqui et al., 2006; Farooqui and Horrocks, 2007).

In addition to COX-, LOX-, and EPOX-derived products, the degradation of ARA and DHA in brain also generates reactive oxygen species (ROS). ROS include oxygen free radicals (superoxide radicals, hydroxyl, and alkoxyl radicals) and peroxides (hydrogen peroxide and lipid

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Table 4.1 ARA-, DHA-, and EPA-derived enzymic and non-enzymic lipid metabolites

Substrate	Enzyme	Lipid mediator	References
ARA	COX-1,-2, and -3	PGE_2, PGI_2, PGF_2, and PGD_2	Wolfe and Horrocks (1994)
	5-LOX, 10-LOX, and 15-LOX	LTA_4, LTB_4, LTC_4, LTD_4, and LTE_4	Wolfe and Horrocks (1994)
	Thromboxane synthases	TXA_2 and TXB_2	Wolfe and Horrocks (1994)
	15-LOX	LXA_4, LXB_4, 15 epi-LXA_4, and 15 epiLXB_4	Chiang et al. (2006)
ARA	Non-enzymic	4-HNE	Esterbauer et al. (1991)
	Non-enzymic	Isoprostanes	Fam and Morrow (2003)
	Non-enzymic	Isofurans	Fam and Morrow (2003)
	Non-enzymic	Isoketals	Fam and Morrow (2003)
DHA	15-LOX	Resolvins/docosatrienes	Hong et al. (2003)
	15-LOX	Protectins/ neuroprotectins	Mukherjee et al. (2004); Serhan (2005)
	Non-enzymic	4-HHE	Kristal et al. (1996)
	Non-enzymic	Neuroprostanes	Yin et al. (2005)
	Non-enzymic	Neuroketals	Bernoud-Hubac et al. (2001)
EPA	COX-1 and -2	PGI_3, PGD_3, PGE_3, and PGF_3	Calder and Grimble (2002)
	5-LOX	LTA_5, LTB_5, LTC_5, LTD_5	Calder and Grimble (2002)
	Thromboxane synthase	TXA_3, TXB_3	Calder and Grimble (2002)
	5-LOX	RvE_1	Serhan et al. (2004)
	5-LOX	RvE_2	Serhan et al. (2004)
	Non-enzymic	F_3-Isoprostane	Nourooz-Zadeh et al. (1997); Gao et al. (2006)

hydroperoxide). Under normal conditions at low levels, ROS function as signaling intermediates in the regulation of fundamental cell activities such as growth and adaptation responses. At higher concentration, ROS diffuse and reach SH groups of many proteins including pump and channel proteins that are embedded in the neural membranes. ROS initiate membrane damage by reacting and shifting the balance between reducing and oxidizing (redox) forces toward oxidative stress (Farooqui et al., 2008a). Other targets of ROS include unsaturated lipids and DNA (Berlett and Stadtman, 1997). The reaction between ROS and proteins or unsaturated lipids in the plasma membrane results in chemical cross-linking of membrane proteins and lipids and a reduction in membrane unsaturation. The depletion of unsaturation in membrane lipids is associated with decreased membrane fluidity and decreased activity of membrane-bound enzymes, ion channels, and receptors (Ray et al., 1994).

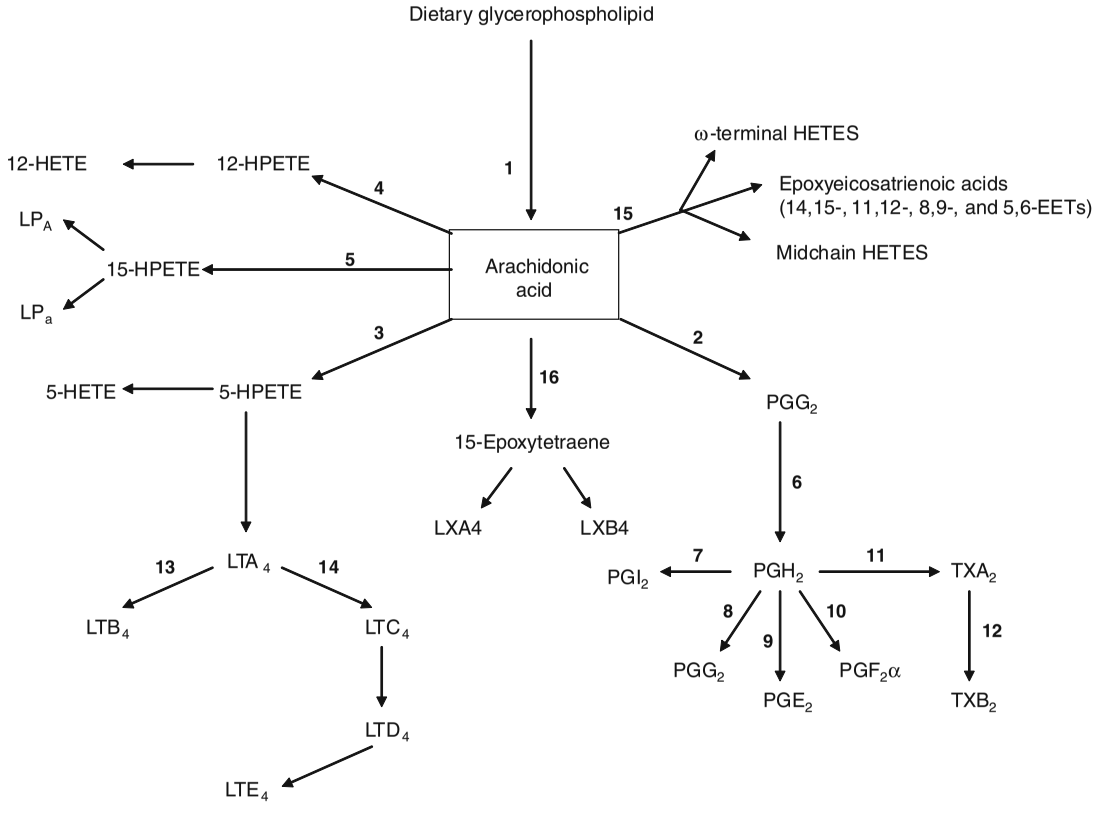

Fig. 4.1 Release of arachidonic acid from neural membrane phospholipids and its metabolism by cyclooxygenases, lipoxygenases, and epoxygenases results in generation of prostaglandins, leukotrienes, thromboxanes, and epoxyeicosanoids. Phospholipase A_2 (1); cyclooxygenases (2); 5-lipoxygenase (3); 12-lipoxygenase (4); 15-lipoxygenase (5); peroxygenase activity of cyclooxygenase (6); PGI_2 synthase (7); PGG_2 synthase (8); PGE_2 (9); PGF_2 synthase (10); thromboxane A_2 synthase (11); thromboxane B_2 synthase (12); LTB_4 synthase (13); LTC_4 synthase (14); cytochrome P450 epoxygenases (15); and 15-LOX (16). Modified from Phillis et al. (2006)

4.2 Arachidonic Acid and Its Enzymic Oxidation in Brain

After its release from neural membrane glycerophospholipids, a small proportion of ARA (10–15%) is converted to eicosanoids, whereas the majority of ARA (85–90%) is reincorporated into brain glycerophospholipids (Rapoport, 1999). Thus, levels of free ARA in brain are very low (<10 μmol/kg). At this level, ARA not only acts as a second messenger itself, but is also a precursor of many lipid mediators (Fig. 4.1). ARA modulates activities of many enzymes including protein kinase C, nitric oxide synthase, diacylglycerol kinase, caspase-3, NADPH oxidase, which are involved in neural cell survival and death (Farooqui and Horrocks, 2006). In addition, ARA modulates ion channels, neurotransmitter release, induction of long-term potentiation, and neural cell differentiation (Katsuki and Okuda, 1995; Farooqui et al., 1997). ARA acts as a facilitatory retrograde neuromodulator in glutamatergic synapses (Katsuki and Okuda, 1995; Farooqui et al., 2008a). ARA also modulates acetylcholine release in rat hippocampus (Almeida et al., 1999). Low levels of ARA are involved in maintaining the structural integrity of neural membranes, optimizing neural membrane fluidity, and thereby regulating neuronal transmission. In the nucleus, ARA may also interact with elements of gene structure, such as promoters, enhancers, and suppressors, to modulate gene expression in a specific manner that is not shared by eicosanoids or other fatty acids (Farooqui et al., 1997; Farooqui and Horrocks, 2007). Under pathological conditions, at elevated levels ARA not only acts as precursor for high levels of eicosanoids but also causes intracellular acidosis and uncouple oxidative phosphorylation, which results in mitochondrial dysfunction. High levels of ARA also produce mitochondrial swelling in neurons and induce changes in membrane permeability by regulating ion channels (Farooqui et. al., 1997; Farooqui and Horrocks, 2007).

The enzymic oxidation of ARA is catalyzed by COX, LOX, and EPOX. Several isoforms of COX, LOX, and EPOX occur in brain. Activities of these enzymes are coupled to various receptors. Under normal conditions, receptor-mediated stimulation of COX, LOX, and EPOX is sufficient to generate enough lipid mediators that maintain normal cellular functions. Under pathological conditions, overstimulation of various receptors results in the generation of high levels of lipid mediators, which may cause neural cell injury (Farooqui and Horrocks, 2007; Farooqui et al., 2008b).

4.2.1 Isoforms of Cyclooxygenases in Brain

COXs are heme-containing bifunctional enzymes with two catalytic sites. A cyclooxygenase site adds two oxygens to ARA to form the hydroxy endoperoxide called PGG_2. The other peroxidase active site reduces PGG_2 to PGH_2 (Jiang et al., 2004; Simmons et al., 2004). PGH_2 is a precursor for several

prostaglandins, thromboxanes (TXA_2), and prostacyclins (PGI_2). These lipid mediators and their related molecules interact with their specific receptors to modulate cell function (O'Banion, 1999). Three forms of COX enzymes designated as COX-1, COX-2, and COX-3 occur in brain tissue. COX-1 is a constitutive enzyme responsible for the physiological production of prostaglandins. It is involved in several homeostatic processes and therefore called a "housekeeping" enzyme. Normally, very low levels of COX-2 are detectable in brain tissue, but this enzyme is induced by inflammatory mediators such as cytokines, growth factors, and bacterial endotoxin (Hoffmann, 2000). COX-1 and COX-2 are homodimers, which share about 60% homology at both the cDNA and the amino acid levels and have similar structural and kinetic properties (Smith et al., 2000; Simmons et al., 2004). The active site of COX-2 is larger and more accommodating than that of COX-1, and COX-1 displays negative allosterism at low concentrations of ARA. This property may be responsible for greater eicosanoid production by COX-2 when the ARA concentration is low (Smith et al., 2000). Thus, despite the structural similarity between COX-1 and COX-2, these isoforms differ substantially in the regulation of their expression and their roles in normal neurological and neuropathological processes. ARA is the preferred substrate for COX-1 and COX-2. However, these enzymes also oxygenate γ-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid (Serhan et al., 2004).

In brain tissue, COX-1 and COX-2 immunoreactivities are present in cerebral cortex, midbrain, and hippocampus. In contrast, COX-1 immunoreactivity is enriched in midbrain, pons, and medulla (Breder et al., 1995). COX-2 immunoreactivity is concentrated in neurons and glial cells of hippocampus, hypothalamus, and amygdala (Andreasson et al., 2001). At the subcellular levels in neurons, astrocytes, and microglial cells, COX-2 immunoreactivity is localized to the perinuclear regions (Tomimoto et al., 2000). The expression of COX-2 is markedly increased in microglial cells after intraperitoneal injection of lipopolysaccharide (LPS), whereas neuronal COX-1 remains unchanged (Elmquist et al., 1997). Upon stimulation by LPS, COX-2 expression and its ability to generate PGE_2, TXA_2, and TXB_2 in microglial cells are several times higher than in astrocytes, but lower than in peripheral macrophages (Minghetti and Levi, 1995). COX-2 induction in microglia by proinflammatory stimuli is apparently similar to peripheral macrophages, and plays important roles in inflammatory and immune responses.

COX-3 is an acetaminophen-sensitive isoform of the COX family. Although different pharmacological properties have been described for COX-3 compared with COX-1 or COX-2 enzymes, many investigators consider COX-3 to be a splice variant of COX-1 (Chandrasekharan et al., 2002; Davies et al., 2004). Thus, COX-3 is a product of the COX-1 gene, but retains intron 1 in its mRNA. Rat COX-3 is a cytosolic basic glycoprotein with a pI value of 12.40. In addition to the abundance of basic amino acids, the COX-3 protein is also very rich in proline (11.81%) (Snipes et al., 2005).

The expression of COX-3 mRNA is highest in choroid plexus and spinal cord followed by pituitary gland, hypothalamus, hippocampus, medulla, cerebellum, and cortex. COX-3 mRNA levels are high in major brain arteries and brain microvessels. The expression pattern of COX-3 mRNA in the rat CNS primarily relates to the vascular density of a given region (Kis et al., 2003). The expression studies of COX-3 mRNA in primary cultures of neurons, astrocytes, endothelial cells, pericytes, and choroidal epithelial cells indicate that the mRNA of COX-3 is present in all of these cell types except neurons. Cerebral endothelial cells show the highest COX-3 expression (Kis et al., 2003). COX-3 mRNA has been cloned and sequenced from rat cerebral endothelial cells.

4.2.2 Roles of Eicosanoids in Brain

Eicosanoids are involved in many processes including fever, sensitivity to pain, sleep, inflammation, and oxidative stress, and are the target of aspirin-like drugs. These metabolites are not stored in neural cells, but are generated in response to receptor-mediated stimulation. Because of their amphiphilic nature, eicosanoids can cross cell membranes and leave the cell in which they are synthesized to act on neighboring cells. Eicosanoids act through specific superficial or intracellular receptors (G protein-coupled receptors), modulating signal transduction pathways and gene transcription. Identification and cloning of specific prostaglandin transporters have also been reported. Prostaglandins also activate nuclear receptors, supporting the view that these lipid mediators also participate in intracrine signaling (Helliwell et al., 2004). Some prostaglandins (Fig. 4.2) modulate neurotransmitter release, whereas others regulate circulatory function, sleep, fever and pain, and vasodilation and vasoconstriction (Phillis et al., 2006).

The generation and accumulation of eicosanoids under pathological conditions modulate cerebrovascular blood flow and promote oxidative stress. Their active production by platelets and leukocytes may contribute to the onset of alterations in the microcirculation and ultimately to CNS dysfunction (Phillis et al., 2006). High levels of prostaglandins have neurodegenerative effects on differentiated murine neuroblastoma cell cultures. In vivo, prostaglandins are associated with the regulation of cytokines and maintenance of the inflammatory cascade (Phillis et al., 2006; Farooqui et al., 2007).

Thromboxanes are eicosanoids that not only mediate platelet aggregation but modulate vasoconstriction and produce vasospasm. They also act as a hypertensive agent and play an important role in Prinzmetal's angina. Two thromboxanes, namely thromboxanes 1 and 2 (TXA_1 and TXA_2), and their receptors are known to occur in brain tissue. Studies on cultures of oligodendrocytes and oligodendrocyte progenitor cells reveal that the expression of COX-1, TXA_2 synthase, and TXA_2 receptor as well as TXA_2 synthesis increases

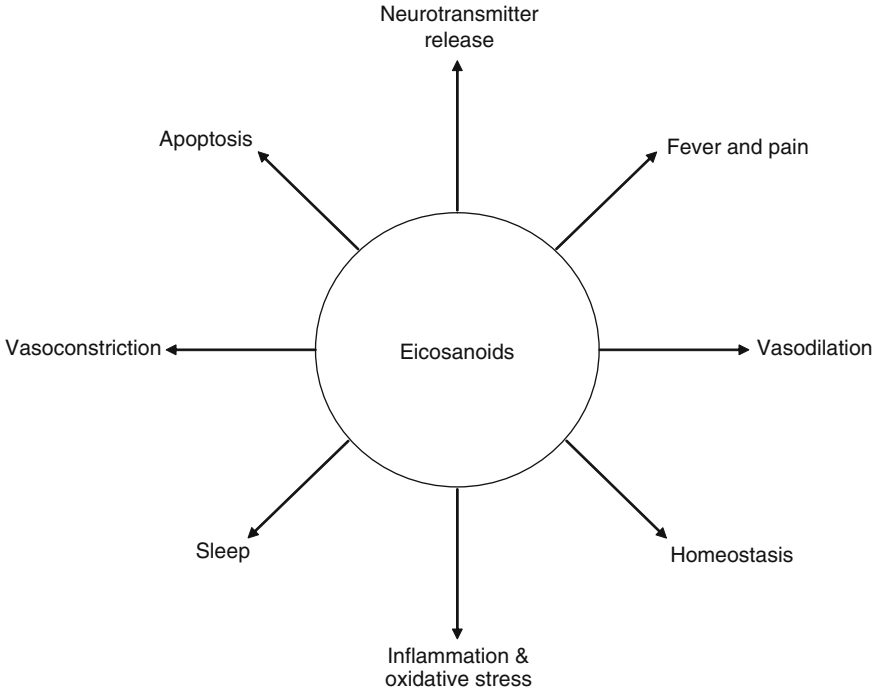

Fig. 4.2 Roles of eicosanoids in brain

during oligodendrocyte maturation (Ramamurthy et al., 2006). Immunocytochemical localization studies indicate that TXA_2 receptors are localized in the plasma membrane and perinuclear compartments in oligodendrocyte progenitor cells. However, during oligodendrocytes differentiation, TXA_2 receptors shift their localization pattern and also become associated with the nuclear compartment. This shift to nuclear localization is confirmed by biochemical analysis in cultured cells and by immunocytochemical analysis in the developing rat brain. Activation of TXA_2 receptor by the agonist U46619 increases intracellular calcium in both oligodendrocyte progenitor cells and oligodendrocytes. U46619-mediated stimulation of TXA_2 receptors in maturing oligodendrocytes results in upregulation of myelin basic protein expression and regulation of MBP expression. The inhibition of endogenous TXA_2 receptor results in the downregulation of signaling pathway and reduction in MBP expression. Collective evidence suggests a novel TXA_2 receptor signaling pathway in oligodendrocytes and a potential role for this signaling during oligodendrocyte maturation and myelin production (Ramamurthy et al., 2006).

Prostacyclin, another eicosanoid, acts not only through its surface receptors but also via its nuclear receptors in physiological and pathological processes. Prostacyclins interact with peroxisomal proliferator-activated receptors and modulate

many biological functions. Similarly, leukotrienes are lipid mediators of ARA metabolism, involved in autocrine and paracrine signaling (Phillis et al., 2006). Similarly, leukotrienes are potent inflammatory mediators synthesized locally within the cerebrovascular system and neural cells through the 5-LOX pathway of ARA metabolism. The leukotrienes, consisting of dihydroxy leukotriene LTB_4 and the cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4, act by targeting not only neural cell surface receptors but also their receptors on inflammatory cells and on structural cells of cerebrovascular vessel walls (Phillis et al., 2006).

4.2.3 Isoforms of Lipoxygenases in Brain

LOXs are a family of heme-containing enzymes that catalyze the dioxygenation of ARA. At least three isoforms of LOXs occur in mammalian tissues. They insert oxygen at carbon 5, 12, or 15 position of ARA, generating 5S-, 12S-, or 15S-hydroperoxyeicosatetraenoic acid (5-, 12-, or 15-HPETE), which are further reduced by glutathione peroxidase to the hydroxy forms (5-, 12-, 15-HETE) (Funk, 2001; Brash et al.,1999; Kuhn and Borngraber, 1999). 5-LOX contains two distinct enzymic activities leading to the formation of LTA_4. First activity catalyzes the incorporation of molecular oxygen into ARA (oxygenase activity), producing HPETE and subsequently formation of unstable epoxide LTA_4 (LTA_4 synthase activity) (Funk, 2001). The second activity catalyzes the insertion of molecular oxygen at position C-5, converting LTA_4 to either 5(S)-hydroxy-6-trans-8,11,14-*cis*-eicosatetranoic acid (5-HETE) or leukotrienes. LTA_4 is also metabolized to LTB_4 by LTA_4 hydrolase or to LTC_4 by conjugation of glutathione at the sixth carbon by the action of LTC_4 synthase. LTA_4 is the precursor for the family of cysteinyl leukotrienes (cysLTs: LTC_4, LTD_4, and LTE_4) (Wolfe and Horrocks, 1994). Leukotrienes exert their effects through the activation of specific cellular membrane receptors. Several leukotriene receptors have been identified in mammalian tissues. They include G protein-coupled $cysLT_1$ (earlier name LTD_4), $cysLT_2$ (earlier name LTC_4), and hydroleukotriene BLT (earlier name LTB4) receptors. With the identification of receptor-selective reagents and development of mice lacking critical biosynthetic enzymes, transporter proteins, and $CysLT_1$ and $CysLT_2$ receptors, diverse functions of cys-LTs and their receptors in immune and inflammatory responses have been discovered (Capra et al., 2006). Thus, in non-neural tissues, cysteinyl-LTs and their receptors have been implicated in the pathophysiology of many inflammatory conditions, such as cancer, atopic dermatitis, idiopathic chronic urticaria, and cardiovascular diseases (Capra et al., 2006).

12-LOX and 15-LOX act on 12- and 15-positions of ARA, generating 12- and 15-HPETE. These pathways lead to the generation of 12- and 15-HETE and lipoxins. 12-LOX is the most common LOX in the brain (Fig. 4.1). Its mRNA is found in rat cortical neurons, astrocytes, and oligodendrocytes (Bendani et al., 1995). Some 15-LOX mRNA along with 15-HETE is also

found in rat brain (Wolfe and Horrocks, 1994). These enzymic pathways can provide lipid mediators that are both constrictors and dilators. They modulate cerebral vasculature and thus are capable of regulating the cerebral circulation in a comparable manner to COX-1 and 2 pathways (Wolfe and Horrocks, 1994). LOXs have a molecular mass of 75–78 kDa. They consist of a single polypeptide chain that is folded into two domains (Brash, 2001; Kuhn and Borngraber, 1999). Rat brain 12-LOX has been characterized and cloned. It displays the highest degree of identity to porcine leukocyte 12-LOX (71%) and to human 15-LOX (75%), but has less resemblance with human platelet 12-LOX (59%) or rat leukocyte 5-LOX (41%).

5-LOX is present in brain tissue and neural cells in the cytoplasm. Upon cell activation, 5-LOX translocates from cytoplasm to the nuclear envelope. This enzyme also requires FLAP, a nuclear envelope-bound 18-kDa protein that acts as an ARA transfer protein. It is essential for leukotriene B_4 (LTB_4) and leukotriene C_4 (LTC_4) synthesis (Manev et al., 2000). LTB_4 acts through BLT_2 receptors and induces release of proinflammatory cytokines. FLAP shows homology with LTC_4 synthase and other microsomal glutathione transferases, but has no enzymic activity itself. 5-LOX also contains a Src homology 3 (SH3) binding motif, which may be involved in the interaction of 5-LOX protein with growth factor receptor-bound protein 2 (Grb2). In cerebellar granule neurons, dexamethasone, a glucocorticoid, upregulates 5-LOX mRNA and protein contents, and this upregulation is blocked by glucocorticoid antagonist, RU486 (Uz et al., 2001). It is also suggested that dexamethasone increases the stability of 5-LOX mRNA. 5-LOX and 15-LOX have been sequenced from several non-neural sources (Dixon et al., 1988). They have a molecular mass of 78 kDa, and share considerable homology with 5-LOX and 15-LOX from soybean.

4.2.4 Roles of Lipoxins in Brain

Lipoxins (LXA_4, LXB_4, 15 epi-LXA_4, and 15 epiLXB_4) are endogenous eicosanoids derived from ARA (Fig. 4.3). They inhibit neutrophil trafficking and stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. They are generated by the action of LOXs on HPETE and HETE. Lipoxins participate in the resolution phase of acute inflammation. They promote resolution of inflammation by modulating key steps in leukocyte trafficking, namely phagocytosis of apoptotic polymorphonuclear neutrophil (PMN) by monocyte-derived macrophages, and preventing neutrophil-mediated acute tissue injury (Serhan et al., 2004; Kantarci and Van Dyke, 2003; Chiang et al., 2006). Synthesis of lipoxins is a transcellular process, which involves interactions among leukocytes, platelets, and endothelium. In monocyte-derived macrophages, LXA_4-mediated phagocytosis does not provoke IL-8 or monocyte chemoattractant protein-1 release (Godson et al., 2000).

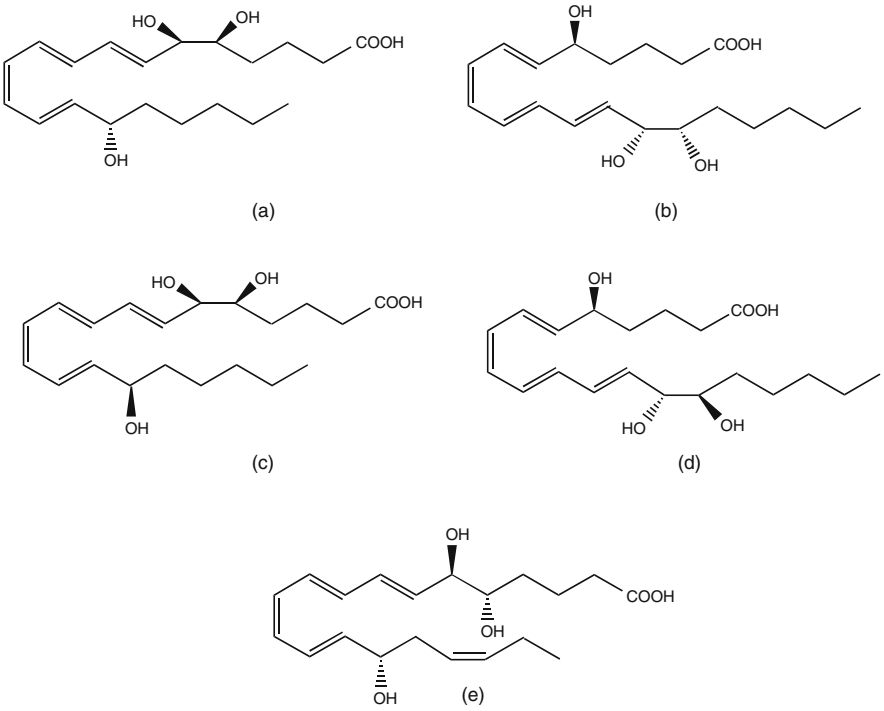

Fig. 4.3 Structures of ARA- and EPA-derived lipoxins. Lipoxin A_4 (LXA_4); lipoxin B_4 (LXB_4); aspirin-triggered lipoxin A_4 (epi-LXA_4); aspirin-triggered lipoxin B_4 (epi-LXB_4); and EPA-derived lipoxin A_5 (LXA_5)

LXA_4-mediated phagocytosis can be attenuated by anti-CD36, αvβ3, and CD18 mAbs. LXA_4-mediated PMN uptake can also be abolished by pertussis toxin and by 8-bromo-cAMP and mimicked by Rp-cAMP, a protein kinase A inhibitor. LXA_4 attenuates PGE_2-stimulated protein kinase A activation (Maderna et al., 2002). In addition, lipoxins stimulate RhoA- and Rac-dependent cytoskeleton reorganization, a process that may lend additional support to the potential role of lipoxins in the resolution of inflammation. Collectively, these studies suggest that LXA_4 is an endogenous stimulus for PMN clearance during inflammation and provide a novel rationale for using stable synthetic analogs as antiinflammatory compounds in vivo (Gordon et al., 2000).

In neural and non-neural tissues, lipoxins bind with high affinity to G protein-coupled ALX and LXA receptors that transduce counter-regulatory signals in part via intracellular polyisoprenyl phosphate remodeling (Norel and Brink, 2004; Serhan et al., 2007). Activation of ALX receptors leads to down-regulation of many endogenous events such as leukocyte migration, TNF-α-mediated production of chemokines, expression of chemokine receptors and adhesion molecules, and superoxide formation. At the molecular level, LXA_4

signaling attenuates activator protein-1 and NF-κB activation in both polymorphonuclear and mononuclear leukocytes and inhibits IL-8 mRNA expression, blocks phosphorylation of p38, inhibits extracellular signal-regulated kinase and *c*-Jun N-terminal kinase, and prevents IL-8 release mediated by lipopolysaccharide (Jozsef et al., 2002). Aspirin, a drug that inhibits prostaglandin and thromboxane generation, facilitates the synthesis of lipoxins and promotes resolution of neuroinflammation through the formation of lipoxins (Serhan et al., 2007; Chiang et al., 2006; Levy et al., 2007). At the molecular level, acetylation of COX-2 enables it to act as a LOX producing the lipoxin precursor 15-HETE from ARA, which is then converted to 15-epi-LXA_4 or 15-epi-LXB_4 by leukocyte 5-LOX. These lipoxins are more potent antiinflammatory agents than LXA_4 and LXB_4. Collective evidence suggests that anti-inflammatory activities of lipoxins, such as inhibition of agonist-induced polymorphonuclear cell chemotaxis and upregulation of β-2 integrins, require the expression of a G-protein-coupled high-affinity ALX and LXA receptors.

Treatment of human bronchial epithelial cells with LXA_4 results in a dose-dependent increase in intracellular $Ca^{2+}{}_i$ followed by a recovery to basal levels (Bonnans et al., 2003). The LXA_4-mediated increase in $Ca^{2+}{}_i$ is completely blocked by the pre-treatment of bronchial epithelial cells with pertussis toxin (G-protein inhibitor). The $Ca^{2+}{}_i$ response is not affected by the removal of external Ca^{2+} but completely blocked by thapsigargin, a Ca^{2+}-ATPase inhibitor treatment. Pre-treatment of the bronchial epithelial cells with either MDL hydrochloride (*cis*-*N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine.HCl), an adenylate cyclase inhibitor or R_p-cAMP, a cAMP-dependent protein kinase inhibitor, prevents the Ca^{2+} response to LXA_4 (Bonnans et al., 2003). In bronchial epithelial cells, LXA_4 also produces a sustained stimulation of the Cl^- secretion. Molecular mechanism and functional consequences of LXA_4-mediated intracellular $Ca^{2+}{}_i$ and Cl^- secretion are not fully understood. However, a non-genomic stimulation of thapsigargin-sensitive Ca^{2+}-ATPase may contribute to increase in intracellular $Ca^{2+}{}_i$ and Cl^- secretion (Bonnans et al., 2003).

Lipoxins regulate coagulation cascade by modulating the expression of tissue factor (TF), a key regulator of coagulation (Maderna et al., 2000). Thus, LXA_4 facilitates the induction of TF activity in a dose-dependent manner. This process is cycloheximide-sensitive and associated with increased TF mRNA levels. Induction of TF activity is specific for LXA_4, and other lipoxins such as LXB_4 have no effect on coagulation cascade (Maderna et al., 2000). Furthermore, 15(*R*/*S*)-methyl-LXA_4 or 16-phenoxy-LXA_4, synthetic analogs of LXA_4, have no effect on TF expression in ECV304 cells. LXA_4-mediated TF expression is inhibited by pertussis toxin as well as GF-109203X, an inhibitor of protein kinase C. This inhibition does not involve the degradation of IκBα. Collective evidence suggests that LXA_4 mediates the induction of TF activity via cell signaling pathways with different structural and receptor requirements from those described for inhibition of leukocyte–endothelial cell interactions. It also supports the role of LXA_4 as a modulator of TF-associated vascular events

during inflammation and thrombosis (Maderna et al., 2000). In addition, LXA_4 inhibits LTD_4-mediated stimulation of phosphatidylinositol 3-kinase (PtdIns 3-kinase) activity in parallel to inhibition of LTD_4-induced mesangial cell proliferation (McMahon et al., 2000). LXA_4 also modulates the activation of erk and p38. LXA_4-mediated activation of ERK is insensitive to PTX and PtdIns 3-kinase inhibition. In contrast, LXA_4-mediated activation of p38 is sensitive to PTX and can be prevented by the LTD_4 receptor antagonist SKF 104353. These data suggest that LXA_4-mediated stimulation of the MAP kinase superfamily involves two distinct receptors: one shared with LTD_4 and coupled to a PTX-sensitive G protein (G_i) and a second coupled via an alternative G protein, such as G_q or G_{12}, to ERK activation (McMahon et al., 2000).

Lipoxins also modulate nociception. LXA_4 receptors are expressed on spinal astrocytes. The delivery of LXA_4 and its stable analogs to spinal cord attenuates inflammation-mediated pain process (Svensson et al., 2007). Furthermore, activation of extracellular signal-regulated kinase and *c*-Jun N-terminal kinase in astrocytes play an important role in spinal nociceptive process, which is attenuated in the presence of lipoxins. This observation suggests the possibility that lipoxins regulate spinal nociceptive processing though their actions upon astrocytic activation. Targeting mechanisms that downregulate the spinal consequences of persistent peripheral inflammation provide a novel endogenous mechanism by which chronic pain may be controlled (Svensson et al., 2007).

4.2.5 Isoforms of Cytochrome P450 Epoxygenases in Brain

Multiple cytochromes P450 epoxygenases (EPOX or CYP450) catalyze the conversion of ARA in three eicosanoid products (Coon, 2005; Kroetz and Xu, 2005). Allylic oxidation of ARA forms several midchain conjugated dienols (5-, 8-, 9-, 11-, 12-, and 15-HETEs), ω-terminal hydroxylation of ARA forms $C_{16}-C_{20}$ alcohols of ARA (16-, 17-, 18-, 19-, and 20-HETEs), and olefin epoxidation of ARA results in the synthesis of four *cis*-epoxyeicosatrienoic acids (14, 15-, 11, 12-, 8, 9-, and 5, 6-EETs) (Fig. 4.1) (Zeldin, 2001). In addition, autooxidation of ARA by cytochrome P450-derived ROS produces lipid hydroperoxides as primary oxidation products. CPY450 epoxygenases have been detected in brain tissues (Alkayed et al., 1996), with isoforms expressed in discrete regions (Hagemeyer et al., 2003). The levels of various cytochrome P450 enzymes in brain are low, but these enzymes are expressed at relatively higher levels in astrocytes (Peng et al., 2004). Astrocyte-generated EETs induce cerebral capillary endothelial cell mitogenesis and tube formation (Munzenmaier and Harder, 2000). In addition, adenovirus-mediated CYP2C9 gene transfection and EPOX overexpression in endothelial cells result in endothelial cell tube formation by stimulating COX-2 expression and prostacyclin production (Michaelis et al., 2005). The blockade of EETs formation with different epoxygenase inhibitors decreases endothelial tube formation in co-cultures of

astrocytes and capillary endothelial cells. The molecular mechanism of endothelial tube formation is not fully understood. However, inhibition of EPOX retards serum-mediated endothelial cell proliferation, and exogenously added EETs rescue cell proliferation from EPOX inhibition (Pozzi et al., 2005). Activation of p38 MAPK is necessary for proliferative responses to 8,9- and 11,12-EETs. 5,6- and 14,15-EET-mediated cell proliferation, involving the activation of PtdIns 3-kinase. Among the 5,6-, 8,9-, 11,12-, and 14,15-EETs, only 5,6- and 8,9-EET promote endothelial cell migration and the formation of capillary-like structures. These processes depend on EET-mediated activation of ERK and PtdIns3K (Pozzi et al., 2005). It is also reported that 5,6- and 8,9-EET are pro-angiogenic in mice and that their neo-vascularization effects are enhanced by the co-administration of an inhibitor of EET enzymic hydration, presumably because of reduced EET metabolism and inactivation. Thus, 5,6- and 8,9-EET are powerful and selective angiogenic lipids. They provide a functional link between the EET proliferative chemotactic properties and their angiogenic activity suggesting their physiological roles in angiogenesis and de novo vascularization (Pozzi et al., 2005). Furthermore, certain EPOX metabolites such as 20-HETE are potent vasoconstrictors. They may produce detrimental effects in brain and heart tissues during ischemic injury and pro-inflammatory effects during reperfusion. On the other hand, a group of regioisomers resulting from the epoxidation of ARA, including 5,6-, 8,9-, 11,12-, and 14,15- EETs, inhibit ischemic and/or reperfusion injury in cardio- and cerebrovasculatures (Gross et al., 2005; Seubert et al., 2007).

Treatment of human umbilical vein endothelial cells (HUVECs) with 11,12-EET markedly stimulates sphingosine kinase activity (Yan et al., 2008). Inhibition of sphingosine kinase by a specific inhibitor, SKI II (4-[[4-(4-Chlorophenyl)-2-thiazolyl]amino]phenol), markedly attenuates 11,12-EET-mediated endothelial cell proliferation. Moreover, 11,12-EET-mediated activation of Akt kinase and transactivation of the EGF receptor are also blocked by sphingosine kinase 1-2. Inhibition of sphingosine kinase 1 by the expression of dominant-negative sphingosine kinase 1 (G82D) substantially attenuates 11,12-EET-mediated endothelial cell proliferation, migration, and tube formation in vitro and Matrigel plug angiogenesis in vivo. Furthermore, blockage of sphingosine kinase 1 expression by specific siRNA inhibits 11,12-EET-mediated endothelial cell proliferation and migration (Yan et al., 2008). In contrast, sphingosine kinase 2 siRNA knockdown has no effect. Collective evidence suggests that sphingosine kinase 1 is an important mediator of the 11,12-EET-mediated angiogenic effects in human endothelial cells (Yan et al., 2008). Collectively, these studies suggest that EPOX-derived EETs modulate angiogenesis via a nitric oxide-dependent mechanism as well as via activation of PtdIns3-kinase and MARK pathways (Wang et al., 2003). EPOX have not been purified and characterized from brain tissue, and no information is available on their physicochemical properties.

4.2.6 cis-*Epoxyeicosatrienoic Acids*

In non-neural tissues, EETs are associated not only with ion transport and gene expression but also with vasodepressor and natriuretic activities. Although the role of EETs in brain function is not fully understood, it is proposed that EETs contribute to the regulation of the cerebral blood flow and are therefore crucial to cerebrovascular homeostasis. EPOXs are also found throughout the cardiovascular system where their lipid products (EETs) regulate vascular tone, cell proliferation, migration, inflammation, and cardiac function. EETs undergo chain elongation and β-oxidation. Action of soluble epoxide hydrolase on EETs converts them to dihydroxyeicosatrienoic acids (DHETs) (Spector et al., 2004; Spector and Norris, 2007). Inhibition of soluble epoxide hydrolase results in accumulation of partial β-oxidation products.

EETs activate smooth muscle, large-conductance, Ca^{2+}-activated K^+ channels, producing hyperpolarization and vasorelaxation. Many of the functional effects of EETs are mediated through the activation of signal transduction pathways and modulation of gene expression through a putative cell surface EET receptor (Spector and Norris, 2007). However, EETs are rapidly taken up by cells and are incorporated into and released from glycerophospholipids, supporting the view that some functional effects may occur through a direct interaction between the EET and an intracellular effector system (Spector and Norris, 2007). 20-Iodo-14,15-epoxyeicosa-8(*Z*)-enoic acid (20-I-14, 15-EE8ZE), a radiolabeled EET agonist, enhances cAMP generation in U937 cells. Maximum cAMP synthesis (4.2-fold) occurs at 9 nM. Like 14,15-EET, 20-I-14,15-EE8ZE relaxes bovine coronary arteries (Yang et al., 2008). The effect of 20-I-14,15-EE8ZE is blocked by the EET antagonist 14,15-epoxyeicosa-5(*Z*)enoic acid (14,15-EE5ZE). The 20-(125)I-14,15-EE8ZE binding is inhibited by eicosanoids with potency order of 11,12-EET >14,15-EE5ZE approximately 14,15-EET >>15-hydroxyeicosatetraenoic acid > 14,15-EET-thiirane >14,15-dihydroxyeicosatrienoic acid. Collective evidence suggests that 20-(125)I-14,15-EE8ZE is a good radiolabeled EET agonist that can be used to identify specific high-affinity EET-binding site not only in U937 cell membranes but also in neural cell membrane. It is likely that this binding site may represent a specific EET receptor, which is probably coupled to a G protein (Yang et al., 2008).

EETs also interact with PPARs. Thus, EETs and their metabolites activate both PPARα and PPARγ in a dose- and time-dependent manner. These receptors are found in the nucleus, and are activated by lipid metabolites. Similar to the longer-term effects of EETs, activation of PPARα and PPARγ induce the inhibition of vascular cell proliferation, migration, and inflammation (Spector and Norris, 2007; Wray and Bishop-Bailey, 2008). It is proposed that EET-PPAR pathway may therefore represent a novel endogenous protective pathway by which short-lived lipid mediators control vascular cell activation (Wray and Bishop-Bailey, 2008). EETs have also been shown to activate β-endorphin

and Met-enkephalin, which may subsequently act on μ-and δ-opioid receptors to produce antinociception (Terashvili et al., 2008).

4.3 Non-enzymic Oxidation of Arachidonic Acid

It is well known that under pathological conditions, the accumulation of free fatty acids triggers an uncontrolled "arachidonic acid cascade." This sets the stage for increased production of ROS. ROS include oxygen-free radicals (superoxide radicals, hydroxyl and alkoxyl radicals, and lipid peroxy radicals) and peroxides (hydrogen peroxide and lipid hydroperoxide). Free radical scavengers including superoxide dismutase, catalase, and glutathione control, in part, the elimination of ROS. In cerebrovascular arteries, ROS promote vascular tone and facilitate cerebral blood flow. Although the major enzymic sources of ROS production in cerebral arteries have not been identified, NADPH oxidases, along with COXs and LOXs, seem to be the primary contributors (Phillis et al., 2006; Sun et al., 2007).

As stated earlier, biological targets of ROS are neural membrane lipids, proteins, and DNA (Farooqui and Horrocks, 2007). ROS may also modulate the expression of cytokines in the nucleus. These cytokines not only stimulate the activities of isoforms of PLA_2 and COX but also modulate their expression in reactive astrocytes located in the penumbra (Lin et al., 2004). Thus, an uncontrolled arachidonic cascade increased calcium influx, enhanced lipolysis, proteolysis, and disaggregation of microtubules, resulting in neural membrane injury (Farooqui and Horrocks, 2007). Non-enzymic oxidation of ARA generates a large number of products in brain tissue. They include 4-hydroxynonenal, acrolein, isoprostanes, isoketals, and isofurans.

4.3.1 4-Hydroxynonenal, Acrolein, and Malondialdehyde

Non-enzymic peroxidation of ARA produces 4-hydroxynonenal (4-HNE), a nine carbon α, β-unsaturated aldehyde that covalently modifies molecular components of neural membranes and disrupts cellular functions (Figs. 4.4 and 4.5) (Esterbauer et al., 1991; Lin et al., 2005; Farooqui and Horrocks, 2006, 2007). 4-HNE contains three functional groups, which confer 4-HNE reactivity toward thiol and amino groups. Thus, 4-HNE interacts with lysine, cysteine, and histidine residues in proteins, deoxyguanosine, and aminoglycerophospholipids (Esterbauer et al., 1991; Guichardant et al., 2002). 4-HNE also undergoes a Michael addition reaction with cellular thiols and forms adducts with glutathione or proteins containing thiol groups (Fig. 4.6). 4-HNE modulates key membrane proteins including glucose transporter, glutamate transporter, and sodium, potassium ATPases (Mark et al., 1997; Keller and Mattson, 1998; Lauderback et al., 2001). Inhibition of Na^+, K^+-ATPase

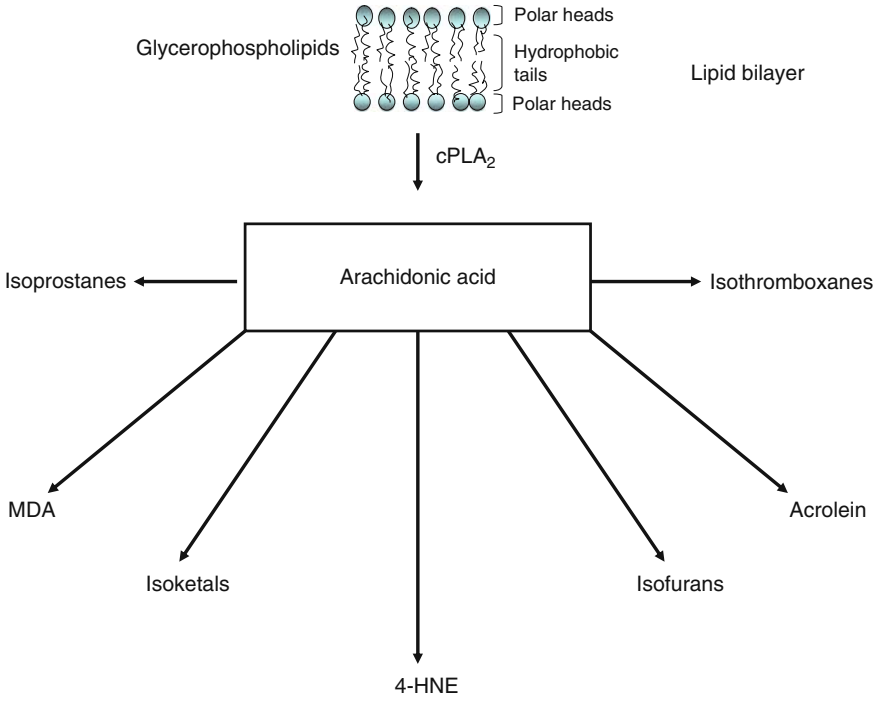

Fig. 4.4 Non-enzymic products of arachidonic acid metabolism

by 4-HNE induces neuronal membrane depolarization resulting in the opening of NMDA receptor channels and additional calcium influx into neurons (Kadoya et al., 2003). 4-HNE also blocks neurite outgrowth, disrupts neuronal microtubules, and modifies cellular tubulin, which may contribute to the cytoskeletal changes in neurons undergoing a neurodegenerative process (Neely et al., 1999; Farooqui et al., 2004). 4-HNE also reduces cellular ATP levels by impairing glucose transport and by depressing mitochondrial function (Keller and Mattson, 1998). Collectively, these studies indicate that in neural cells 4-HNE produces many deleterious effects, such as inhibition of DNA and RNA synthesis, disturbance in calcium homeostasis, and mitochondrial dysfunction, resulting in the inhibition of enzymic activities and suppression of ADP and ATP transport through the inner mitochondrial membrane (Picklo et al., 1999). These events play a substantial role in the disruption of mitochondrial function. In neural and non-neural cell cultures, 4-HNE produces apoptotic cell death that involves activation of caspases, proteolysis of downstream caspase targets, increase in levels of protein carbonyls and ubiquitinated proteins, decrease in proteasomal function and nucleosomal DNA fragmentation through the activation of poly(ADP-ribose) polymerase (Kruman et al., 1997; Hyun et al., 2002). Induction of apoptotic cell death in sympathetic neurons by 4-HNE is

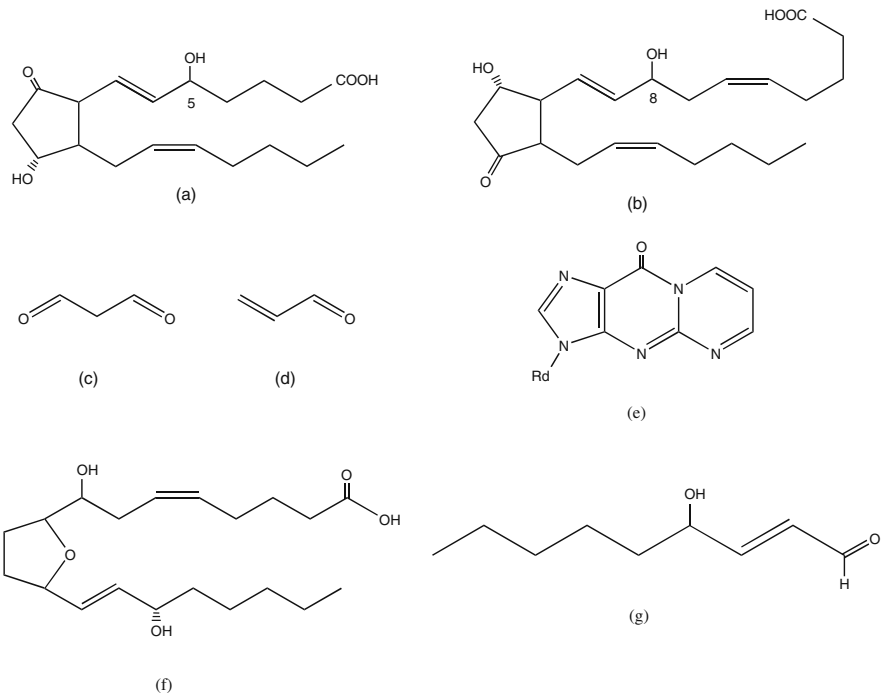

Fig. 4.5 Chemical structures of non-enzymic products of arachidonic acid metabolism. $5E_{21}$-Isoprostane (**a**); 8-D_{21}-isoprostane (**b**); malondialdehyde (**c**); acrolein (**d**); M_1A (**e**); isofuran (**f**); and 4-HNE (**g**)

improved by the deletion of neuronal *c*-Jun N-terminal kinase (JNK3), indicating that JNK pathway is crucial for 4-HNE-mediated apoptosis (Bruckner and Eatus, 2002).

In non-neural cells, at low concentrations 4-HNE significantly enhances the proliferation index, whereas at higher concentrations progressively inhibit cell proliferation. Low concentrations of 4-HNE increase caspase 3 activity, whereas high concentrations inhibits it. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione reductase (GSH-Rd) are also increased progressively with 4-HNE concentrations, particularly GSH-Px. Glucose-6-phosphate dehydrogenase (G6PDH) displays a different pattern, increasing at low 4-HNE concentrations and rapidly declined thereafter (Larini et al., 2004). Collective evidence suggests that 4-HNE activates signaling pathways that control cell death. Association of 4-HNE with the proteasome increases levels of protein carbonyls and ubiquitinated proteins and down-regulates proteasomal function. 4-HNE also inhibits interleukin-6 production in rat cortical neurons by inhibiting the activation of NF-κB and suppressing IκB phosphorylation (Lim et al., 2006).

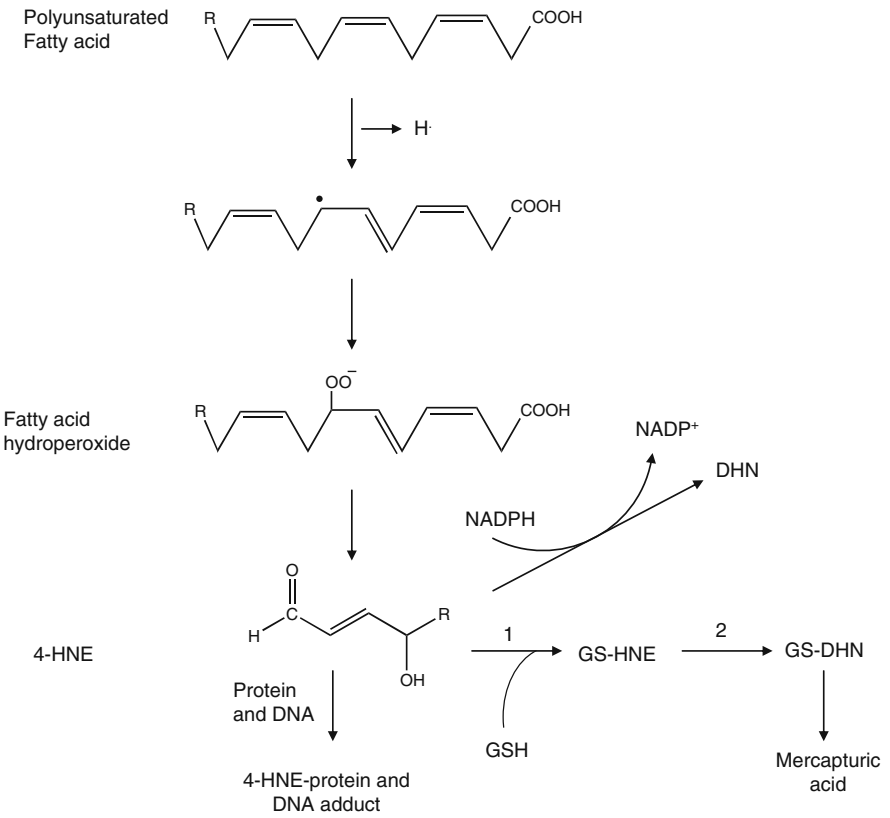

Fig. 4.6 Generation of 4-HNE through non-enzymic oxidation of polyunsaturated fatty acid, 4-HNE detoxification pathway, and protein and DNA adduct formation. Glutathione S-transferase (1); aldose reductase (2); 4-hydroxynonenal (4-HNE); 1,4-dihydroxy-2-nonene (DHN); 4-HNE-glutathione adduct (GS-HNE); glutathione-dihydroxynonene adduct (GS-DHN); and R (CH_3)

In the cerebral cortex, 4-HNE exists as two enantiomers, (*R*)-4-HNE and (*S*)-4-HNE. (*R*)-4-HNE is enantioselectively oxidized to the non-toxic 4-hydroxy-trans-2-nonenoic acid (HNEA) by NAD-dependent aldehyde dehydrogenases in the rat cerebral cortex (Murphy et al., 2004). Subcellular distribution studies have indicated that mitochondria have the highest specific activity. HNEA competes for the specific binding of the γ-hydroxybutyrate (GHB) receptor ligand ^{3}H-NCS382 (30 nM) and displaces it in membrane preparations of human frontal cerebral cortex and whole rat cerebral cortex with IC_{50} of 3.9±1.1 and 5.6±1.2 μM, respectively. Inhibition is attenuated when the carboxyl group of HNEA is replaced with an aldehyde or an alcohol. HNEA neither forms adduct with proteins nor affects the binding of β-adrenergic receptor and $GABA_B$ receptor antagonists, indicating the selectivity of HNEA

for the GHB receptor. Based upon the structural similarity of HNEA to ligands of the GHB receptor, it is proposed that HNEA may act as an endogenous ligand for the GHB receptor (Murphy et al., 2004).

4-HNE also has genotoxic properties. It produces its mutagenic effects through its clastogenic action at superphysiological concentrations. Treatment of mouse lymphoma cells with 4-HNE results in dose-dependent induction of mutations. Thus, 4-HNE triacetate (17 nM) produces 33% cytotoxicity with the mutant frequency of 719×10^{-6} (>sevenfold higher than the spontaneous mutant frequency). The loss of heterozygosity analysis in the thymidine kinase (Tk) mutants indicates that the majority of 4-HNE triacetate-induced mutations are caused by clastogenic events that affect a large segment of the chromosome (Singh et al., 2005).

4-HNE inhibits cell growth and induces differentiation in different leukemic cell lines (Barrera et al., 2004). The onset of 4-HNE-mediated differentiation is accompanied by the suppression of c-myc expression. In HL-60 cells, cell cycle progression is regulated by three protein classes, the cyclins, the cyclin-dependent kinases (CDKs), and the CDK inhibitors (CKIs). 4-HNE suppresses the expression of cyclin D1, D2, and A, and increases p21 expression, but has no effect on CDK expressions (Barrera et al., 2005). The molecular mechanism associated with these processes is not fully understood. However, cyclins D/CDK2 and cyclin A/CDK2 phosphorylates pRB; 4-HNE produces an increase of hypophosphorylated pRb. Hypophosphorylated pRb binds and inactivates the E2F transcription factors. It is proposed that 4-HNE produces a decrease of "free" E2F, as well as an increase of pRb bound to E2F with consequent repression of the transcription. Thus, 4-HNE reduces E2F transcriptional activity by modifying a number of genes involved in regulation of the pRb/E2F pathway (Barrera et al., 2004, 2005). Accumulating evidence suggests that 4-HNE modulates many signaling pathways in a concentration-dependent manner. Levels of 4-HNE are modulated by glutathione S-transferases (GSTs), which account for the metabolism of most cellular 4-HNE through its conjugation to glutathione (Awasthi et al., 2005).

Non-enzymic peroxidation of ARA also produces acrolein (2-propenal) (Figs. 4.4 and 4.5), an unsaturated aldehyde (Esterbauer et al., 1991). The toxicity of acrolein is reflected in its ability to alkylate nucleophilic centers in macromolecules. Thus, acrolein binds to proteins, DNA, and glycerophospholipids, and disrupts the functions of these molecules. Acrolein reacts with cysteine, histidine, and lysine residues of proteins. Its incorporation into proteins generates carbonyl derivatives (Uchida, 2003). Interactions between acrolein and DNA result in modification of DNA bases through the formation of exocyclic adducts. Acrolein produces toxic effects in brain tissue by binding to proteins and inducing mitochondrial oxidative stress (Luo and Shi, 2004).

Acrolein binds to synaptosomal membrane proteins in a dose-dependent manner and increases the levels of protein carbonyl groups. However, pretreatment of synaptosomes with glutathione ethyl ester (GEE) significantly ameliorated both the conformational alterations and the protein carbonyls induced by

acrolein (Pocernich et al., 2001). Acrolein is a potent inhibitor of brain mitochondrial respiration (Picklo and Montine, 2001). Acrolein-mediated toxicity is accompanied by significant impairment of adenine nucleotide translocase activity (Luo and Shi, 2004) and of glucose and glutamate uptake in primary neuronal cultures (Lovell et al., 2000).

Malondialdehyde is a major product of non-enzymic oxidation of ARA as well as prostaglandin biosynthesis (Marnett, 2002) (Figs. 4.4 and 4.5). It has mutagenic and carcinogenic properties. It reacts with DNA to form adducts to deoxyguanosine, deoxyadenosine, and deoxycytidine. The major adduct to DNA is a pyrimido[1,2-α]purin-10-(^{3}H)one nucleoside derived from deoxyguanosine (Wang et al., 2004). It is called M_1G. Its incorporation into viral genomes and replication in *Escherichia coli* result in high mutagenic activity. M_1G has been detected in liver, white blood cells, pancreas, and breast from healthy human beings at levels ranging from 1 to 120 per 108 nucleotides. Malondialdehyde-mediated DNA damage represents one of the most serious forms of DNA damage. Very little is known about M_1G in brain tissue. Collective evidence suggests that malondialdehyde-modified DNA adducts in animal and human tissues may serve as a marker of animal and human cancer (Zhang et al., 2002).

4.3.2 Isoprostanes

Isoprostanes are prostaglandin-like molecules that are specific and reliable markers of in vivo lipid peroxidation. They are formed non-enzymically by free radical-catalyzed peroxidation of esterified ARA (Figs. 4.4 and 4.5) (Basu, 2004). The molecular mechanism by which isoprostanes are formed is analogous to the production of prostaglandins by COXs. Unlike prostaglandins, the formation of isoprostanes in situ is initiated at the esterified ARA on the glycerophospholipid molecule (Fam and Morrow, 2003). At least two mechanisms have been proposed for isoprostane synthesis. One mechanism involves the generation of positional peroxyl radical isomers of ARA that undergo endocyclization to form PGG_2-like compounds. These compounds are reduced to PGF_2-like compounds. F_2-Isoprostane (F_2-IsoP) is subsequently released in free form by the action of PLA_2 (Fam and Morrow, 2003; Montuschi et al., 2007). According to second mechanism, the generation of isoprostane starts with a 4-exocyclization of a peroxyl radical leading to an intermediate dioxetane (Durand et al., 2005). Several isoprostanes have been identified in brain tissues, including biological fluids, such as 8-iso prostaglandin $F_2\alpha$ (8-iso-$PGF_2\alpha$, 8-epi-$PGF_2\alpha$, i$PF_2\alpha$-III, 15-F_2t-IsoP), and its metabolite, 2,3-dinor-4,5-dihydro-8-iso-$PGF_2\alpha$. The isoprostanes are in vivo markers of lipid peroxidation in humans: they are endogenously generated, have characteristic structures, ubiquitous in nature, stable in- and ex vivo, and can be reliably determined. The determination of

F_2-isoprostanes is one of the most reliable and authentic biomarker for assessing oxidative stress in vivo.

Isoprostanes act as vasoconstrictor in brain microvasculature. They exert their effects through receptor-dependent and receptor-independent mechanisms. F_2-IsoPs exert their effects through the thromboxane receptors, TPα and TPβ (Farooqui and Horrocks, 2007). Their effects can be blocked by thromboxane receptor antagonists (Takahashi et al., 1992; Morrow et al., 1996; Opere et al., 2005; Morrow, 2006). Thromboxane receptors, like prostaglandin receptors, are linked to different sets of G proteins resulting in distinct biological effects on brain and other body tissues (Lahaie et al., 1998). F_2-IsoP-mediated effect on vascular beds promotes interactions between endothelial cells and monocytes (Fam and Morrow, 2003). Isoprostane-mediated monocyte adhesion involves protein kinases such as PKA and mitogen-activated protein kinase kinase 1. F_2-IsoP also modulates the p38 MAPK pathway during monocyte adhesion (Cracowski, 2004). It is proposed that F_2-IsoP not only affects vascular and bronchial smooth muscles function but also modulates cellular proliferation (Fam and Morrow, 2003). These processes may be associated with inflammation and atherosclerosis. Receptor-independent action of F_2-IsoP is due to adduct formation. These compounds contain reactive α, β-unsaturated carbonyl group on the prostane ring, which readily reacts with thiol-containing compounds to produce adducts that have many biological effects.

Recently, new class of IsoPs, known as A_2- and J_2-IsoPs or cyclopentenone IsoPs, which are highly reactive electrophiles, has been identified (Milne et al., 2005; Musiek et al., 2007a). They form adducts with thiol-containing molecules such as cysteine residues in proteins and glutathione. Cyclopentenone IsoPs, which are very reactive α,β-unsaturated aldehydes, are favored products of the IsoP pathway in the brain and are formed abundantly after oxidant injury (Musiek et al., 2007a). In non-neural cells, cyclopentenone IsoPs prevent lipopolysaccharide-mediated IκBα degradation and subsequent NF-κB nuclear translocation and transcriptional activity (Musiek et al., 2007b). Expressions of inducible nitric oxide synthase and cyclooxygenase-2 along with nitrite and prostaglandin synthesis are also blocked by cyclopentenone IsoPs. 15-J_2-IsoPs potently stimulate PPARγ nuclear receptors, whereas 15-A_2-IsoP does not, although the antiinflammatory effects of both molecules are PPARγ-independent. 15-A_2-IsoPs mediated oxidative stress in RAW cells that is inhibited by the antioxidant 4-hydroxy-TEMPO (TEMPOL) or the mitochondrial uncoupler carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (Musiek et al., 2007b). TEMPOL also abrogates the inhibitory effect of 15-A_2-IsoPs on lipopolysaccharide-mediated NF-κB activation, inducible nitric oxide synthase expression, and nitrite generation, suggesting that 15-A_2-IsoPs prevent the NF-κB pathway at least partially via a redox-dependent mechanism. 15-J_2-IsoP, but not 15-A_2-IsoP, also mediates the RAW cell apoptosis again via a PPARγ-independent mechanism. These compounds also mediate neuronal apoptosis by a mechanism that involves glutathione depletion, ROS generation,

and activation of several redox-sensitive pathways. At biologically relevant concentrations, cyclopentenone IsoPs also enhance neurodegeneration caused by other insults. In addition to the F_2-IsoPs, E_2- and D_2-IsoPs can also be formed by rearrangement of H_2-IsoP endoperoxides. Collective evidence suggests that cyclopentenone IsoPs may serve as negative feedback regulators of inflammation and have important implications for defining the role of oxidative stress in the inflammatory response, which may contribute to neurodegeneration following metabolic, traumatic injuries and neurodegenerative diseases (Milne et al., 2005; Musiek et al., 2007a,b).

4.3.3 Isoketals

Isoketals (isolevuglandins) differ from isoprostanes in having a characteristic aldehydic group in a 1,4-dicarbonyl array, making them extremely reactive toward primary amino groups in proteins (Boutaud et al., 2005). Isoketals produce their effects by interacting and modifying biologically important proteins (Davies et al., 2004). Isoketals have highly reactive γ-ketoaldehydes that form pyrrole adducts with the ε-amino group of lysine residues in protein (Davies et al., 2004; Davies, 2008). These pyrrole adducts are unstable in the presence of oxygen and are further converted to lactam and hydroxylactam adducts (Fig. 4.7), which accumulate as stable end products. Collective evidence suggests that isoketals are some of the most reactive products derived from the peroxidation of lipids, and exert their biological effects by rapidly adducting to primary amines such as the lysyl residues of proteins. They also have remarkable ability to cross-link proteins through oxidation of the pyrrole. Oxidative stress-mediated stimulation of COX generates PGH_2, which may be converted into two specific isomers of IsoK [levuglandin (LG) D_2 and E_2]. Conjugation of IsoK with model proteasome substrates significantly inhibits their rate of degradation by the 20S proteasome (Davies et al., 2002; Davies, 2008). The ability of IsoK to block proteasome function directly is observed only at very high concentrations. However, at lower concentrations, an IsoK-adducted protein (ovalbumin) and peptide (Ab1-40) significantly retards chymotrypsin-like activity of the 20S proteasome. Moreover, IsoK inhibits proteasome activity in dose-dependent manner in P19 neuroglial cultures resulting in cell death. These findings suggest that these γ-ketoaldehydes can inhibit proteasome activity, and overproduction of these metabolites may have some relevance to neural cell death in neurodegenerative diseases (Davies et al., 2002). Intra-hemispheric injections of 15-E_2-IsoK are known to disrupt the blood–brain barrier. In human embryonic kidney (HEK)-293 cells and cultured atrial (HL-1) myocytes, E_2-isoketal potentiates inactivation of cardiac Na^+ channels (Fukuda et al., 2005). Furthermore, E_2-isoketals are generated in the epicardial border zone of the canine healing infarct, an arrhythmogenic focus where Na^+ channels exhibit similar

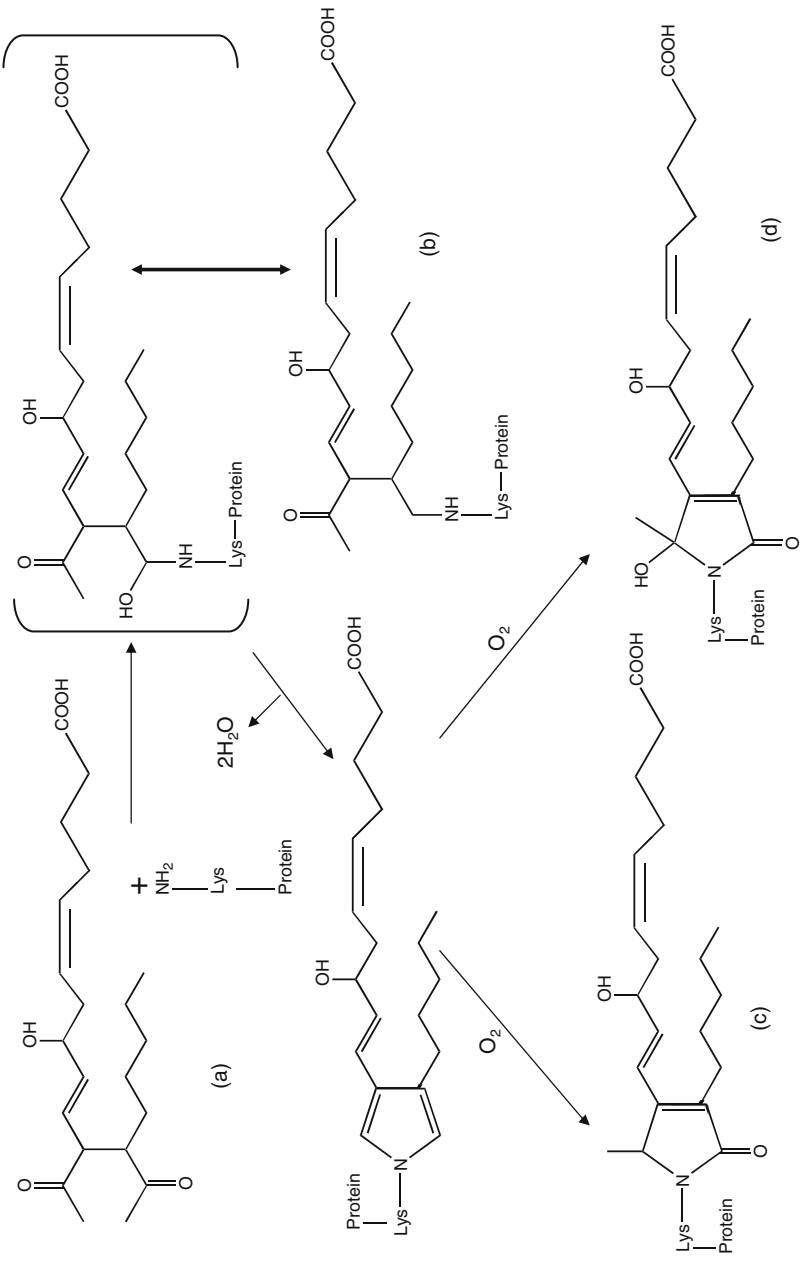

Fig. 4.7 Formation of lactam, hydroxylactam, and Schiff base lysine adducts through the reaction between isoketal and protein lysine residues. Isoketal (**a**) reacts with lysine residue in proteins and forms an intermediate, which either forms Schiff base adduct (**b**) or transform into lactam (**c**) and hydroxylactam adducts (**d**)

inactivation defects. Based on these results, it is proposed that E_2-isoketal-mediated Na^+ channel dysfunction may be implicated in ischemia-related conduction abnormalities and arrhythmias (Fukuda et al., 2005).

4.3.4 Isofurans

Oxidation of ARA under high oxygen tension generates substituted tetrahydrofuran derivatives (Fessel et al., 2002). These compounds are called as isofurans (IsoF) (Figs. 4.4 and 4.5). Two mechanisms have been proposed for IsoF generation: a cyclic peroxide cleavage mechanism and an epoxide hydrolysis mechanism. The synthesis of IsoF is modulated by oxygen tension. Increase in oxygen tension promotes the formation of isofurans and retards the synthesis of IsoP. IsoF are readily detectable in normal body fluids and tissues, and their levels are dramatically increased in animal model of oxidant injury and chronic neurodegenerative diseases (Fessel et al., 2002). Combined measurement of IsoFs and IsoPs not only provides a reliable index of oxidant stress severity but can also be used as an important parameter for evaluating the effectiveness of antioxidant therapies (Roberts et al., 2005).

Mitochondrial dysfunction in neurodegenerative diseases may not only lead to oxidative damage to brain tissue but also result in increased intracellular oxygen tension, thereby modulating the generation of F_2-IsoPs and IsoFs. Thus, elevated levels of IsoFs but not F_2-IsoPs are observed in the substantia nigra of patients with Parkinson disease (PD) and Lewy body disease (DLB) compared to age-matched controls. No changes have been reported in levels of IsoFs and F_2-IsoPs in the substantia nigra of patients with multiple system atrophy (MSA) and Alzheimer disease (AD). This preferential increase in IsoFs in the substantia nigra of patients with PD or DLB not only indicates a unique mode of oxidant injury in these two diseases but also suggests different underlying mechanisms of dopaminergic neurodegeneration in PD and DLB from those of MSA (Fessel et al., 2003).

4.4 Enzymic and Non-enzymic Oxidation of DHA

As stated earlier, DHA is a major component of plasmalogens and phosphatidylserine. DHA provides neural membranes with an optimal lipid microenvironment for interactions between membrane proteins and lipids that are associated with signal transduction processes (Horrocks and Farooqui, 2004). DHA has hypolipidemic, antithrombotic, and antiinflammatory effects in neural and non-neural systems. It is metabolized by neural and non-neural tissues by enzymic and non-enzymic mechanisms. The enzymic oxidation of DHA produces resolvins and docosatrienes (Hong

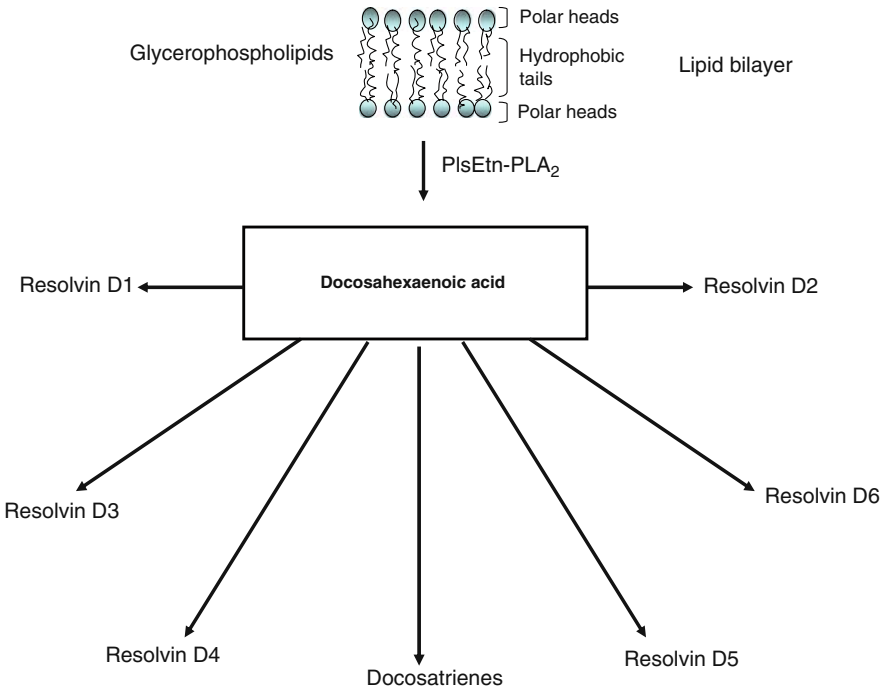

Fig. 4.8 Enzymically derived metabolites of docosahexaenoic acid metabolism

et al., 2003; Marcheselli et al., 2003; Serhan, 2005; Bazan, 2008) (Fig. 4.8). Resolvins and docosatrienes have antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, vasodilatory, and neuroprotective effects in neural and non-neural tissues. The non-enzymic oxidation of DHA generates 4-hydroxy 2-hexenal (4-HHE), neuroprostanes (NPs), neuroketals (NKs), and neurofurans (Fig. 4.9). These metabolites induce oxidative stress.

4.4.1 Enzymic Oxidation of DHA

Oxidation of DHA by a 15-LOX-like enzyme generates 10,17S-docosatrienes and 17S-resolvins (Hong et al., 2003; Marcheselli et al., 2003; Serhan, 2005; Ariel and Serhan, 2007). These second messengers not only antagonize the effects of eicosanoids but also modulate leukocyte trafficking and downregulate the expression of cytokines in glial cells. They are collectively called as docosanoids. They possess potent antiinflammatory, neuroprotective, and pro-resolving properties (Hong et al., 2003; Marcheselli et al., 2003; Serhan, 2005).

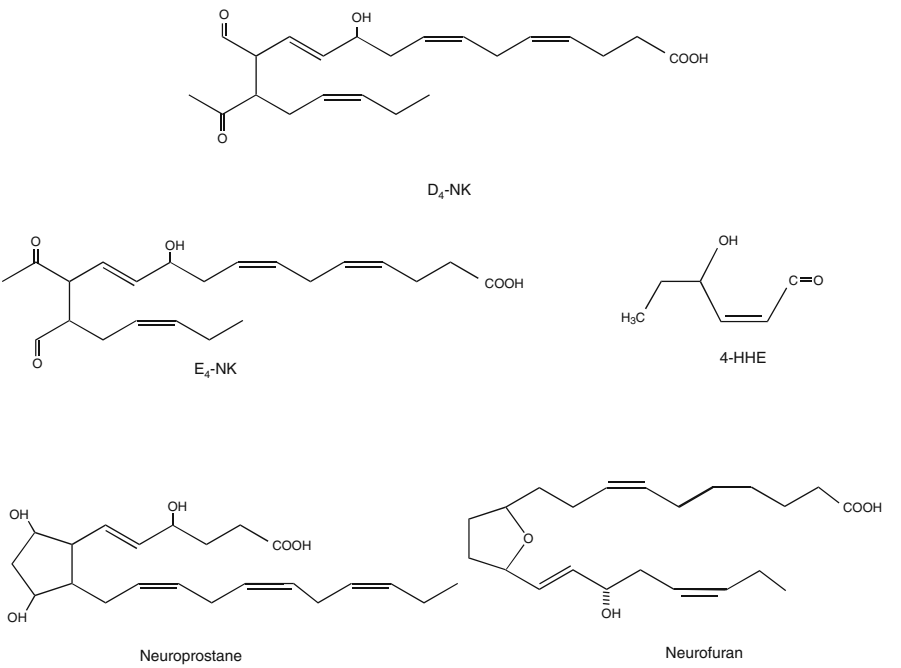

Fig. 4.9 Chemical structures of non-enzymic products of docosahexaenoic acid metabolism

4.4.2 17S D Series Resolvins

15-LOX-catalyzed oxidation of DHA generates resolvins of the D series (RvDs) and protectins neuroprotectin D_1/protectin D_1 (NPD_1/PD_1) (Serhan and Chiang, 2008; Bazan, 2008). Aspirin impinges on these systems, triggering formation of the epimeric 17R-series RvDs – denoted as "aspirin-triggered-RvDs" – which possess bioactivity in vivo equivalent to that evoked by their 17S-series counterparts (i.e. RvDs). They include RvD_1, RvD_2, RvD_3, RvD_4, RvD_5, and RvD_6 (Figs. 4.8 and 4.10). These metabolites exert potent agonist actions on macrophages and vascular endothelial cells that can control the magnitude of the local inflammatory response. They act through specific receptors found in neural and non-neural cells. These receptors are called as resolvin D receptors ($resoDR_1$) and resolvin E receptors ($resoER_1$) (Serhan and Chiang, 2008). These receptors have neither been fully characterized in non-neural tissues nor in brain. Thus, detailed investigations on link between enzymic activities (PLA_2, COX, LOX, and EPOX and their downstream enzymes) and $resoDR_1$ and $resoER_1$ in neural and non-neural cells are urgently required.

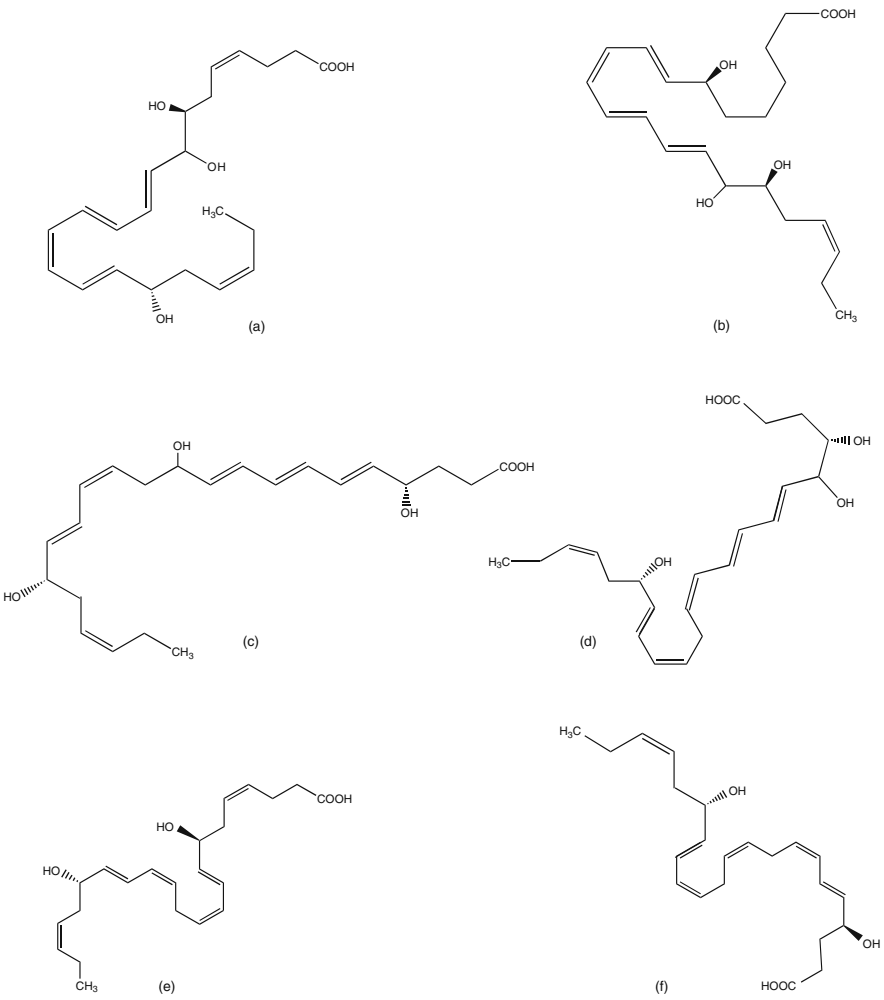

Fig. 4.10 Chemical structures of DHA-derived resolvins. Resolvin D_1 (**a**); resolvin D_2 (**b**); resolvin D_3 (**c**); resolvin D_4 (**d**); resolvin D_5 (**e**); and resolvin D_6 (**f**)

4.4.3 Docosatrienes

Oxidation of DHA by 15-LOX-like enzyme also generates NPD_1 (10,17S-docosatriene) (Figs. 4.10 and 4.11). This metabolite accumulates during reperfusion. The synthesis of this metabolite is enhanced by calcium ionophore A23187, IL-1β, or the supply of DHA. The infusion of NPD_1 during reperfusion has neuroprotective effects. Thus, the expression of antiapoptotic Bcl-2 proteins (Bcl-2 and BclxL) is upregulated by NPD_1 whereas the expression of pro-apoptotic proteins, Bax and Bad, is downregulated by NPD_1. Moreover,

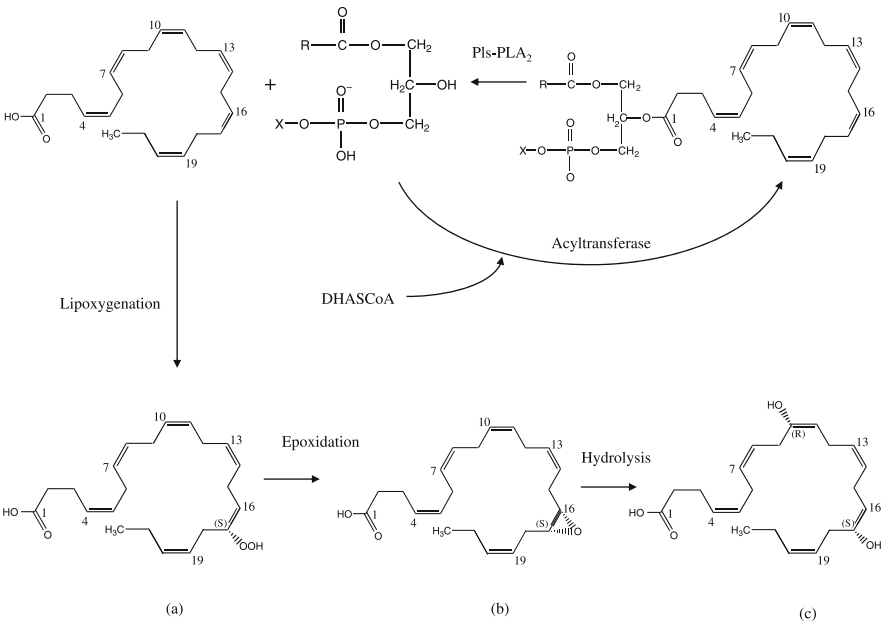

Fig. 4.11 Release of docosahexaenoic acid from glycerophospholipids and its conversion into neuroprotectin D_1.17-*S*-hydroperoxy-DHA (**a**); 16,17-epoxydocosatriene (**b**); and neuroprotectin D (**c**)

neuroprotectin D1 inhibits oxidative stress-mediated activation of caspase-3 and COX-2 (Bazan, 2005a,b). The precise molecular mechanisms involved in the above process are not fully understood. However, it is known that Bcl-2 family proteins regulate apoptotic signaling at the level of mitochondria, causing the release of cytochrome *c* and activating effector enzyme caspase-3. Soluble amyloid precursor protein-α stimulates the synthesis of NPD_1 (Lukiw et al., 2005). In retina, epithelium-derived factor acts as an agonist and induces the synthesis of NPD_1 and thus facilitates NPD_1-mediated paracrine and autocrine signal transduction processes. Also, DHA and epithelium-derived factor synergistically activate NPD_1 synthesis and antiapoptotic protein expression and downregulate proapoptotic Bcl-2 protein expression and activation of caspase 3 during oxidative stress (Mukherjee et al., 2007). Based on many elegant studies, it is proposed that NPD_1 promotes homeostatic regulation of the integrity of neural cells, particularly during oxidative stress, and this protective signaling may be relevant not only in retinal diseases but also in neurodegenerative diseases (Bazan, 2008). Moreover, neurotrophins are NPD_1-synthesis agonists, and NPD_1 contents are reduced in the CA1 region of the hippocampus of Alzheimer disease patients. Overall, NPD_1 promotes neural cell survival through the induction of antiapoptotic and neuroprotective gene-expression programs that suppress amyloid ss peptide (Ass), composed of 42

amino acids (Ass42), generation and its neurotoxicity (Bazan, 2008). NPD_1 synthesis is activated by growth factors and neurotrophins. Although receptors for NPD_1 have not been characterized in brain tissue, their occurrence has been suggested (Hong et al., 2003; Marcheselli et al., 2003; Mukherjee et al., 2004). Collectively, these studies suggest that the generation of resolvins and neuroprotectins may be an internal protective mechanism for preventing apoptotic cell death-mediated brain damage (Serhan, 2005; Bazan, 2005b) and NPD_1 through its receptors may act by inducing and modulating gene-expression programs that are neuroprotective through downregulation of proapoptotic and proinflammatory factors and upregulation of Bcl-2-family antiapoptotic proteins, which are crucial modulators of cell survival (Serhan, 2005; Bazan, 2005b; Mukherjee et al., 2007).

4.5 Non-enzymic Oxidation of Docosahexaenoic Acid

DHA is highly vulnerable to autoxidation because of the presence of six double bonds. Its autoxidation includes 4-HHE, NP, and neurofuran (Yin et al., 2005) (Fig. 4.9**)**. Generation of NP esterified in glycerophospholipids may induce changes in biophysical properties of neural membranes. This is particularly important for neurons, because one of DHA's function is to provide and maintain neural membrane fluidity and permeability that facilitate interactions between DHA and proteins for optimal membrane function. Increasing evidence suggests that protein modifications by 4-HHE may be involved in various neurological diseases associated with abnormal glycerophospholipid metabolism.

4.5.1 4-Hydroxyhexenal

Oxidation of DHA produces 4-HHE (Fig. 4.9**)**. 4-HHE contains a conjugated double bond between the α and β carbons; the γ carbon of 4-HHE is electron-deficient and reacts readily with nucleophiles such as thiols and amines, while the carbonyl group forms Schiff bases with amino groups such as the N-termini of proteins and the ε-amino group of lysine. Thus, 4-HHE binds to proteins and 4-HHE protein complexes are potential markers of oxidative stress (Yamada et al., 2004). In vivo protein-bound 4-HHE can be detected with monoclonal antibody HHE53 (MAb HHE53). Treatment of cortical neuronal cultures with 4-HHE produces neurotoxicity, which can be blocked by the addition of thiol scavengers. Like 4-HNE, 4-HHE not only depletes neuronal reduced glutathione but modifies specific proteins of 75, 50, and 45 kDa in dose- and time-dependent manners. The time-dependent generation of 4-HHE differs from that of F_4-NPs, indicating that different oxidation pathways may be involved in DHA oxidation (Long et al., 2008).

Since there are structural similarities between 4-HNE and 4-HHE, one can expect identical modulation of neurochemical processes. The biological activities and efficacies of DHA- and ARA-derived aldehydes differ significantly. For example, 4-HHE induces mitochondrial permeability transition (MPT) more effectively than 4-HNE. 4-HNE is inactive at concentrations <1 μM, but induces its biphasic effects on mitochondrial membrane at 1 and 200 μM. In contrast, 4-HHE consistently enhances calcium-mediated induction of the MPT, even at femtomolar concentrations. 4-HHE-mediated induction of MPT can be prevented by cyclosporin A and glutathione. Thus, 4-HHE treatment results in a rapid disruption of calcium homeostasis (Kristal et al., 1996). The exquisite specificity and sensitivity of MPT toward 4-HHE indicate that 4-HHE-mediated modulation of mitochondria may play an important role in neural cell survival. Furthermore, 4-HHE more effectively blocks the mitochondrial ATP translocator than does 4-HNE (Picklo et al., 1999). 4-HHE modulates endothelial nitric oxide synthase (iNOS) through NF-κB activation (Lee et al., 2004b). In contrast, 4-HNE inhibits NF-κB activation. In addition, 4-HHE oxidizes purified mitochondrial aldehyde dehydrogenase (ALDH5A) 6.5-fold less efficiently than HNE (Long et al., 2008). During aging, 4-HHE regulates redox balance producing vascular alterations due to endothelial cell deterioration and dysfunction. The molecular mechanism of 4-HHE-mediated endothelial dysfunction is not known. However, it is proposed that 4-HHE regulates redox balance through the upregulation of iNOS and participation of nuclear factor κB (NF-κB) (Lee et al., 2004b). Pretreatment of endothelial cells with NF-κB inhibitors, Bay 11-7082, and *N*-acetyl cysteine (NAC) blocks the upregulation of iNOS by blunting IκB degradation and NF-κB-binding activity. Furthermore, Bay 11-7082, NAC, and caffeic acid methyl ester significantly decreases 4-HHE-mediated generation of NO. Collective evidence suggests that in endothelial cells, 4-HHE induces iNOS gene expression through NF-κB activation, which can lead to vascular dysfunction by the activation of various proinflammatory processes (Lee et al., 2004b). This is tempting to speculate that 4-HHE and 4-HNE may produce different effects on transcription factor and enzymic activities of neural and non-neural tissues (Camandola et al., 2000; Lee et al., 2004b).

4.5.2 Neuroprostanes

Non-enzymic oxidation of DHA results in generation of NPs (Roberts et al., 2008; Yin et al., 2005) (Fig. 4.9). NPs have 22 carbons and 4 double bonds and are analogous to isoprostanes (Fig. 4.5). During NP synthesis, oxygen-mediated DHA radicalization generates peroxyl radicals, which undergo endocyclization followed by the addition of molecular oxygen and reduction to form the F ring of NP. NP-containing glycerophospholipids

may produce changes in neural membrane fluidity and permeability resulting in impairment in optimal neuronal function (Fam and Morrow, 2003; Yin et al., 2005).

Nothing is known about PLA_2 activity that releases NP from NP-bound glycerophospholipids. F_4-NP is the first characterized NP. Synthetic NP strongly suppresses lipopolysaccharide-mediated expression of iNOS and cyclooxygenase-2 in macrophages (Musiek et al., 2008). It inhibits lipopolysaccharide-mediated NF-κB activation by retarding Iκ kinase-induced phosphorylation of IκBα. Mutation on Iκ kinase β cysteine 179 markedly reduces the effect of NP, suggesting that NP acts by blocking this thiol residue (Musiek et al., 2008). Effects of NP are independent of PPARγ and are dependent on an intact reactive cyclopentenone ring. It is interesting to note that free radical-mediated oxidation of DHA greatly enhances its antiinflammatory potency, an effect that closely parallels the formation of NPs; and chemical reduction or conjugation to glutathione blocks the anti-inflammatory effects of oxidized DHA (Musiek et al., 2008). Collective evidence suggests that NPs are potent inhibitors of NF-κB signaling, and they may contribute to the antiinflammatory actions of DHA. NPs occur in cerebrospinal fluid (CSF) from normal individuals. The levels of F_4-NP are significantly increased in CSF from patients with Alzheimer disease (Reich et al., 2001). E_4-NP and D_4-NP are also detected in normal rat and human brain. Levels of E_4/D_4-NP in normal brain were one-third compared to levels of F_4-NP (Roberts et al., 2005).

4.5.3 Neuroketals

Non-enzymic oxidation of DHA also generates NKs (Bernoud-Hubac et al., 2001) (Fig. 4.9). NKs not only form lactam and Schiff base adducts but also generate lysine adducts, suggesting that these metabolites may be involved in protein–protein cross-linking in brain tissue under oxidative stress. Because NKs have either a 1,4-pentadiene or 1,4,7-octatriene side chain structure, it is speculated that they may undergo further oxidation to form NKs with an additional hydroxyl group. NK lysyl-lactam protein adducts are detected in non-oxidized rat brain synaptosomes at a level of 0.09 ng/mg of protein, which increase 19-fold following oxidation in vitro (Bernoud-Hubac et al., 2001). NK lysyl-lactam protein adducts are also detected in vivo in normal human brain at a level of 9.9 ± 3.7 ng/g of brain tissue, but very little is known about their toxic and injurious effects on brain neuropathologies. It is suggested that generation of NPs and NKs may intensify oxidative stress in ischemic and traumatic injuries and neurodegenerative diseases (Roberts et al., 2005; Farooqui and Horrocks, 2006). High levels of NKs and NPs are reliable indices of oxidative stress in vivo (Roberts et al., 1998, 2005; Fam and Morrow, 2003).

4.5.4 Neurofurans

Neurofurans are 22-carbon tetrahydrofuran ring-containing compounds formed non-enzymically by free radical-mediated peroxidation of DHA. The neurofurans are analogous to the isofurans and are formed during oxidative stress. Levels of neurofurans are higher than isoprostanes, and were first characterized in rat liver CCl_4 toxicity by mass spectrometry. Neurofurans are increased in the brain cortex of a mouse model of Alzheimer disease, and are decreased in mouse brain cortex by deletion of p47(phox), an essential component of the phagocyte NADPH oxidase (Song et al., 2008). It is proposed that determination of the neurofurans may be useful in diagnosis, timing, and selection of dose in the treatment and chemoprevention of neurodegenerative disease.

4.6 Enzymic Oxidation of EPA in Brain

The oxidized EPA, rather than native EPA, possesses antiatherosclerotic, anti-inflammatory, and antiproliferative effects. The action of COX enzymes on EPA generates the 3-series of prostaglandins and thromboxanes and the 5-series of leukotrienes (Fig. 4.12). These eicosanoids have different biological properties than the corresponding analogs produced by the metabolism of ARA. For example, TXA_3 is less active than TXA_2 in aggregating platelets and constricting blood vessels (James et al., 2000; Calder and Grimble, 2002). In vitro studies on specificities of COX enzymes or PG endoperoxide H synthases associated with oxidation of EPA and ARA indicate that although these fatty acids compete for the same enzymes, some differences are observed. Under optimal conditions, purified COX-1 oxygenates EPA with only 10% of the efficiency of ARA, and EPA significantly inhibits ARA oxygenation by COX-1 (Wada et al., 2007). Two- to threefold higher activities or potencies with 2-series versus 3-series compounds have been reported for COX-2, PGD synthases, microsomal COX-1, and EP_1, EP_2, EP_3, and FP receptors (Wada et al., 2007). Surprisingly, oxygenation of ARA by COX-2 is only modestly inhibited by EPA, and TxA_3 has equal affinity for TP-α receptor. These studies indicate that increasing glycerophospholipid EPA/AA ratios in cells may slow down eicosanoid signaling, with the largest effects being on COX-1 pathway (Wada et al., 2007). In addition, oxidation of EPA by 15-LOX-like enzyme generates resolvins of the E series (Arita et al., 2006, 2007). Thus, resolvin E_1 (RvE_1, 5S,12R,18R)-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid) is an oxygenase product derived from EPA that displays potent antiinflammation/proresolution actions in vivo (Fig. 4.12) (Arita et al., 2006). RvE_1 is an initial metabolite. The conversion of RvE_1 to the oxo product inactivates RvE_1. RvE_1 blocks the activation of NF-κB by TNF-α. Antiinflammatory action of RvE1 has been studied in human polymorphonuclear (PMN) leukocyte (Arita

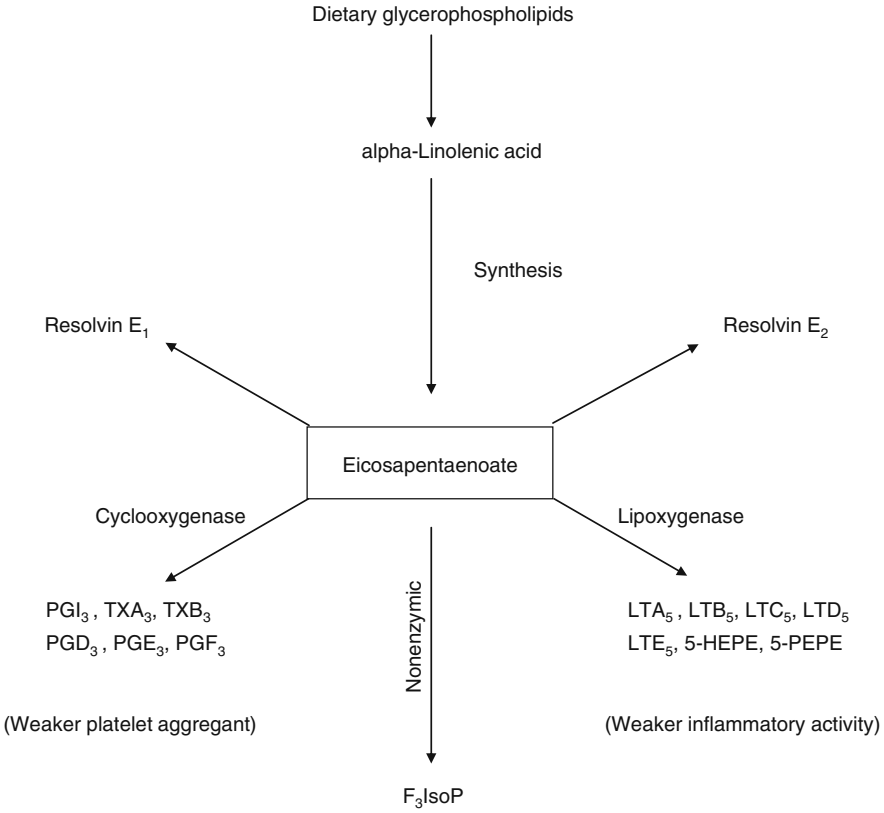

Fig. 4.12 Enzymic oxidation of EPA via COX-2 and 5-LOX pathways

et al., 2007). Human PMN show specific binding with RvE_1. [^{3}H]RvE_1-specific binding to human PMN can be displaced by leukotriene B_4 (LTB_4) and U-75302 (LTB_4 receptor 1, BLT_1) antagonist, but not by chemerin peptide, a ligand specific for another RvE1 receptor ChemR23. Recombinant human BLT_1 show specific binding with [^{3}H]RvE_1. RvE_1 selectively blocks adenylate cyclase with BLT_1, but not with BLT_2 (Arita et al., 2007). RvE_1 partially mediates calcium mobilization, and retards subsequent stimulation by LTB_4. RvE_1 also attenuates LTB_4-mediated NF-κB stimulation in BLT_1-transfected cells. In vivo antiinflammatory actions of RvE_1 are markedly downregulated in BLT_1 knockout mice when given at low doses in peritonitis. In contrast, RvE_1 at higher concentrations significantly prevents PMN infiltration in a BLT_1-independent manner (Arita et al., 2007). RvE_1 also bind with ChemR23 receptors. Treatment of dendritic cells with small interference RNA specific for ChemR23 blocks RvE_1-mediated regulation of IL-12, demonstrating novel counterregulatory responses in inflammation initiated by EPA-derived RvE_1 and its receptor. Collectively, these studies suggest that RvE_1 binds to BLT_1 as a

partial agonist, potentially serving as a local damper of BLT_1 signals on leukocytes along with other receptors (e.g., ChemR23-mediated counterregulatory actions) to mediate the resolution of inflammation (Arita et al., 2006, 2007).

Isolation, identification, and bioactions of another resolvin, 5S,18-dihydroxy-eicosapentaenoic acid, (resolvin E_2, RvE_2) have also been reported (Tjonahen et al., 2006). RvE_2 blocks zymosan-induced polymorphonuclear (PMN) leukocyte infiltration, and elicits potent antiinflammatory properties in murine peritonitis. Similar to RvE_1, human recombinant 5-LOX generates RvE_2 from a common precursor of E series resolvins, namely 18-hydroxyeicosapentaenoate (18-HEPE). Collective evidence suggests that RvE_2, together with RvE_1, may contribute to the beneficial actions of $n-3$ fatty acids in human diseases. It is stated that the 5-LOX in human leukocytes is a pivotal enzyme that generates both pro- and antiinflammatory chemical mediators.

EPA modulates lipoprotein metabolism, and, as stated above, EPA downregulates the synthesis of proinflammatory cytokines (IL-1β and TNF-α). The molecular mechanism associated with downregulation of cytokine secretion by EPA is not fully understood. However, inhibition of the TNF-α expression may be closely associated with downregulation. EPA appears to prevent NF-κB activation by preventing the phosphorylation of I-κB-α. In addition, suppression of ARA-derived eicosanoids by EPA may also play an important role, because the EPA competes with ARA for COX- and LOX-mediated oxidation (Zhao et al., 2004; Phillis et al., 2006).

4.7 Non-enzymic Oxidation of EPA

The non-enzymic oxidation of EPA generates F_3 isoprostane (Nourooz-Zadeh et al., 1997; Gao et al., 2006).The amounts F_3-IsoP formed are extremely large and greater than the levels of F_2-IsoPs generated from ARA. EPA supplementation markedly reduces the levels of arachidonate-derived F_2-IsoPs by up to 64%. These studies provide the evidence that F_3-IsoPs is a novel in vivo oxidation product of EPA (Gao et al., 2006). Like non-enzymic production of J_2-isoprostanes from ARA in vivo, the non-enzymic peroxidation of EPA produces J_3-IsoPs both in vitro and in vivo (Fig. 4.13). Levels of J_3-IsoPs are increased approximately 200-fold with oxidation of EPA. Thus, basal levels (0.8 ± 0.4 ng/mg EPA) are increased to 196 ± 23 ng/mg EPA after 36 h. J_3-IsoPs are also detected in fresh liver tissue from EPA-fed rats. In control rats, basal levels of J_3-IsoPs are very low. The molecular mechanism of EPA non-enymic oxidation is not fully understood. However, it depends upon the site of oxygen insertion on EPA molecule and results in the generation of one of eight different hydroperoxides. After further oxidation, ring arrangement, and dehydration, six different series of A_3 and J_3 isoprostanes are generated from EPA. During

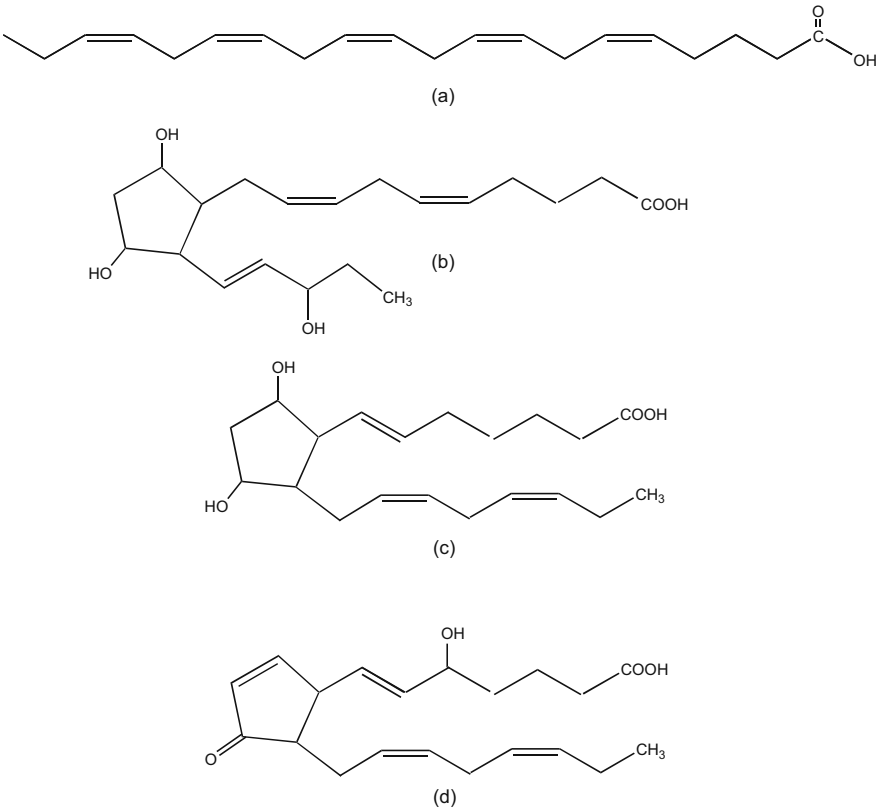

Fig. 4.13 Products of non-enzymic oxidation of EPA. Eicosapentaenoic acid (**a**); F_3-isoprostane 18-series (**b**); F_3-isoprostane 5-series (**c**); and J_3-isoprostane 5-series (**d**)

oxidative stress, in vivo levels of J_3-IsoPs are markedly increased (Brooks et al., 2008). These observations suggest that dietary $n-3$ fatty acids may influence the formation of bioactive peroxidation products derived from $n-6$ fatty acids by channeling the free radical pathway away from the F_2-IsoPs (Brooks et al., 2008). Although the molecular mechanism associated with the effect of J_3 isoprostanes is not understood, these mediators have been reported to induce Nrf2-directed gene expression (Gao et al., 2007). Under the normal conditions, Nrf2-dependent transcription is repressed by a negative regulator Keap1. When tissues or cells are subjected to oxidative stress, Nrf2 escapes Keap1-mediated repression and activates antioxidant responsive element (ARE)-dependent gene expression to maintain cellular redox homeostasis. In addition, Nrf2 has recently been recognized as a key factor regulating an array of genes that defend cells against the deleterious effects of environmental insults (Zhang, 2006).

4.8 Conclusion

ARA and DHA are found on *sn*-2 position of glycerol moiety of PtdCho and PlsEtn. Under physiological conditions, acylation/deacylation reactions maintain PtdCho and PlsEtn levels in neural membranes. Under pathological conditions, metabolism of ARA and DHA produces a variety of lipid mediators. ARA-derived lipid metabolites include prostaglandins, leukotrienes, thromboxanes, and lipoxins. Prostaglandins, leukotrienes, and thromboxanes are vasoconstrictive and proinflammatory, and their accumulation contributes to neural cell injury. Lipoxins are antiinflammatory, and their accumulation may protect cells from neural cell injury. In contrast, DHA breakdown through 15-LOX-like enzymes produces docosatrienes, resolvins, protectins, and neuroprotectins. These lipid mediators are collectively called as docosanoids, and their generation protects cells from neural injury. ARA- and DHA-derived lipid mediators modulate cellular function by acting through extracellular as well as intracellular receptors. The enzymic oxidation of EPA not only results in formation of weaker platelet aggregants, less vasoconstrictive and proinflammatory prostaglandins, and thromboxanes of 3 series prostaglandins and 5 series leukotrienes, but also generates resolvins E_1 and E_2 that cause antiinflammatory effects. DHA and ARA are ligands/modulators for the nuclear receptors NF-κB, PPAR, and SREBP-1c, which control various genes of inflammatory signaling and lipid metabolism. DHA and EPA downregulate inflammatory genes and lipid synthesis, and stimulate fatty acid degradation. In addition, the DHA/ARA contents of cell and organelle membranes as well as membrane microdomains strongly influence membrane function and numerous cellular processes, such as cell death and survival. Enzymically generated metabolites of ARA, DHA, and EPA are involved in modulation of neurotransmitter release, long-term potentiation, membrane repair, and cell cycle regeneration. In contrast, non-enzymic lipid metabolites of ARA, DHA, and EPA produce oxidative stress, and may contribute to cell injury and neurodegeneration. Accumulating evidence suggests that DHA and EPA not only suppress the synthesis of ARA-derived eicosanoids and increase neural membrane fluidity but also modulate gene expression, transcription factor activity, signal transduction pathways, and downregulate the production of free radicals and ROS.

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Chapter 5
Roles of Docosahexaenoic and Eicosapentaenoic Acids in Brain

5.1 Introduction

Docosahexaenoic acid (22:6 *n*−3, DHA) is an important essential polyunsaturated fatty acid (PUFA) that contains six *cis* double bonds located at positions 4, 7, 10, 13, 16, and 19. Eicosapentaenoic acid (20:5 *n*−3, EPA) is a 20-carbon fatty acid with five *cis* double bonds located at positions 4, 7, 10, 13, and 16. These essential fatty acids are found in fish and fish oil. Plant-derived sources of DHA and EPA precursors include flaxseed, flaxseed oil, walnuts, canola oil, and soybean oil. DHA and EPA incorporate into neural membrane glycerophospholipids, where they support neural cell membrane integrity and functions. DHA contents of brain vary considerably from one region to another, with the highest levels in the frontal cortex and the lowest in the substantia nigra/ventral tegmental area (Levant et al., 2006). Increased availability of DHA produces elevation in DHA content only in the olfactory bulb, parietal cortex, and substantia nigra/ventral tegmental area. In contrast, treatments that lower whole-brain DHA levels reduce DHA content in all brain regions except the thalamus, dorsal midbrain, and the substantia nigra/ventral tegmental area. Alterations in DHA level can be caused by changes in docosapentaenoic acid (*n*−6 DPA, 22:5 *n*−6) content; however, the change in DHA and *n*−6 DPA is nonreciprocal in some brain regions (Levant et al., 2006). Collective evidence indicates that the fatty acid compositions of specific brain regions are differentially affected by variation in DHA availability during development. DHA is a major structural component of neuronal membranes, and changes in DHA content of neuronal membranes lead to functional changes in the activity of receptors and other proteins embedded in the membrane glycerophospholipid bilayer. As stated earlier, DHA is selectively esterified in ethanolamine plasmalogen and phosphatidylserine, and therefore it is highly prevalent at the cytofacial site of the plasma membrane, where it may specifically participate in intracellular signaling. EPA can be converted into DHA, and low levels of EPA are found in neural membranes. EPA plays important physiological functions that can affect neuronal activity, signaling, and neuronal blood flow

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(Das, 2003; Lonergan et al., 2004). In brain astrocytes and endothelial cells, the synthesis of DHA is very low (Horrocks and Yeo, 1999; Horrocks and Farooqui, 2004).

Dietary intake of DHA and EPA results in suppression of proinflammatory cytokines and decrease in adhesion molecule expression. These effects occur at the level of gene expression and may be responsible for antagonism of the effects of ARA-derived mediators or through more direct actions on the intracellular signaling pathways, which lead to activation of transcription factors such as NF-κB. DHA and EPA downregulate the activity of NF-κB (Calder, 2003, 2004). Collective evidence suggests that DHA and EPA are major bioactive $n-3$ fatty acids that are not synthesized in human body and can only be obtained directly from the diet, particularly by consuming fish and fish oil. These fatty acids not only influence the biophysical state of the neural cell membranes but may also, via direct and indirect routes, act on multiple pathways including signaling, gene and protein activities, protein modifications, and probably play an important role in modulating protein aggregation (Caramia, 2008). Dietary deficiency of DHA and EPA has consequences on cerebral development, modifying the activity of enzymes of the cerebral membranes and decreasing the efficiency in learning tasks (Horrocks and Farooqui, 2004; Farooqui and Horrocks, 2007).

5.2 Comparison of Biochemical Activities of DHA and EPA

As stated above, EPA and DHA resemble each other in many biochemical effects, including the decrease in production of the key immunoregulatory cytokines (IL-10, TNF-α, and INF-γ) and in prevention of LPS toxicity (Lonergan et al., 2004; Zhao et al., 2004; Verlengia et al., 2004a). DHA and EPA differ from each other not only in modulation of specific genes expression but also in many biochemical and physicochemical effects (Das, 2003). For example, EPA is oxidized by COXs and 5-LOX, generating 3-series prostaglandins, thromboxanes, and 5-series leukotrienes (Calder, 2004) (Fig. 5.1). In contrast, DHA is not a substrate for COX. It is oxidized by 15-LOX-like enzyme that produces neuroprotectins and docosatrienes. EPA has hypotriglyceridemic and hypocholesterolemic effects, but DHA has no effect on plasma triglycerides (Table 5.1) (Hashimoto et al., 1999). EPA, but not DHA, increases mitochondrial fatty acid oxidation and upregulates 2,4-dienoyl-CoA reductase gene expression in rats (Willumsen et al., 1996). EPA, but not DHA, not only decreases mean platelet volume but also reduces platelet activation, an early step in platelet aggregation in normal subjects (Park and Harris, 2002). DHA is less effective than EPA in inhibiting vascular smooth muscle proliferation. DHA is a more potent inhibitor of lymphocyte adhesion to endothelial cells than EPA (Hashimoto et al., 1999). Enrichment of DHA, but not EPA, in

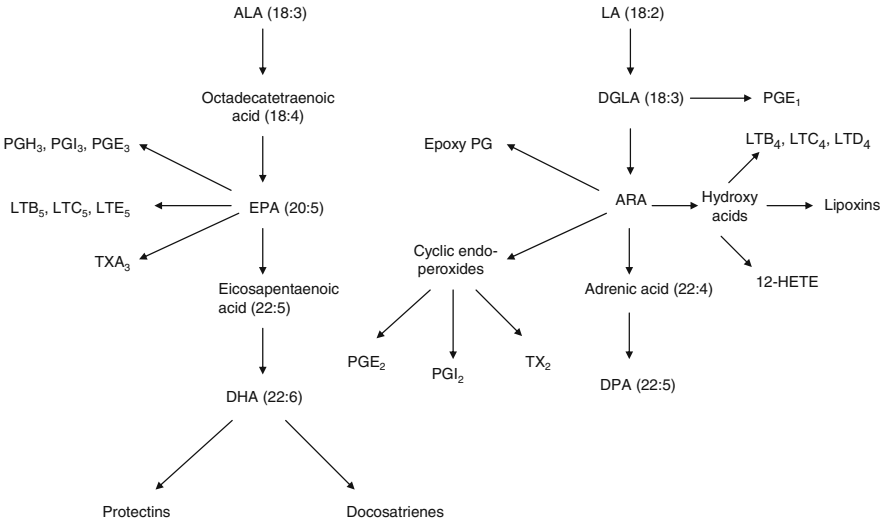

Fig. 5.1 Generation of eicosanoids and docosanoids from precursors of ARA, EPA, and DHA

human skin fibroblasts alters membrane biophysical characteristics and upregulates the activities of 5′-nucleotidase and adenylate cyclase (Brown and Subbaiah, 1994). Furthermore, EPA and DHA differ from each other on their effect on neural membrane capacitance. Thus, EPA increases PC12 cells

Table 5.1 Differences in properties and activities of EPA and DHA in brain

EPA	DHA	References
Essential for neural cell signaling	Component of neuronal membrane	Mitchell et al. (1998)
Precursor for 3-series PG and 5-series LT	Precursor for docosatrienes and neuroprotectins	Calder (2004); Bazan (2005a,b); Serhan (2005a,b)
Increases membrane capacitance in PC12 cells	No effect on membrane capacitance	Ong et al. (2006)
No effect on Na^+ and K^+ channels	Blocks on Na^+ and K^+ channels	Gerbi et al. (1994)
Produces antidepressant and antipsychotic effects	No antidepressant and antipsychotic effects	Edwards et al. (1998); Peet et al. (1998)
No effect on glucose transport	Modulates glucose transport	Pifferi et al. (2007)
3-Thia fatty acids have more pronounced effect	3-Thia fatty acids have less pronounced effect	Madsen et al. (1998)
Converted to DHA	Not converted to EPA	Farooqui and Horrocks (2007)
Hypotriglyceridemic and hypocholesterolemic	Very little effect on triacylglycerol	Farooqui et al. (2007)

3-Thia fatty acids are tetradecylthioacetic acid and 3,10-dithiadicarboxylic acid.

membrane capacitance whereas DHA has no effect (Ong et al., 2006). The reason for the stimulatory effect of EPA on membrane capacitance is not understood. However, it is likely that EPA interacts with the external ion channel domain of PC12 membranes differently than DHA. DHA blocks voltage-activated sodium channels whilst EPA has no effect on membrane excitability and sodium channels in hippocampal neurons (Xiao and Li, 1999). Similarly, DHA modulates certain voltage-gated K^+ channel in Chinese hamster ovary cells, whereas EPA has no effect on K^+ channels. EPA modulates DHA synthesis in SH-SY5Y neuroblastoma cell cultures. DHA levels are an important regulator of brain glucose uptake, possibly through their affect on some but not all the glucose transporters (Pifferi et al., 2007). EPA has no such affect. DHA plays important roles in neurogenesis, neurotransmission, and protection against oxidative stress, whereas EPA produces antidepressant and antipsychotic activity by modulating neuronal activity (Table 5.1) (Maes et al., 2007). DHA, through the generation of docosanoids, has stronger ability to antagonize ARA-dependent cell signaling than EPA-derived eicosanoids (3-series PG and 5-series LT) (Obajimi et al., 2005; Farooqui and Horrocks, 2007) (Fig. 5.2). Furthermore, EPA alters the rate of uptake and release of ARA from glycerophospholipids in an exposure time-dependent manner, whereas DHA has no or little effect. DHA and EPA supplementation has opposite effects on the norepinephrine-stimulated cyclic AMP accumulation in rat pinealocytes (Delton-Vandenbroucke et al., 1996). DHA deficit is associated with dysfunctions of neuronal membrane stability and transmission of serotonin, norepinephrine, and dopamine, which might connect to the pathogenesis of mood and cognitive dysfunction. In contrast, EPA is important in balancing the immune function, antidepressant, and antipsychotic activities associated with the depression and physical health of neural cells by reducing ARA-derived lipid mediators. Collective evidence suggests that both fatty acids contribute to the modulation of neural cell function not only by generating different lipid mediators and regulating physicochemical properties of neural membranes but also by modulating different genes and enzymic activities. The greater effect of DHA on neural and non-neural membranes compared to EPA may also be due to its higher levels than EPA and its greater ability to inhibit HMG-CoA reductase and reduce membrane cholesterol content or the cholesterol/glycerophospholipid molar ratio, or both (Hashimoto et al., 1999; Farooqui et al., 2006). Balance in DHA and EPA is important for homeostasis and normal brain developments.

Studies on the effect of 3-thia fatty acids (tetradecylthioacetic acid and 3,10-dithiadicarboxylic acid) in non-neural tissues provide more evidence on differences in metabolic utilization of EPA and DHA. Thus, administration of 3-thia fatty acids to rats produces a significant decrease in hepatic level of DHA (17−24%) and EPA (40–80%) levels (Madsen et al., 1998). This decrease is accompanied by increased gene expression of mitochondrial 2,4-dienoyl-CoA reductase and enoyl-CoA isomerase. The mitochondrial oxidation of EPA is increased more than fourfold after administration of 3-thia fatty acids. EPA-CoA is a good substrate for mitochondrial carnitine acyltransferase-I, and treatment

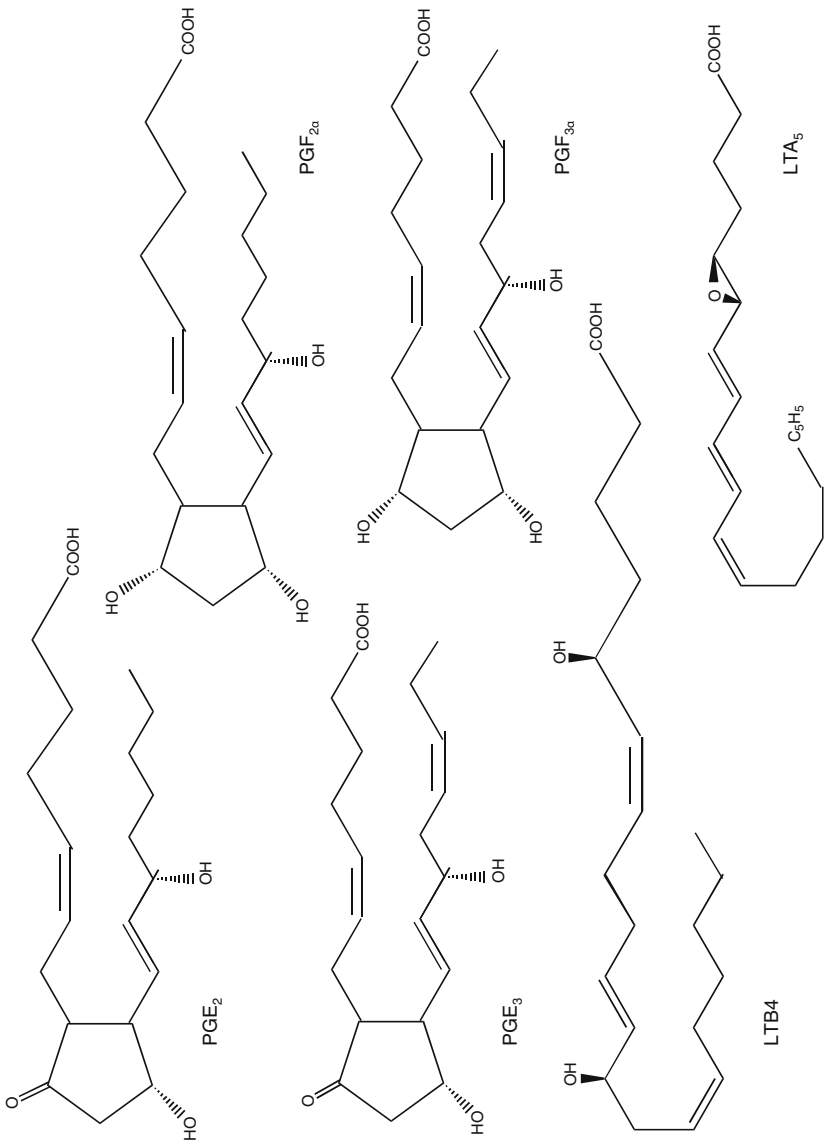

Fig. 5.2 Chemical structures of ARA- and EPA-derived eicosanoids

with 3-thia fatty acids increases its activity by 1.7-fold (Madsen et al., 1998). In contrast, DHA is a poor substrate for both mitochondrial and peroxisomal β-oxidation. DHA-CoA is a very poor substrate for mitochondrial carnitine acyltransferase-I and -II, and its activity does not respond to 3-thia fatty acid treatment. However, the peroxisomal DHA-CoA oxidase is increased 10-fold after 3-thia fatty acid treatment. In contrast, peroxisomal EPA-CoA oxidase is increased only fivefold (Madsen et al., 1998). In isolated hepatocytes, 16% of total metabolized EPA is oxidized and 76% is incorporated into glycerolipids, whereas there is very little effect on DHA oxidation. It is suggested that during 3-thia fatty acid-mediated mitochondrial and peroxisomal proliferation increase in $n-3$ fatty acid oxidation results in the decreased levels of hepatic EPA and DHA. DHA is oxidized by the peroxisomes at higher rate, whereas EPA, which can be oxidized in both organelles, is mainly oxidized by mitochondria (Madsen et al., 1998). DHA differs from EPA in its mechanism of inhibition of platelet redox status (Lagarde et al., 2003). DHA is a potent inhibitor of TxA_2-mediated platelet aggregation at the receptor level. EPA is inactive at the receptor level (Swann et al., 1990; Lagarde et al., 2003). Collectively, these studies suggest that DHA and EPA differ from each other in many biochemical and biophysical properties.

5.3 Role of DHA in Brain Tissue

Dietary DHA maintains functional maturation of the retina and visual cortex, resulting in optimal visual acuity and brain development. DHA-deficient animals have reduced visual acuity and impaired learning ability. DHA regulates neurotransmitter release, modulates genes associated with brain development, effects physicochemical properties of neural membranes, and regulates the activities of many enzymes associated with signal transduction processes (Horrocks and Farooqui, 2004). During brain development, DHA induces neurite outgrowth and promotes optimal signal transduction (Yamashima, 2008; Ma et al., 2008). In hippocampus, it improves long-term potentiation and learning ability of aged rats, and promotes cognitive function of humans with memory deficits, but the underlying mechanisms associated with beneficial effects of PUFA remain unknown. Recent studies indicate that PUFAs act as endogenous ligands for G-protein-coupled receptor 40 (GPR40), which are located throughout the brain tissue. It is proposed that polyunsaturated-GPR40 interaction may be crucial for adult neurogenesis and/or memory formation in hippocampus (Yamashima, 2008; Ma et al., 2008).

5.3.1 DHA-Mediated Modulation of Physiocochemical Properties of Membranes

Incorporation of DHA alters physicochemical properties of neural membranes, including bilayer thickness, acyl chain packing, free volume, and phase transition temperature (Mitchell et al., 1998; Wassall et al., 2004). DHA and cholesterol are

involved in microdomain (raft) formation (Wassall et al., 2004; Shaikh et al., 2003, 2004). Lipid rafts play an important role in the compartmentalization and modulation of cell signaling. DHA-containing lipid bilayer can be distinguished from ARA-containing bilayer by extremely high water permeability, minimal interactions with cholesterol, and loose acyl chain packing (Mitchell et al., 1998). These physicochemical properties may facilitate functioning of the G-protein-coupled receptors associated with vision, taste, and odor receptors. Collectively, these studies suggest that DHA incorporation in neural membranes causes changes in neural membrane fatty acid composition, which may lead to alterations in many membrane properties such as permeability, receptor affinities, ion fluxes, and activities of membrane-bound enzymes.

5.3.2 DHA-Mediated Modulation of Neurotransmission

DHA-mediated changes in neural membrane also modulate neurotransmission. Thus, DHA modulates and facilitates neurotransmission through its effect on a variety of receptors including dopaminergic, noradrenergic, glutamatergic, and serotonergic neurotransmitter receptors and insulin, retinoid, and TGF-β receptors (Fig. 5.3)

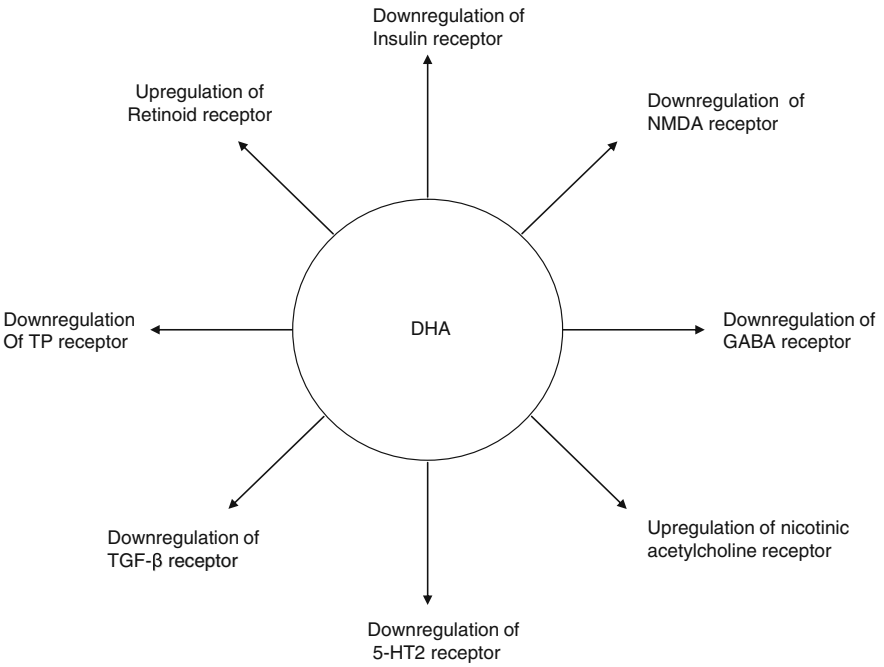

Fig. 5.3 Modulation of receptors by docosahexaenoic acid. *N*-Acetyl-D-asparatate receptor (NMDA receptor); γ-aminobutyric acid receptor (GABA receptor); serotonin receptor (5-HT_2 receptor); transforming growth factor- β receptor (TGF-β receptor); TxA_2 receptor (TP receptor); and retinoic acid receptor (retinoid receptor)

(Zimmer et al., 2000; Chalon et al., 1998; Högyes et al., 2003). DHA modulates dopaminergic and serotonergic neurotransmission in rat frontal cortex, and a chronic deficiency of DHA changes dopamine metabolism by altering dopamine receptor and transporters in the nucleus accumbens (Zimmer et al., 2000; Chalon, 2006). Dietary supplementation not only changes the endogenous dopamine levels in frontal cortex but also elevates binding of dopamine to the D_2 receptors (Chalon et al., 1998, 2006). DHA also modulates glutamatergic neurotransmission. It is a component of PtdSer, a neural membrane phospholipid needed for maintenance of NMDA receptor-mediated processes (Calon et al., 2005). DHA differentially modulates glutamate transporters (GLT1, GLAST, and EAAC1) through different mechanisms (Berry et al., 2005). Thus, DHA stimulates GLT1 and EAAC1 through a mechanism that requires extracellular Ca^{2+}, CaM kinase II, and protein kinase C, but not protein kinase A. In contrast, the inhibitory effect of DHA on GLAST does not require extracellular Ca^{2+} and does not involve CaM kinase II (Berry et al., 2005). Collectively, these studies suggest that DHA status not only improves cognitive development and behavior but also increases neuroplasticity of neural membranes. These processes contribute to synaptogenesis and may be involved in synaptic transmission.

5.3.3 DHA-Mediated Modulation of Gene Expression

DHA interacts with the genome and modulates the expression of many transcription factors (LXR, HNF-4, NF-κB, and SREBP) and proteins (Jump, 2004). DHA and its metabolites bind directly to specific transcription factors to regulate gene transcription. Using protein microarrays procedure, it is shown that DHA not only downregulates protein kinase Cβ, protein kinase Cγ, and phospholipase Cγ, but alters the expression of many proteins associated with signal transduction processes in the brain (Puskas and Kitajka, 2006). Collective evidence suggests that supplementation of DHA in animal diet produces the overexpression of genes associated with signal transduction, synaptic plasticity, energy metabolism, and membrane trafficking (Kitajka et al., 2002; Puskás et al., 2003). Interactions of DHA with transcription factors results in upregulation of β-oxidation enzymes and downregulation of desaturase expression. These enzymes modulate the synthesis of DHA from α-linolenic acid (Price et al., 2000; Nakamura and Nara, 2003).

DHA selectively promotes alterations in the subcellular distribution of lipidated cytosolic proteins (Ras- and Src-related tyrosine kinases), depending on their trafficking route by modifying membrane glycerophospholipid composition. The effect of DHA seems to be potentially universal to lipidated signaling proteins, regardless of the functional state of proteins and the type of lipid modifications (Seo et al., 2006). For example, DHA inhibits the association of Ras with membranes and reduces Ras-dependent signaling and cell proliferation. It is proposed that DHA may modulate Ras membrane association not only by modifying membrane lipid composition but effecting the subcellular distribution of Ras isoforms (N-Ras, H-Ras, and K-Ras4B)

(Seo et al., 2006). Although, some Ras is present in the plasma membrane, but the major Ras-signaling platform is in endomembranes including the endoplasmic reticulum and the Golgi (Apolloni et al., 2000; Choy et al., 1999; Seo et al., 2006). In plasma membranes, Ras-signaling proteins play critical roles in various cellular functions by relaying extracellular signals from surface receptors to downstream signaling networks. The overactivation of these proteins by overexpression and chronic activation of upstream receptors may be associated with oncogenesis and immune disorders (Yu et al., 2003). It is proposed that diet with lower ARA/DHA ratio can directly influence the intracellular trafficking and subcellular localization of lipidated proteins, revealing the importance of DHA in diet. The dramatic influence of DHA on plasma membrane targeting of N-Ras, H-Ras, and Lck also underscores the pharmacological potential of DHA, including antioxidant, antiinflammatory, and antiapoptotic effects that have long been appreciated (Seo et al., 2006; Farooqui et al., 2007).

DHA is a ligand for the retinoid X receptor (RXR) in brain (Lengqvist et al., 2004; Farooqui et al., 2004; de Urquiza et al., 2000). RXR activation is closely associated with nuclear signaling and gene expression. RXR also serves as a fatty acid sensor in vivo. A number of proteins act as co-activators (Jump, 2002b). DHA supplementation not only modulates gene but also mediates changes in the stability of the mRNA for several lipogenic enzymes. As stated earlier, a deficiency of DHA results in downregulation of brain glucose transporter expression, resulting in a decrease in glucose utilization in the cerebral cortex of DHA-deficient rats (Pifferi et al., 2005). DHA also interacts with the PPAR, SREBP, and carbohydrate response element-binding protein (ChREBP) (Clarke, 2000; Jump, 2002b; Nakamura et al., 2004). In addition, DHA modulates the expression of genes for cytokines and their receptors, cell adhesion molecules, cytoskeleton proteins, and hormone receptors. The effect of DHA is sustained as long as DHA remains in the diet. Thus, DHA acts like a hormone to control the activity of key transcription factors, and modulates a variety of neural cell functions (Jump, 2002a,b; Valentine and Valentine, 2004). Collective evidence suggests that DHA modulates the expression of a number of genes with such broad functions as DNA binding, transcriptional regulation, transport, cell adhesion, cell proliferation, raft formation, membrane protrusions, and membrane localization. These effects, in turn, may significantly modify cell function, development, and/or maturation.

5.3.4 DHA-Mediated Modulation of Enzymic Activities

DHA modulates the activities of a number of enzymes, including COX-2, PLA_2, and PKC (Table 5.2). COX-2 catalyzes the overproduction of prostaglandins (PG) at inflammatory sites. DHA effect is mediated through the NF-κB-binding site in the COX-2 promoter. DHA prevents nuclear p65 NF-κB subunit translocation by decreasing cytokine-mediated ROS and regulating ERK1/2, NADPH oxidase, and

Table 5.2 Effect of DHA and EPA on enzymic activities

Enzymic activity (DHA)	Effect	References
Phospholipase A_2	Inhibition	Farooqui et al. (2006)
Phospholipase C		Sanderson and Calder (1998)
Phospholipase D1		Diaz et al. (2002)
Protein kinase C		Padma and Das (1999)
Cyclooxygenase	Inhibited	Calder (2004)
Glutathione peroxidase	Increased	Delton-Vandenbroucke et al. (2001)
MAP kinase	Inhibited	Hirafuji et al. (2003)
Cyclin-dependent kinase	Inhibited	Hirafuji et al. (2003)
Caspase-3	Inhibited	Akbar et al. (2005)
Neutral sphingomyelinase	Inhibited	Wu et al. (2005)
HMG-CoA reductase	Inhibited	Duncan et al. (2005)
EPA		
SPC-induced Rho-kinase	Inhibited	Yoneda et al. (2008)
Protein kinase A	Activated	Szentandrassy et al. (2007)
Endothelial NOS	Activated	Li et al. (2007)
MMP-1	Inhibited	Kim et al. (2005)
Super oxide dismutase	Activated	Sohma et al. (2007)
Carnitine palmitoyl transferase	Activated	Guo et al. (2005)

PKCϵ activities (Massaro et al., 2006). In addition, DHA inhibits nitric oxide synthase and stimulates the activities of antioxidant enzymes of glutathione peroxidase (GSH-Px) and glutathione reductase (GR). However, DHA does not alter the levels of glutathione (GSH) as compared to the control. Collective evidence suggests that DHA may act as a potent neuroprotector (Wang et al., 2003). DHA also modulates Na^+, K^+ ATPase, an integral membrane protein associated with nodes of Ranvier in neurons (Marszalek and Lodish, 2005). This enzyme facilitates and maintains Na^+ and K^+ gradients necessary for resting neural membrane potential. Supplementation of DHA results in increased Na^+,K^+-ATPase activity in sciatic nerve (Gerbi et al., 1998). In contrast, DHA significantly reduces Na^+, K^+-ATPase activity in human endothelial cells.

DHA modulates phosphorylation by upregulating ERK kinase in photoreceptors. This process is blocked by 1,4-diamino-2,3-dicyano-1,4-*bis*(2-aminophynyltio) butadiene (U0126), a specific MEK inhibitor. U0126 not only retards DHA-mediated mitochondrial depolarization but also prevents the DHA-mediated increase in opsin expression. DHA upregulates the early expression of Bcl-2, and downregulates Bax expression. At the same time, DHA also decreases caspase-3 activation in photoreceptors. Collectively, these studies suggest that DHA readily incorporates into cell membranes and lipid rafts, and modulates membrane-associated signaling proteins such as Ras, Akt, and Her-2/neu. DHA exclusively activates the ERK/MAPK pathway to promote photoreceptor survival during early development in vitro and upon oxidative stress (German et al., 2006).

5.3.5 DHA-Mediated Modulation of Inflammation and Immunity

The immune system protects the host against pathogenic invaders. However, dysregulation of the immune system components can be directed against host tissues, so causing damage. Thus, the immune function, along with inflammatory reactions, is a fundamental component of host defense against pathogenic invaders. These immune function and inflammatory reactions involve interactions amongst many different cell types dispersed throughout the body. Immune function involves phagocytosis, processing of antigens derived from intracellular and extracellular pathogens, activation of T cells with proliferation, and the synthesis of cytokines that elicit effector cell functions such as antibody production and killing cell activity. Brain is an immunologically active organ. It is in direct communication with the immune and endocrine systems (Wilson et al., 2002). Inflammatory reactions are protective mechanisms that isolate the damaged brain tissue from uninjured area, destroy affected cells, and repair the extracellular matrix (Correale and Villa, 2004). All neural cells, including microglia, astrocytes, neurons, and oligodendrocytes, participate in inflammatory reactions and responses.

Immune function is regulated by PUFAs. The $n-3$ PUFAs reduce both resistance to bacterial infection and host survival. Both neural and immune cells are rich in ARA and DHA. ARA, EPA, and DHA contents can be altered through oral intake of diet enriched in these fatty acids. Low ARA intake enhances some immune functions, whereas high ARA intake decreases certain immune functions (Calder, 2003; Harbige, 2003). Similarly, dietary intake of DHA and EPA increases cell survival, and reduces disease severity in spontaneous autoantibody-mediated diseases. Thus, intake of ARA, DHA, and EPA not only results in alterations in neural membrane composition but also changes the pattern and levels of eicosanoids, lipoxins, and docosanoids generation (Figs. 5.4 and 5.5). Changes in the fatty acid composition of neural and immune cells also affect phagocytosis, T cell signaling, and antigen presentation capability and downregulation of inflammatory reactions (Calder, 2003, 2004, 2007; Siddiqui et al., 2007). These effects are mediated at the membrane level, suggesting important roles of fatty acids in membrane order, membrane trafficking lipid rafts, which are plasma membrane microdomains in which many receptors are localized. The incorporation of DHA in membrane lipid rafts changes the lipid environment of membrane microdomains not only changing the localization of receptors, such as interkeukin-2-receptor, STAT5a and STAT5b, but also suppressing the expression of JAK1, JAK3, and tyrosine phosphotyrosine in soluble membrane preparation. DHA not only modulates immune responses and exert beneficial immunosuppressive effects, but modulates the molecular mechanisms inhibiting T cell activation (Li et al., 2006).

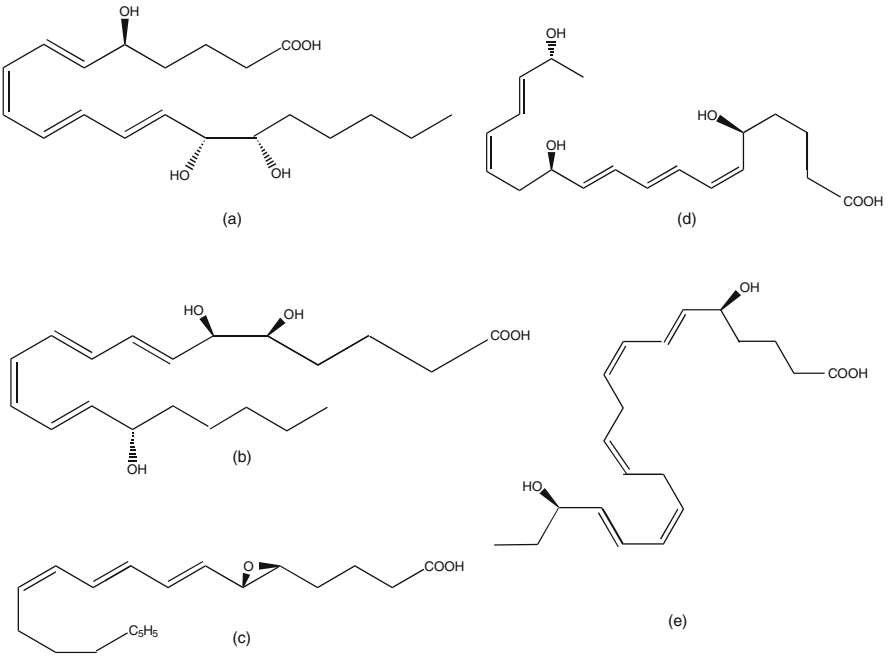

Fig. 5.4 Chemical structures of ARA-derived lipoxins and EPA-derived resolvins. Lipoxin A_4, LXA_4 (**a**); lipoxin B_4, LXB_4 (**b**); EPA-derived leukotrienes, LTA_5(**c**); resolvin E_1, RvE_1(**d**); and resolvin E_2, RvE_2 (**e**)

DHA reduces the expression of proinflammatory cytokines such as interleukin-1, interleukin-2, interleukin-6, and tumor necrosis factor-α (Caughey et al., 1996; Wu and Meydani, 1998), and modulates the expression of interferon-γ and adhesion molecule, reflecting faster maturation of the immune system. A variety of other molecular mechanisms may also explain how DHA interferes with immune cell function. These mechanisms include alterations in eicosanoid synthesis, orphan nuclear receptor activation (e.g., PPARs, liver X receptors), and T lymphocyte signaling by changing the molecular composition of lipid rafts (Stulnig, 2003). In addition, *n*−3 fatty acids modulate changes in the concentrations and actions of several orexigenic and anorexigenic neuropeptides in the brain, including neuropeptide Y and α-melanocyte-stimulating hormone (α-MSH) (Goncalves et al., 2005), suggesting that in cancer anorexia, *n*−3 fatty acids are associated with quantitative reversal of hypothalamic NPY, α-MSH, and serotonin receptor ($5\text{-}HT_{1B}$-receptor) enhancing food intake. Patients with acute and chronic inflammatory conditions have low tissue levels of *n*−3 fatty acids and high levels of proinflammatory cytokines, in association with anorexia and decreased food intake. Thus, *n*−3 fatty acid supplementation suppresses proinflammatory cytokine production and improves food intake by normalizing hypothalamic orexigenic peptides and neurotransmitters (Goncalves et al., 2005).

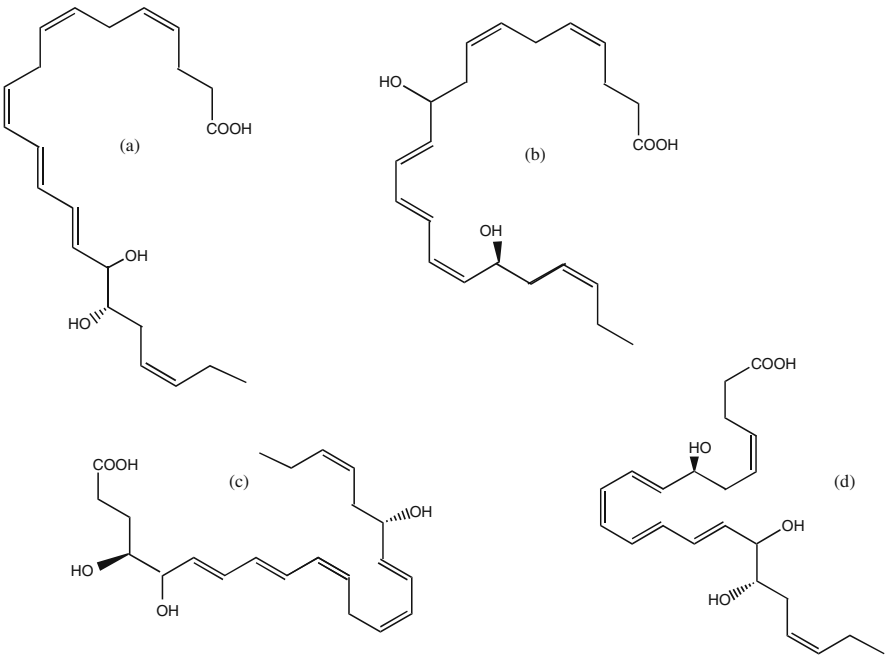

Fig. 5.5 Chemical structures of DHA- and EPA-derived protectins and docosatrienes. 16,17 S-docosatriene (**a**); 10,17 S-docosatriene (**b**); 4S5,17 S-resolvin (**c**); 7,16,17S-resolvin (**d**). These metabolites retard the actions of eicosanoids

5.3.6 DHA-Mediated Modulation of Learning and Memory

Long-term potentiation (LTP) and long-term depression (LTD) of hippocampal synaptic transmission are two forms of activity-dependent synaptic plasticity that underlie learning and memory. DHA is crucial for the induction and maintenance of LTP (Fujita et al., 2001; Izaki et al., 1999). DHA also blocks the induction of LTD. These processes require the involvement of NMDA receptors in hippocampal CA1 neurons (Young et al., 1998). The molecular mechanism of these processes remains unknown. However, it is possible that DHA not only acts on postsynaptic sites but also incorporates into PtdSer, which maintains activity of NMDA receptor (Zucker, 1989). DHA modulates NMDA-independent long-lasting modulation in the Schaffer collateral-CA1 synapses of hippocampal slices by inhibiting K^+ channels (Poling et al., 1995; Honore et al., 1994). In addition, DHA blocks GABA-induced chloride channel activity and potentiates NMDA responses of dissociated rat substantia nigra neurons (Fig. 5.3) (Nabekura et al., 1998). Based on these observations, it is proposed that DHA may improve memory formation by blocking the inhibitory actions of GABA on LTP (Das, 2003). Collectively, these studies suggest that the complete blockade of NMDA receptors prevents both LTP and LTD

induction, whereas a moderate concentration of the NMDA antagonist, D-APV, shifts the direction of synaptic plasticity to the induction of LTD (Young et al., 1998).

Incorporation of DHA in neural membranes from transgenic mice over-expressing the Tg2576 gene partly protects the mice from NMDA receptor subunit loss. Thus, DHA stabilizes the structure of NMDA receptors (Calon et al., 2005). Collectively, these studies suggest that DHA not only improves cognitive development but also increases neuroplasticity of neural membranes. These processes contribute to synaptogenesis and may be involved in synaptic transmission.

5.3.7 DHA-Mediated Modulation of Apoptosis

Antiapoptotic effect of DHA is observed in neural cell and non-neural cell cultures (Fig. 5.6). DHA prevents apoptosis in serum-deprived Neuro 2A cells (Kim et al., 2000; Kishida et al., 1998; Rotstein et al., 1997, 1998). The molecular mechanism of DHA-mediated antiapoptotic effect is not known. However, the translocation of Raf-1 kinase, downregulation of caspase-3 activity, and inhibition of the phosphatidylinositol 3-kinase pathway by DHA may be associated with the prevention of apoptosis induced by staurosporine in Neuro 2A cells (Kim et al., 2000; Akbar and Kim, 2002; Akbar et al., 2005). DHA promotes neuronal survival by facilitating translocation/activation of Akt (a serine/threonine kinase involved in cell survival) through its capacity to increase PtdSer. DPA (22:5 $n-6$), which replaces DHA during $n-3$ deficiency, is less effective in accumulating PtdSer and translocating Akt, and thus less

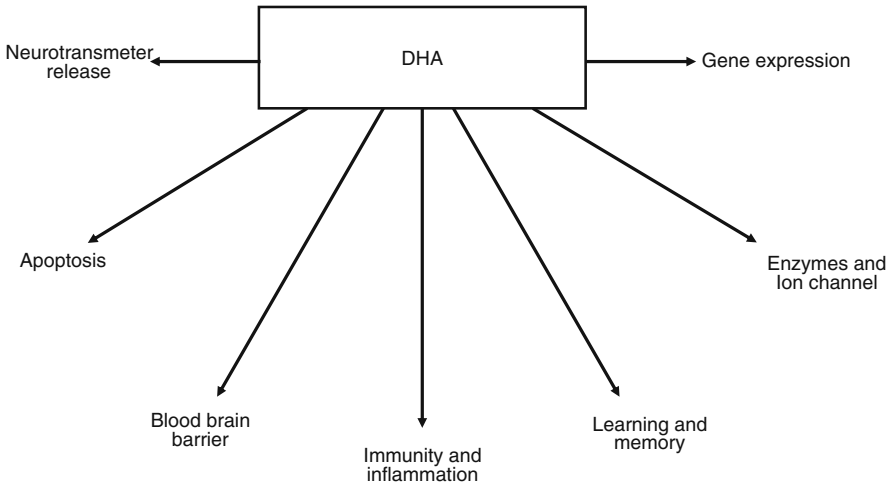

Fig. 5.6 Roles of DHA in brain

effective in preventing apoptosis (Akbar et al., 2005). The extent of the anti-apoptotic effect of DHA correlates with a time-dependent increase in the PtdSer content. Treatment of Neuro 2A cells with DHA in serine-free culture medium has no effect on caspase-3 and phosphatidylinositol 3-kinase activities. Thus, an increase in PtdSer content is necessary for staurosporine-induced apoptosis (Kim et al., 2000; Akbar and Kim, 2002; Akbar et al., 2005).

In contrast, in non-neural tumor cells, Ramos cells, and HL-60 cells, DHA induces apoptosis (Siddiqui et al., 2001; Miura et al., 2004). Although the molecular mechanism associated with DHA-mediated cell death remains elusive, it is becoming increasingly evident that in CaCo-2 colon cancer cells, DHA upregulates caspases including caspases 5, 8, 9, and 10 and downregulates cyclooxygenase 2 expression and several cell cycle-related genes (Narayana et al., 2003).

In addition, DHA modulates PPARγ, a key regulator of lipid metabolism, which also modulates proliferative activity in a variety of cells including mammary cells. Expression of PPARγ in the nucleus is activated by second messengers such as J-series prostaglandins, which have been shown to cause apoptosis in vivo in explants of human breast cancer cells in immunosuppressed mice. Thus, DHA-mediated apoptosis may occur via ligand-dependent transcription factor, PPARγ (Zand et al., 2008). The activation of PPARγ results in the induction of apoptosis through NF-κB inhibition. Treatment of Ramos cells and p53-deficient Burkitt's lymphoma cell line with low doses of DHA and γ-IR produces marked phosphorylation of IκB and translocation of p65/NF-κB to the nucleus in parallel with increase in apoptosis (Zand et al., 2008). Preincubation of the cells with GW9662, a selective antagonist for PPARγ, significantly retards NF-κB activation. Collective evidence suggests that low concentration of DHA inhibit γ-IR-mediated activation of NF-κB and sensitized Ramos cells to IR-mediated cytotoxicity. Pretreatment of Ramos cells with GW9662 blocks the ability of DHA to inhibit γ-IR-mediated activation of NF-κB, and retards the DHA radiosensitizing effect, indicating that PPARγ may act as a mediator of DHA in inhibition of NF-κB (Zand et al., 2008). In addition, DHA may modify the expression of β-catenin, a protein, whose expression is associated with induction of apoptosis in colon cancer cells (SW480 and HCT116) (Calviello et al., 2007). Proteasomal inhibitors, MG132 and lactacystin, block DHA-mediated β-catenin decrease, suggesting that DHA may regulate the proteasomal degradation of β-catenin. The reduced levels of β-catenin are accompanied by decreased translocation of β-catenin into the nucleus, where it acts as a transcription factor in concert with T-Cell Factor (TCF). Collective evidence suggests that DHA may exert proapoptotic and antitumoral effects not only through proteasomal regulation of β-catenin levels but also through alterations in the expression of TCF-beta-catenin target genes (Calviello et al., 2007).

Another mechanism of apoptosis induction in non-neural cells may involve the participation of ceramide. DHA increases ceramide levels and reduces the amount of phosphorylated retinoblastoma protein (pRb), a protein which is

involved in cell cycle and growth (Siddiqui et al., 2001, 2003, 2004). DHA also mediates cell cycle arrest and apoptosis by activating protein phosphatases. Besides dephosphorylation of retinoblastoma protein, these protein phosphatases interact with bcl2, an apoptotic protein that regulates the release of cytochrome *c* from mitochondria, and eventually activation of caspase-3 (Siddiqui et al., 2004; Bougnoux, 1999).

5.3.8 DHA and Generation of Docosanoids

COXs do not oxidize DHA. The action of an enzyme resembling 15-LOX on DHA produces docosatrienes and neuroprotectins (Fig. 5.6) (Hong et al., 2003; Marcheselli et al., 2003; Serhan et al., 2004; Serhan, 2005a). As stated earlier, docosatrienes and neuroprotectins antagonize the effects of eicosanoids, modulate leukocyte trafficking, and downregulate the expression of cytokines in glial cells. They also modulate interactions among neurons, astrocytes, oligodendrocytes, microglia, and cells of the microvasculature (Hong et al., 2003; Marcheselli et al., 2003; Serhan et al., 2004; Bazan, 2006). The neuroprotectins comprise docosatrienes and neuroprotectins (Serhan, 2005a; Bazan, 2005a). Following ischemic reperfusion injury or during oxidative stress in cell culture, the infusion of neuroprotectin D_1 (NPD_1) downregulates oxidative stress and apoptotic DNA damage. NPD_1 also upregulates the antiapoptotic Bcl-2 proteins, Bcl-2 and bclxL, and decreases the expression of the proapoptotic proteins, Bax and Bad (Bazan, 2005a). Furthermore, this metabolite inhibits caspase-3 activity, and blocks IL-1-mediated expression of COX-2.

In non-neural cells, resolvins of D series (RvD_1 to RvD_6) are generated from DHA. Administration of RvDs and RvD_1 protects kidney tissues from ischemia/reperfusion injury. RvDs and RvD_1act through reducing the number of infiltrating leukocytes and blocked TLR-mediated activation of microglial cell macrophages in kidney (Duffield et al., 2006). The generation of RvDs and RvD_1 may be an internal neuroprotective mechanism for preventing tissue damage (Hong et al., 2003; Marcheselli et al., 2003; Serhan, 2005b; Mukherjee et al., 2004; Bazan, 2005a; Lukiw et al., 2005; Muckova et al., 2006).

5.3.9 DHA-Mediated Generation of Neurite Outgrowth

DHA is needed for optimal brain development. A deficiency of DHA in the hippocampus not only decreases neuronal soma size in this region (Ahmad et al., 2002) but also reduces levels of PtdSer and PlsEtn (Hamilton et al., 2000; Farooqui and Horrocks, 2001). These processes interfere with learning and memory (Moriguchi et al., 2000). Restoration of memory formation in DHA-deficient animals occurs when these animals were fed a DHA-adequate diet. Similarly, supplementation of DHA in cell culture media not only induces

neurite outgrowth in PC12 cells (Ikemoto et al., 1997), hippocampal cells (Calderon and Kim, 2004), and rat cortical primary neuronal cultures (Cao et al., 2005), but also promotes branching. DHA inclusion in the culture medium increases the neurite length of DHA-deficient neurons to the level of DHA-adequate neurons (Calderon and Kim, 2004). The molecular events associated with DHA-mediated neuritogenesis and differentiation are not fully understood. However, the overexpression of long-chain acyl-CoA synthetase 6 specifically promotes DHA internalization, activation to DHA-CoA, and accumulation in differentiating PC12 cells (Marszalek et al., 2005), suggesting that DHA plays an important role in the brain development (Calderon and Kim, 2004; Cao et al., 2005).

In addition to glycerophospholipid synthesis, neurite outgrowth requires the synthesis of protein (Ikemoto et al., 1999; Kawakito et al., 2006). In vitro treatment of neurons with DHA results in strong growth-associated protein-43 (GAP-43) immunoreactivity, and higher PtdSer and EtnGpl contents but a lower PtdCho content than control neurons (Cao et al., 2005). Fatty acid analyses of DHA-supplemented neurons display significant increase in DHA contents in PtdSer and EtnGpl, supporting the view that these lipids are immediate precursors of lipid mediators associated with the signal transduction processes involved in the induction of neurite outgrowth in DHA-supplemented cell cultures. Supplementation of serum-free medium with hormones and DHA results in the complete development of synapses of cultured mouse fetal hypothalamic cells as attested by the increase in number and the regular shape and diameter of synaptic vesicles.

Additionally, DHA induces BDNF protein expression in rat cortical astrocytes (Rao et al., 2007). It is suggested that BDNF plays an important role in synaptic plasticity and neuronal survival. The expression of BDNF changes with age, and the impact of these alterations may have functional consequences for neuronal pathways (for example, the reductions in inhibitory and excitatory synaptic terminals). BDNF protein expression and levels of DHA are reduced in the normal aging brain, indicating that this decrease in BDNF and DHA levels may be related to reduction in neural membrane plasticity, resulting in alterations in cognitive function (Farooqui, 2009).

DHA supplementation also modulates the morphology of astrocytes in primary cultures. The three populations of astrocytes are seen in DHA-treated astrocytic cultures (Champeil-Potokar et al., 2006). They differ from each other in their $n-3$:$n-6$ fatty acid ratios in glycerophospholipids. DHA-treated astrocytes have a physiologically high $n-3$:$n-6$ fatty acid ratio (1.58); untreated astrocytes show a low $n-3$:$n-6$ fatty acid ratio (0.66). In contrast, ARA-treated astrocytes have a very low $n-3$:$n-6$ fatty acid ratio (0.36), with excess $n-6$ fatty acids. DHA-treated astrocytes have greater gap junction capacity than unsupplemented cells or ARA-treated astrocytes. The elevated gap junction coupling of DHA-enriched astrocytes may be involved in functional distribution of connexin 43 at cell interfaces. These findings suggest that the high $n-3$:$n-6$ fatty acid ratio that occurs naturally in

astrocyte membranes is required for optimal gap junction coupling and astrocyte neuronal communication (Champeil-Potokar et al., 2006).

5.3.10 DHA-Mediated Modulation of Visual Function

DHA is a major constituent of photoreceptor membranes (Neuringer and Connor, 1986; Anderson et al., 1976). It accumulates in rod outer segment disks, plays an important role in disordering disk membranes, creates an adequate environment for conformational rhodopsin changes, and modifies the activity of retinal enzymes. Biophysical and biochemical properties of DHA modulate photoreceptor membrane function by altering permeability, fluidity, thickness, and lipid phase properties (SanGiovanni and Chew, 2005). DHA-containing glycerophospholipids enhance the formation of the active metarhodopsin II conformation of photoactivated rhodopsin.

Studies on the reconstitution of visual signal transduction system components (rhodopsin, G protein, and phosphodiesterase) indicate that DHA is required for efficient coupling between receptor and G protein, and an equimolar mixture of phosphatidylethanolamine and phosphatidylcholine containing 50 mol% docosahexaenoyl chains results in optimal photochemical function (Wiedmann et al., 1988; Mitchell et al., 2003).

In retina, DHA also modulates gene expression, cellular differentiation, and cellular survival. DHA activates a number of nuclear hormone receptors including the PPARα and the retinoid X receptor. Interactions of DHA with PPARα prevent endothelial cell dysfunction and vascular remodeling through inhibition of vascular smooth muscle cell proliferation, inducible nitric oxide synthase production, interleukin-1-induced COX-2 production, and thrombin-induced endothelin 1 production. DHA also modulates the production and activation of angiogenic growth factors, eicosanoids. Changes in DHA levels alter the electro-retinogram and visual acuity tests in human and nonhuman primates (Connor et al., 1990). Thus, DHA-mediated changes in neural membrane fatty acid composition may facilitate the restoration of many membrane properties such as membrane fluidity, permeability, receptor affinities, ion fluxes, and activities of membrane-bound enzymes.

5.3.11 DHA-Mediated Modulation of Nociception (Pain)

Nociception is a complex process. It is characterized by peripheral and central mechanisms (Svensson and Yaksh, 2002; Broom et al., 2004). The induction of nociception is accompanied by an increase in the expression of COX-2 activity and generation of PGI_2 and PGE_2 (Svensson and Yaksh, 2002). Intrathecal or local injections of $PLA_2$and COX-2 inhibitors prevent the development of nociception (Yeo et al., 2004; Phillis et al., 2006).EPA and DHA interact directly with transient

receptor potential cation channel V1 (TRPV1), an ion channel expressed in nociceptive neurons and brain (Matta et al., 2007). It is subject to polymodal activation via various agents including capsaicin, noxious heat, low extracellular pH, and direct phosphorylation by PKC. ARA-derived LOX and EPOX products directly activate TRPV1 and TRPV4, respectively. In contrast, TRPV3 is a thermosensitive channel with an intermediate temperature threshold of 31–39°C. EPA and DHA not only activate TRPV1 in a phosphorylation-dependent manner but increase responses to extracellular protons, and displace binding of the ultrapotent TRPV1 ligand ^{3}H resiniferatoxin. In contrast to their agonistic properties, EPA and DHA competitively block the responses of vanilloid agonists at 1–10 nM. Among n–3 fatty acids, DHA exhibits highest efficacy as an agonist, whereas EPA and ALA are markedly more effective inhibitors (Matta et al., 2007). Similarly, EPA, but not DHA, profoundly blocks capsaicin-mediated pain behavior in mice. These effects are not related to membrane elasticity because Triton X-100, a non-ionic detergent, has very little effect on properties of TRPV1. Thus, EPA and DHA differentially modulate TRPV1, and this signaling may contribute to their biological effects. Collectively, these studies suggest that dietary supplementation with DHA can be most beneficial for the treatment of pain (Matta et al., 2007).

5.3.12 DHA, Plasma Membrane Targeting, and Raft Formation

Interactions between specific lipid anchors and membranes modulate membrane localization of lipidated cytosolic signaling proteins. Although very little is known about the regulatory role of membrane composition in lipidated protein membrane targeting, DHA has been shown to selectively inhibit plasma membrane targeting and increase the endomembrane localization of lipidated proteins that are cytoplasmic cargo in the exocytic pathway, without affecting the exocytic pathway itself (Seo et al., 2006). Lipidated proteins have different subcellular distribution profiles in membranes from various tissues, which contain a distinct membrane lipid composition (Chapkin et al., 2008). In addition, it is becoming increasingly evident that subcellular localization of proteins with a certain trafficking pathway can be selectively regulated by dietary manipulation. This form of regulated plasma membrane targeting of a selected subset of upstream signaling proteins may provide cells with the flexibility to coordinate the arrangement of signaling translators on the cell surface, allowing neural and non-neural systems to respond and adapt to the vagaries of an everchanging extracellular environment (Seo et al., 2006; Chapkin et al., 2008). Collectively, these studies indicate that membrane lipid composition modulates cell signaling by modulating intracellular trafficking and localization of membrane proteins, supporting a potential molecular mechanism for the health benefits of DHA (Farooqui, 2009).

5.4 Roles of EPA in Brain

Small amount of dietary EPA incorporates into neural membrane glycerophospholipids and supports neuronal function. Thus, neural membranes contain much more ARA than EPA. This makes the local synthesis of cerebral EPA-derived eicosanoids questionable. Levels of EPA in brain can be increased by feeding rats with EPA-supplemented diet. At present, information on the release of EPA from neural membrane glycerophospholipids is not available. However, it is proposed that calcium-independent phospholipase A_2 ($iPLA_2$) may be involved in EPA release (Fig. 5.7). It is well known that EPA competes with ARA for COXs and LOXs. The released EPA is metabolized to less active eicosanoids, which retard the action of ARA-derived eicosanoids. ARA-derived eicosanoids act through specific superficial or intracellular receptors called as the DP, EP, FP, IP, and TP receptors (Coleman et al., 1994). Eicosanoid receptors are typically G-protein-coupled receptors with seven transmembrane segments that have an extracellular amino terminus and an intracellular carboxyl terminus. These receptors are involved in the generation of cyclic AMP, diacylglycerol, and phosphatidyl 1,4,5-trisphosphate and the modulation of calcium ion influx. The binding of ARA-derived eicosanoids with eicosanoids receptors on astrocytes also regulates glutamate release in the

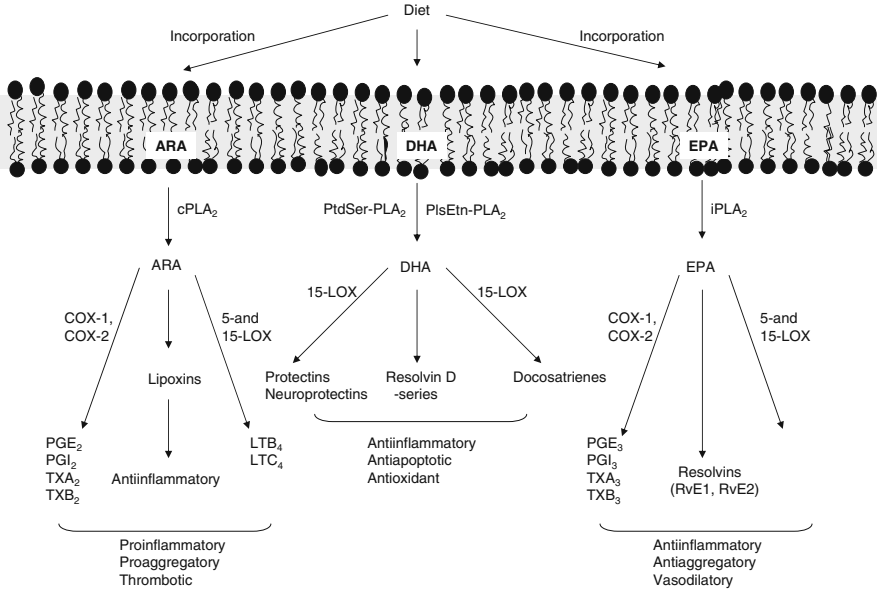

Fig. 5.7 Physiological properties of ARA, EPA, and DHA-derived lipid mediators. ARA-derived eicosanoids are PGE_2, PGI_2, LTB_4, LTC_4, TX_2, EPA-derived eicosanoids are PGI_3, PGE_3, LTB_5, LTC_5, LTE_5, TXA_3, and DHA-derived are protectins and docosatrienes. Lipoxins are ARA-derived antiinflammatory lipid mediators

synaptic cleft (Bazan, 2003; Farooqui et al., 2008). The released glutamate is associated with the modulation of neuronal excitability and synaptic transmission at the presynaptic level. In contrast, glutamate uptake by astrocytes avoids its potential neurotoxic accumulation in the synaptic cleft (Farooqui et al., 2008). Although the occurrence of 3-series PG and 5-series LT receptors in brain tissue has been reported in brain, very little is known about their affinities and interactions with DP, EP, FP, IP, and TP receptors (Coleman et al., 1994) and associated signal transduction processes that are less inflammatory.

5.4.1 EPA-Mediated Modulation of Inflammation and Immunity

COX and LOX-mediated oxidation of EPA generate less active eicosanoids (3-series PG and 5-series LT) (Figs. 5.1 and 5.4). EPA inhibits the release of ARA into neural membrane glycerophospholipids. It also inhibits PLA_2 activity of neuronal cultures in a dose- and time-dependent manner (Table 5.3), and this inhibition is reversed by bovine serum albumin, preventing the release of ARA and generation of ARA-derived eicosanoids. These processes decrease the production of PGE_2 and LTB_4 (Calder and Grimble, 2002; Calder, 2004, 2007). In non-neural cells, EPA incorporates into T cell membrane glycerophospholipids and produces changes in lipid homeostasis by shifting the metabolic pathways toward energy supply. EPA optimizes the function of immune cells. Through the modulation of inflammatory processes and immune cell activation, EPA induces positive effects on various states of immune deficiencies and diseases with hyper-inflammatory character (Fan et al., 2004; Grimm et al., 2002).

Table 5.3 Effect of EPA and DHA on partially purified $cPLA_2$ activity from bovine brain

Fatty acid concentration (μM)	$cPLA_2$ activity in the presence of DHA	$cPLA_2$ activity in the presence of EPA
Control	100±11	100±13
10	95±8	98± 6
20	90±7	97±5
50	60±8	80±6
75	50±7	60±7
100	45±5	55±5
100 + 0.25% bovine serum albumin	85±8	98±3
100 + 0.5% bovine serum albumin	92±7	97±4

Neuron-enriched culture extract is used as enzyme source, and enzymic activity is determined by the method of Hirashima et al. (1992). Specific activity is expressed as pmol/min/mg protein. Enzymic activity is expressed as relative activity (%).

Very little is known about the possible role of dendritic cells (DC) in the resolution of inflammatory processes. Treatment of DC with EPA-derived RvE_1 during differentiation results in apoptosis of activated T cells through

the induction and activity of indoleamine 2,3-dioxygenase (Vassiliou et al., 2008). The exposure of DC to RvE_1 promotes the maintenance of an immature chemokine receptor expression pattern even following TLR stimulation, with high CCR5 and no CCR7 expression. This observation indicates, in RvE1-treated DC cells, the presence of pathogens at the inflammatory site, instead of migrating to lymph nodes, and mediates apoptosis in effector T cells infiltrating the inflammatory site (Vassiliou et al., 2008). Collectively, these studies suggest that EPA modulates immunosuppressive and autoimmune effects in neural and non-neural tissues in humans and animals (Li et al., 2005).

5.4.2 EPA-Mediated Modulation of Depression

A growing number of observational and epidemiological studies indicate that low levels of $n-3$ fatty acids play a role in the pathophysiology of psychiatric disorders including Attention Deficit Hyperactivity Disorder (ADHD), Alzheimer disease (AD), schizophrenia, and depression. Supplementation studies, using individual or combination $\omega-3$ fatty acids, decrease symptoms of depression associated with some of these conditions (Logan, 2003; Peet and Strokes, 2005; Young and Conquer, 2005). Intake of purified EPA is known to significantly reduce blood pressure without altering heart rate during the 6-month treatment (Matsumura, 2007). EPA suppresses sympathetic nerve activity without inducing any parasympathetic nerve activity. EPA has been used to treat several psychiatric and neurodegenerative diseases due to its antiinflammatory and neuroprotective effects (Peet, 2003; Song and Zhaq, 2007). A total of six out of seven clinical trials have indicated that EPA significantly improves depressive symptoms when compared to either depressed subjects treated with DHA or with the placebo-treated populations. Several investigations have also reported that EPA could effectively treat schizophrenia. Thus, EPA treatment can reverse both the glycerophospholipid abnormalities and the cerebral atrophy in schizophrenia (Puri et al., 2000). Clinical trials also indicate that EPA is beneficial for the management of most symptoms of Huntington disease (HD), and a more extensive clinical investigation has demonstrated that EPA only improves motor functions (Song and Zhaq, 2007). The molecular mechanism associated with the beneficial effect of EPA currently remains unknown. However, recent evidence suggests that EPA-mediated improvement in motor function not only correlates with downregulation of ARA metabolism and reduction in prostaglandin-mediated signaling in schizophrenic subjects but is also related with alterations in NF-κB pathway signaling and decrease in brain-derived neurotrophic factor levels and/or alterations in blood flow to the brain tissue (Song and Zhaq, 2007; Murck and Manku, 2007; Stahl et al., 2008).

5.4.3 EPA-Mediated Modulation of Gene Expression

Few studies have been performed on the involvement of EPA in gene expression (Salvati et al., 2004, 2008). In C6 cell, EPA stimulates the expression of proteolipid protein (PLP) gene via cAMP-mediated pathways (Salvati et al., 2004). Intracerebroventricular injections of EPA or DHA into 2-day-old rats result in increase in PLP, myelin basic protein, and myelin oligodendrocyte protein mRNAs in all brain regions. This affect is more pronounced in EPA-treated rats than DHA-treated rats. The enhancement in PLP transcript levels is followed by an increase in PLP translation in EPA-treated rats. EPA-treated rats also show an increase in 2′-3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) protein levels, a further indication of accelerated myelinogenesis. In EPA-treated rats, upregulation of myelin genes expression coincides with a decrease of cAMP-response element-binding protein (CREB)-DNA binding in the cerebellum and cortex. The downregulation of CREB activity is caused by a decrease in the levels of CREB phosphorylation. EPA lowers the plasma cholesterol levels by suppressing gene expression of cholesterol biosynthesis enzymes and a cholesterol efflux protein from the liver (Sugiyama et al., 2008). Detailed investigations on differences between EPA-and DHA-mediated gene expression in brain have not been performed, but recent studies indicate that these fatty acids modulate many common genes that are involved in signal transduction, myelinogenesis, cell cycle, apoptosis, and cytokine synthesis. Collective evidence suggests that EPA and DHA act as immunomodulators and modulate many genes (Table 5.4).

Table 5.4 Roles of EPA in brain tissue

Role of EPA	References
Modulation of depression	Peet and Strokes (2005)
Modulation of immunity and inflammation	Calder (2004)
Modulation of gene expression	Salvati et al. (2004)
Modulation of enzymic activities	Li et al. (2007); Sohma et al. (2007)
Generation of resolvin	Dona et al. (2008)

Like neural cells, EPA and DHA modulate the expression of genes in non-neural cells (Jurkat cells and Raji cells). These genes include genes associated with signal transduction, cell survival, transcription factors, cell cycle, defense and repair, apoptosis, DNA synthesis, cell adhesion, cytoskeleton, hormone receptors, apoptosis, and cytokine synthesis. EPA and DHA differentially regulate functional parameters and gene expression in different types of non-neural cell. Expression of some genes is increased while others decreased (Verlengia et al., 2004a,b). Intensity of expression may vary considerably with neural and non-neural cells. Among neural cells, during myelinogenesis oligodendrocytes may respond to EPA more intensely than DHA (Salvati et. al., 2004). In non-neural cells, the extent of cell proliferation through upregulation

of cyclin-dependent kinase 10 and GI/S-specific cyclin E for EPA is more than DHA (Verlengia et al., 2004a,b). DHA causes more pronounced inhibition of mesanglial cell proliferation than EPA through the downregulation of extracellular signal-regulated kinase and cyclin E activity and induction of the cell cycle inhibitor p21 expression (Yusufi et al., 2003). Furthermore, DHA inhibits osteoclast differentiation more potently than EPA (Rahman et al., 2008). The differential potential closely correlate with the inhibition of osteoclast-specific genes like tartrate-resistant acid phosphatase, cathepsin K, calcitonin receptor, matrix metalloproteinase-9 expression and osteoclast-specific transcription factor, c-Fos, as well as osteotropic proinflammatory cytokine, TNF-α, to a greater extent with DHA than EPA. Pretreatment of RAW 264.7 cells with DHA also reduces the activation of NF-κB and p38MAPK than EPA (Rahman et al., 2008). Thus, DHA is much more effective than EPA in alleviating RANKL-induced proinflammatory cytokine production, intracellular signaling activation resulting in decreased osteoclast activation and bone resorption (Rahman et al., 2008). Accumulating evidence indicates that significant differences are observed between the effects of EPA and DHA, but it may be an oversimplification to generalize the effects of $n-3$ fatty acids on neural and non-neural cells and more studies are required on this important topic.

5.4.4 EPA-Mediated Modulation of Enzymic Activities

Like DHA, EPA also modulates several enzymic activities (Table 5.4). It inhibits $cPLA_2$and COX-2 activities of neuronal cultures. It protects hippocampus from age-related and irradiation-mediated changes that lead to impairment in synaptic function. This may be due to its anti-inflammatory effects, specifically preventing changes induced by the proinflammatory cytokine, interleukin-1beta (IL-1β). It is suggested that neuroprotective effect of EPA may be dependent on its ability to inhibit the downstream consequences of JNK activation (Lonergan et al., 2004). Rho-kinase (ROK)-mediated Ca^{2+} sensitization of vascular smooth muscle contraction plays a pivotal role in cerebral vasospasm. This Rho-kinase (ROK)-mediated Ca^{2+} sensitization is inhibited by EPA through the selective inactivation of Src family protein tyrosine kinases (Src-PTK) (Shirao et al., 2008). EPA prevents lipopolysaccharide-stimulated DNA binding of activator protein-1 and *c*-Jun N-terminal kinase activity (Zhao and Chen, 2005). It activates PKA (Szentandrassy et al., 2007) and inhibits UV-induced MMP-1 expression in human dermal fibroblasts (Kim et al., 2005).

5.4.5 EPA and Generation of Resolvin E_1

Resolvin E_1 (RvE_1; 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid; RvE_1) and resolvin E_2 (5S,18-dihydroxy-eicosapentaenoic acid; RvE_2)

(Fig. 5.4) are EPA-derived lipid mediators. RvE1 is synthesized during resolution of inflammation and in human vasculature via leukocyte–endothelial cell interactions. It possesses antiinflammatory and proresolving properties (Dona et al., 2008). As stated in Chapter 4, RvE_1 induces partial calcium mobilization and retards subsequent stimulation by LTB_4. RvE_1 also attenuates LTB4-mediated NF-κB stimulation in BLT_1-transfected cells. In vivo RvE_1 synthesis is downregulated in BLT_1 knockout mice. In contrast, at higher concentrations RvE_1 blocks PMN infiltration in a BLT_1-independent manner (Arita et al., 2007). RvE_1 also bind with ChemR23 receptors. Treatment of dendritic cells with small interference RNA specific for ChemR23 prevents RvE_1-mediated regulation of IL-12, indicating novel counterregulatory responses in inflammation initiated by EPA-derived RvE_1 and its receptor. In light of the interaction between RvE_1 and leukotrieneB_4(LTB_4) and their potential impaired immunity regulation hematostasis, it is proposed that RvE_1 may play an antiatherosclerosis and plaque stabilization role through its pro-resolution effect (Wu et al., 2008). The anti-inflammatory effect of RvE_1 can be realized by blocking LTB_4/BLT_1 (receptor of LTB4) pathway. Collectively, these studies suggest that RvE_1 binds to BLT_1 as a partial agonist, potentially serving as a local damper of BLT_1 signals on leukocytes along with other receptors (e.g., ChemR23-mediated counterregulatory actions) to mediate the resolution of inflammation (Arita et al., 2006, 2007; Wu et al., 2008).

Human whole blood RvE_1 regulates leukocyte expression of adhesion molecules. RvE_1 stimulates L-selectin shedding, while reducing CD18 expression in both neutrophils and monocytes. When added to whole blood, RvE1 neither affects ROS production in neutrophils or monocytes nor stimulates cytokine/chemokine production in heparinized blood (Dona et al., 2008). RvE_1 rapidly decreases leukocyte rolling in mice blood. In human platelet-rich plasma, RvE_1 selectively inhibits both ADP-stimulated and thromboxane receptor agonist, U46619-stimulated platelet aggregation, in a concentration-dependent manner. In contrast, Δ^6,14-trans-RvE_1 isomer is inactive. RvE_1 has no effect on collagen-stimulated aggregation. Collective evidence suggests that RvE_1 is a potent modulator of leukocytes as well as selective platelet responses in blood and platelet-rich plasma, respectively. It is proposed that novel agonist-specific antiplatelet actions of RvE_1 are potent and may underlie some of the beneficial actions of EPA in humans (Dona et al., 2008).

RvE_1 is metabolized to several metabolites not only in blood but in several tissues including spleen, kidney, and liver. Newly discovered RvE_1 products include 19-hydroxy-RvE_1, 20-carboxy-RvE_1, 18-oxo-RvE_1, and 10,11-dihydro-RvE (Hong et al., 2008). Presence of these metabolites indicates that both $\omega-1$ hydroxylation and reduction of conjugated double bonds in RvE_1 are associated with RvE_1 metabolism in mammalian tissues. It is interesting to note that 19-hydroxy-RvE1, 20-carboxy-RvE1, 18-oxo-RvE_1, and 10,11-dihydro-RvE_1 show reduced bioactivity in vivo. It is proposed that during inflammation coordinated action of RvE_1 and its metabolic products may facilitate homeostasis by regulating resolution, and RvE_1 metabolome may serve as a biomarker of these processes (Hong et al., 2008).

RvE_2 is generated by human recombinant 5-LOX, and is a common precursor of E series resolvins, namely 18-HEPE. During catalysis, the initial 5-hydroperoxide intermediate is also converted to a 5(6)-epoxide intermediate in RvE_1 formation (Tjonahen et al., 2006). It also blocks zymosan-induced PMN leukocyte infiltration, and possesses potent antiinflammatory properties in murine peritonitis (Tjonahen et al., 2006). Collectively, these studies suggest that RvE_2, together with RvE_1, may contribute to the beneficial actions of $\omega-3$ fatty acids in human diseases (Tjonahen et al., 2006).

5.4.6 EPA-Mediated Modulation of Lipid Rafts

Very little is known about the incorporation of EPA into neural membranes. Studies on the incorporation of EPA in cultured human Jurkat E6-1 T cells indicate that EPA treatment alters glycerophospholipid composition, resulting in a considerable increase of unsaturated fatty acyl chains in glycerophospholipid rafts from EPA-treated T cells compared with control cells (Li et al., 2006). Effective incorporation of EPA to rafts is not only in the exoplasmic but also in the cytoplasmic membrane glycerophospholipid leaflet. Thus, it is likely that by altering lipid raft lipid microenvironment, EPA incorporation may modulate T cell function. Similarly, treatment of intestinal membrane epithelial cells with EPA results in enrichment of unsaturated fatty acyl chains in microdomains of tight junctions. It is proposed that EPA may contribute to the activity of intestinal tight junction barrier (Zhao et al., 2008).

5.4.7 Other Roles of EPA

Studies on the effect of EPA on platelet-activating factor (PAF) have been controversial. Treatment of human monocytes monolayers with EPA at the optimal concentration of 1 μg/ml decreases PAF generation by 28%. However, treatment of monocyte monolayers with DHA has no appreciable affect on PAF generation (Sperling et al., 1987). Further studies have shown that 3 weeks of dietary supplementation with MaxEPA (18 g) daily produces no effect on the PAF generation in calcium ionophore-stimulated human monocyte monolayers. However, 6 weeks of dietary supplementation results in a 47% decrease of total PAF generation, suggesting that EPA downregulates PAF metabolism. It is proposed that dietary EPA modulates monocyte function in healthy volunteers through (a) suppression of LTB_4 synthesis, (b) suppression of PAF synthesis, and (c) delayed kinetics of inhibition of PAF generation and ARA metabolism relative to other cellular lipid alteration (Sperling, 1991). In contrast to above studies, investigations on PAF biosynthesis in neutrophils from individuals on a fish oil-enriched diet and in mast cells enriched with EPA in vitro have shown that EPA incorporates into a PAF precursor pool. However,

this incorporation does not inhibit PAF production because PLA_2 can use EPA- as well as ARA-containing glycerophospholipids in the initial step of PAF biosynthesis (Triggiani et al., 1990).

5.5 Conclusion

EPA and DHA are essential fatty acids that belong to $n-3$ fatty acid family. These fatty acids differ from each other in their metabolism as well as in their neurochemical effects.

EPA is metabolized to 3-series prostaglandins, thromboxanes, and 5-series leukotrienes by COXs and 5-LOX. Metabolism of EPA also generates RvE_1 and RvE_2. In contrast, DHA is not a substrate for COX. It is oxidized by 15-LOX-like enzyme that produces neuroprotectins and docosatrienes. DHA generates RvD series resolvins. EPA- and DHA-derived lipid mediators reduce the risk for cardiovascular and cerebrovascular diseases through their antiinflammatory, antithrombotic, antiaggregatory, antiapoptotic, and antioxidant effects. In cardiovascular and cerebrovascular systems, EPA- and DHA-derived lipid mediators also reduce plasma viscosity, enhance fibrinolysis, and improve vascular endothelial cell function by increasing the availability of nitric oxide, reducing the expression of endothelial cells adhesion molecules, inhibiting smooth muscle cells migration and proliferation, and reducing blood pressure. In brain tissue, EPA competes with ARA for COXs and LOXs, and decreases the generation of ARA-derived lipid mediators. In contrast, DHA-derived lipid mediators produce neuroprotective, antiapoptotic, and antioxidant effects. In addition, $n-3$ dietary fatty acids regulate the expression of numerous genes, either positively or negatively. Such nutrient–gene interactions have important effects on cell metabolism, differentiation and growth, and ultimately on disease processes.

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Chapter 6
Status of Docosahexaenoic Acid Levels in Aging and Consequences of Docosahexaenoic Acid Deficiency in Normal Brain

6.1 Introduction

Aging is defined as a time-dependent progressive functional impairment process that leads to mortality. The most prominent characteristics of aging include the progressive loss of physiological capability, decrease in ability to respond adaptively to environmental stimuli, increased susceptibility to diseases, and increased mortality. These changes are translated into decrements in neuronal functioning accompanied by behavioral declines, such as decreases in motor and cognitive performance, in both humans and animals (Joseph et al., 2005). Thus, biological aging is a progressive, endogenous, irreversible, and deleterious and highly conserved process that can be modulated by diet, environment, and genes (Spindler 2005; Spindler and Dhahbi, 2007). Many theories have been advanced to explain aging, but the biological mechanisms that underlie aging are still unknown. Major theories of aging include increase in free radical-mediated oxidative stress and changes in gene expression (Harman, 1981; Helfand and Rogina, 2000; Hulbert et al., 2007). Aging produces several changes in human brain. Brain aging is a complex process that involves many independent and interrelated neurochemical factors which cause alterations in neural membrane composition, resulting in gradual loss of neurons in different brain regions (Yehuda et al., 2002; Farooqui and Farooqui, 2009). The ability of brain to create new synaptic connections is significantly diminished in aged brain. Human brain shrinks with aging. The decrease in weight and volume in the aging brain is probably due to the loss of neurons and myelinated axons (Conde and Streit, 2006). Histological studies demonstrate a decrease in myelin density and in the number of myelinated fibers during aging (Peters, 2002). Thus, changes in brain white matter are prominent features of the aging brain (Toews and Horrocks, 1976). This is accompanied by alterations in glycerophospholipid composition of neural plasma membrane, glial cell activation, and reorganization of the molecular components of the node of Ranvier (Hinman et al., 2008). Integrity of myelin and plasma membranes, which are composed of PUFA-containing glycerophospholipids, sphingolipids, cholesterol, and proteins, is very important for optimal function of neural cell body and axons in the brain. Breakdown of myelin results in disintegration of many brain functions. Dietary essential fatty acids (LA, ALA, ARA, EPA, and DHA) play an important role in maintaining the

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stability and functions of myelin and plasma membranes. Large amounts of DHA accumulate in the brain gray matter and in visual elements of the retina during the development. Deficiency of essential fatty acids in neonatal period not only delays myelinogenesis, producing impairment in learning and memory, motor, vision, and auditory abnormalities, but also decreases membrane fluidity and affects activities of membrane-bound enzymes and ion channels (Table 6.1) (Salvati et al., 2000;

Table 6.1 Effect of DHA deficiency on glucose uptake, neurotransmitter release, and neuron size in brain

Neurotransmitter	Brain region	Effect	References
Dopamine receptor	Hippocampus	Decreased	Kuperstein et al. (2008)
Cholinergic	Frontal cortex and hippocampus	Decreased	Aids et al. (2003)
Serotonin	Hippocampus	Increased	Kodas et al. (2004)
Glutamatergic	Hippocampus and forebrain	Decreased	Dyall et al. (2007)
Glutamate transporter	Kuperstein et al. (2008)	Increased	Berry et al. (2005)
Glucose uptake	Kuperstein et al. (2008)	Decreased	Ximenes da Silva et al. (2002); Pifferi et al. (2007)
Glucose transporter	Olfactory bulb and neocortex	Decreased	Hichami et al. (2007)
Membrane fluidity	Neural membranes	Increased	Hashimoto et al. (2006)
Neuron body size	Hippocampus	Decreased	Ahmad et al. (2002a,b)

Stockard et al., 2000). Studies on the effect of low- and high-DHA diet in senescence-accelerated prone 8 (SAMP8) mice indicate that after 8 weeks of feeding DHA-enriched diet, SAMP8 mice show improvement in acquisition and retention in a T-maze foot shock avoidance test and a higher proportion of DHA in hippocampal and amygdala glycerophospholipids (Petursdottir et al., 2008), suggesting that, in mature animals, DHA is incorporated into brain glycerophospholipids and that dietary DHA may be associated with delay in cognitive decline (Petursdottir et al., 2008).

Autopsy and neuroimaging studies have indicated that during aging process changes in white matter are more prominent than cortical changes, at least during certain segments of the age span and in certain regions of the brain (Knight, 2000). Aging produces changes in glycerophospholipid–fatty acid composition, but no significant changes in the content of different glycerophospholipid classes have been observed, except for sphingomyelin, phosphatidic acid, plasmalogens, and cardiolipin (Horrocks et al., 1981; Giusto et al., 1992, 2002; Farooqui and Farooqui, 2009). An increase in microglial activation has also been reported in several brain regions, including hippocampus, during aging (Finch and Cohen, 1997). Possible mechanisms may include microglial reaction to increased production of lipid mediators, oxidation of oxidized

proteins and nucleic acids, and generation of advanced glycation end products (AGEPs) (Morgan et al., 1999). AGEPs activate NFκB in cell of monocyte lineage and induce transcription of proinflammatory cytokines, which accentuate the inflammatory reaction (May and Ghosh, 1998). It has also been proposed that age-dependent gene expression may represent a decline of regulatory function in the organism, resulting in disruption of metabolic homeostasis (Mattson, 2002; Mocchegiani et al., 2006). The fatty acid composition of cell membrane glycerophospholipids varies considerably among species, and may be responsible for variation in their metabolic rate.

Underlying causes of specie differences in maximum life span are unknown. Maintenance of membrane lipid asymmetry and differential vulnerability of PUFAs in membrane glycerophospholipids to peroxidation have been proposed to play important roles (Balasubramanian and Schroit, 2003; Hulbert, 2005). High amounts of PUFAs in neural and non-neural cells and loss of membrane asymmetry impair the lifespan due to an increase in the susceptibility of membranes to lipid peroxidation and its damaging effect on cellular molecules. Also, in vitro studies indicate that PUFAs elevate basal metabolic rate. Thus, differences in the characteristic maximum life span of species can be explained by differences in fatty acid composition and susceptibility of these fatty acids to lipid peroxidation and rate of generation of ROS (Hulbert, 2005; Hulbert et al., 2007). ROS attack lipids, proteins, and nucleic acids. ROS cross-link intracellular proteins and generate ceroid pigment, age pigment, or lipofuscin that accumulates in postmitotic tissues. Age-mediated ROS generation involves an increase in oxidation of DNA and RNA in the cerebellum of male rats (Cui et al., 2007). Oxidation of DNA results not only in generation of 8-hydroxy deoxyguanosine but also DNA protein cross-links in discrete brain regions of young and aged rats.

Furthermore, ROS indirectly contribute to brain damage by activating a number of cellular pathways resulting in the expression of stress-sensitive genes. Moreover, age-related ROS generation upregulates the mechanism that induces glial cell-mediated inflammation. It should be noted that aging and longevity are affected not only by strong genetic component but also by life style and environmental factors.

6.2 DHA and ARA Entry and Metabolism in Developing Brain

Developing brain has requirement for *n*–3/*n*–6 fatty acids, and DHA in particular is essential for the development of the brain and retina. Initially, developing brain cannot synthesize DHA and ARA from its precursors, but it develops capacity to synthesize DHA and ARA as a function of gestational age. At this stage, DHA and ARA and their precursors (ALA and LA) are transported from mother into fetal circulation across placenta. Thus, levels of these fatty acids in fetus depend upon essential fatty acid status of maternal diet (Rump et al., 2001). The molecular mechanism associated with the transfer of DHA and ARA to

fetus is not fully understood. However, several studies suggest that various fatty acid transporters and binding proteins with the preferential DHA transfer and different lipolytic enzymes may play an important role. In addition to the above route, a new route of DHA entry into fetus has also been described. This route involves the gastrointestinal tract to supply DHA to the brain (Green and Yavin, 1995). The postnatal period is critical for the accumulation of PUFAs, which are obtained from mother's milk. Developing brain utilizes considerable amounts of PUFAs for the synthesis of neural plasma membranes. In humans, levels of DHA and ARA in plasma and red blood cells rise with intake of dietary DHA and ARA. DHA levels drop across most CNS structures in neonates consuming formulas with no DHA, but ARA levels are relatively immune to ARA in the diet and supplementation of infant formula with DHA is effective in restoring DHA levels in structures other than the cerebral cortex. These studies on perinatal long-chain PUFA nutrition emphasize the importance of DHA and ARA in the developing brain. Thus, both *n*–6 and *n*–3 PUFAs are needed for neuronal growth, development of synaptic processing of neural cell interaction, and expression of genes regulating cell differentiation and growth. The fetus and placenta are dependent on maternal *n*–6 and *n*–3 fatty acid supply for their growth and development. A balanced intake of *n*–6 and *n*–3 fatty acids is necessary for optimal brain development, and *n*–6 to *n*–3 fatty acid imbalance early in life may lead to persistent reductions in DHA levels in glycerophospholipids in the brain even after long-term *n*–3 fatty acid repletion (Uauy and Dangour, 2006). This may adversely affect the offspring's neural transmission times and postnatal thriving. Similarly, excessive consumption of *n*–3 fatty acids during pregnancy and lactation adversely affect fetal and infant development and the auditory brainstem response, a measure of brain development and sensory function (Church et al., 2008, 2009). Collective evidence suggests that consuming either large or inadequate amounts of *n*–3 fatty acids during pregnancy and lactation may not be proper because of the potential for adverse effects on infant development.

Although, postnatal accretion of long-chain polyenoic fatty acids in cerebellum, frontal and occipital brain lobes is initially low, but the rate of fatty acid accretion increases rapidly as brain weight and fat content of brain increase; and after a 4-week period accretion of long-chain fatty acids is proportional to the increase in brain weight (Clandinin et al., 1980). In the fetal rat brain, a marked accumulation of DHA occurs during the last 3 days of gestation, prior to synaptogenesis (Green and Yavin, 1996, 1998). Under normal conditions, almost 75% of the intrauterine brain DHA accretion takes place in the final days of pregnancy, constituting a "DHA accretion spurt." This "DHA accretion spurt" coincides with the peak of neurogenesis in the rat fetus (Clandinin, 1995). Although fetal brain and liver can synthesize small amount of DHA from its precursors, relative contribution and significance of endogenously synthesized DHA to "DHA accretion spurt" remains unknown. Under stress conditions, "DHA accretion spurt" may produce harmful effect on brain development because of an increase in multiple double-bond targets for lipid peroxidation.

The "DHA accretion spurt" is supported by the maternal supply of DHA or its precursor. Under maternal dietary *n*–3 fatty acid deficiency, DHA content in the fetal brain can be restored by direct intraamniotic injection of millimolar concentrations of ethyl-DHA. This approach may hold a potential advantage in the event of maternal–fetal insufficiency, a stress that may cause intrauterine growth retardation (Yavin et al., 2001). DHA enters into fetal brain in several forms, including coupled with plasma proteins, esterified into glycerophospholipids and triacylglycerols that are present in lipoproteins, attached with fatty acid-binding proteins (Sastry, 1985; Scott and Bazan, 1989; Sellner, 1993; Green and Yavin, 1998). Conversion of DHA into docosahexaenoyl-CoA is catalyzed by docosahexaenoyl-CoA synthetase. This step functions as an intracellular trapping mechanism for DHA (Bazan et al., 1986). The activity of docosahexaenoyl-CoA synthetase correlates not only with the DHA contents of developing brain but also with the levels of DHA in various brain regions and subcellular organelles. Acyl-CoA:lysophospholipid acyltransferase catalyzes the transfer of docosahexaenoyl moiety to a 1-acyllysophospholipid, and thus facilitates the incorporation of DHA into aminoglycerophospholipids such as ethanolamine plasmalogen (PlsEtn) and phosphatidylserine (PtdSer), and therefore it is highly prevalent at the cytofacial site of the plasma membrane where it may specifically participate in intracellular events.

6.3 Alterations in DHA Levels in Various Regions During Aging

As stated earlier, *n*–3 fatty acids are essential dietary nutrients necessary for normal brain growth and function. Inadequate intakes of *n*–3 fatty acids results in the deficiency of DHA and increase in *n*–6 fatty acids in the brain. Deficiency of DHA in the developing brain leads to deficits in neurogenesis, neurotransmitter metabolism, and alterations in learning and memory and visual function in animals. Western diet is deficient in *n*–3 fatty acids. The DHA status of the newborn and breastfed infant depend on the maternal intake of DHA and varies widely (Innis, 2000, 2008). Intervention studies indicate that improvement in maternal DHA levels decrease the risk of poor infant and child visual and neural development. Thus, maternal fatty acid status is important for DHA transfer from mother to the infant before and after birth, with short- and long-term implications for neural function (Innis, 2000, 2008).

The effects of age-related oxidative damage on PUFA content in neural membrane glycerophospholipids in hippocampus and amygdala of 2-, 4-, 12-, and 18-month-old SAMP8 mice indicate that PUFA contents are significantly altered during aging. In comparison to the younger SAMP8 mice, the hippocampus of the 12-month-old mice contains lower proportions of DHA in PtdSer and PtdIns and higher proportions of ARA in PtdSer. Their amygdala has a lower proportion of ARA in PtdCho. In the hippocampus of the oldest age group, the proportions of DHA in PtdSer and ARA in PtdCho and PtdIns were higher than in the younger

age groups. Differences in PUFA content are related to α-tocopherol levels in different brain regions (Petursdottir et al., 2007).

Studies on fatty acid composition in various regions of neonatal and adult rat brain indicate that DHA and ARA are not distributed evenly in various regions of rat brain (Xiao et al., 2005). In weaning and adult rats, highest levels of DHA occur in cortex, whereas medulla has the lowest DHA levels. In the aged rats, cortex and cerebellum contain the highest DHA levels, whereas in neonatal rats the striatum contains highest levels of DHA, and the hypothalamus and hippocampus have the lowest levels of DHA (Xiao et al., 2005a). In contrast, highest levels of ARA are found in hippocampus whereas the medulla contains the lowest level except for the neonatal rats whose cerebellum, hypothalamus, striatum, and midbrain have lower ARA levels than hippocampus and cortex. Depletion of DHA depletion by feeding an *n*–3-deficient diet for two generations indicates that DHA is not proportionally depleted in various regions of rat brain. Cortex, hippocampus, striatum, cerebellum, and hypothalamus show DHA depletion by >71%, whereas the midbrain and medulla show only 64% and 57% DHA depletion, respectively. Feeding DHA-enriched diet results in the complete DHA recovery in all regions, except for the medulla where the recovery is only 62% (Xiao et al., 2005b). Supplementation of diet with fish oil during brain development and adulthood in normal rats produces a significant enhancement of both memories (Chung et al., 2008). Following dietary DHA repletion, the hippocampus and olfactory bulbs accumulate more DHA and are more resistant to dietary DHA deprivation, and show better DHA recovery than the visual cortex, frontal cortex, and cerebellum (Chung et al., 2008).

6.4 DHA in Plasmalogens and Phosphatidylserine

As stated earlier, DHA is selectively esterified in PlsEtn and PtdSer. These glycerophospholipids are associated with inner leaflet of lipid bilayer. In contrast, bulk of ARA-containing PtdCho is located in the outer leaflet of lipid bilayer. The physiological significance of this unusual distribution of lipid head groups, esterified fatty acids, and amide-linked fatty acids are unclear, but the glycerophospholipids and sphingolipids along with cholesterol are asymmetrically distributed between the two leaflets of the plasma membrane lipid bilayer and are likely to contribute to a dynamic lipid substructure associated with a variety of cellular functions (Yamaji-Hasegawa and Tsujimoto, 2006; Farooqui, 2009). Studies on comparisons of molecular species in the choline, ethanolamine, and serine glycerophospholipids in synaptosomal plasma membranes (SPM) and myelin from the cerebral cortex of adult rats indicate that SPM contain more DHA than myelin, and PtdCho in SPM contain more saturated fatty acids. Oleic acid content of all three lipid classes is much higher in myelin than in SPM, while ARA content is higher in ethanolamine glycerophospholipids and lower in serine

glycerophospholipids (Porcellati, 1983; Farooqui and Horrocks, 2007). PtdSer is synthesized from PtdEtn or PtdCho through the replacement of ethanolamine or choline by serine, respectively, in a base-exchange reaction catalyzed by base-exchange enzymes. Supplementation of neural cells with free DHA facilitates the synthesis and accumulation of PtdSer (Garcia et al., 1998, Hamilton et al., 2000, Kim and Hamilton 2000). This process promotes and maintains neuronal survival by translocating and activating Akt and Raf-1. The survival process under sub-optimal involves PIP_3 as survival signal. DHA-mediated Raf-1 translocation promotes neuronal differentiation, which is one of the downstream events of Raf-1 activation. The trophic action of DHA as a free fatty acid may also be important for neuronal differentiation, because DHA has been shown to be an endogenous ligand for the retinoid X receptor, a nuclear receptor that acts as a ligand-activated transcription factor (Lengqvist et al., 2004).

It is also stated that there is also a direct correlation between the level of PtdSer and DHA content, which varies by brain region, age, type of cell, and subcellular organelle (Wen and Kim 2004). Few studies have been performed on the glycerophospholipid composition of neural membranes from different regions of animal and human brains. In order to reconstruct a membrane composition found in vivo under physiological conditions in aging brain, one has to determine the glycerophospholipid composition expressed in terms of mol/surface area for a specific membrane in a particular subcellular organelle of each brain region. A major challenge for the future will be to investigate the composition of the lipid bilayers of different neuronal plasma membranes and identify the effects of DHA-containing glycerophospholipids on the ability of the plasma membranes to perform their many different functions (Farooqui and Horrocks, 2007).

Plasmalogens are vinyl ether (enol ether) linkage-containing glycerophospholipids. They contain a vinyl ether linkage at the *sn*-1 position with 16:0, 18:0, and 18:1 (*n*–7 and *n*–9) side-chains (alk-1-enyl groups), an ester bond linking ARA or DHA at the *sn*-2 position, and a phosphoethanolamine or phosphocholine group at the *sn*-3 position of the glycerol moiety (Farooqui et al., 2008). Levels of PlsEtn increase rapidly during the intense period of myelination, and there is an eightfold increase in PlsEtn levels per gram of brain tissue in white matter during the first year of life. In human brain, there is a steep rise in PlsEtn content, followed by a further rise up to 30 to 40 years of age (Toews and Horrocks, 1976). This is followed by a decline of PlsEtn levels during normal aging. At 70 years of age, the levels of PlsEtn are 18% less than at 40 years of age (Rouser and Yamamoto, 1968; Horrocks et al., 1981). Collectively, these studies suggest that DHA-containing plasmalogens are major glycerophospholipids in brain tissue. Their metabolism may be involved in signal transduction processes associated with neural cell functions such as synaptogenesis, myelination, and ion transport (Farooqui and Horrocks, 2001). Like PlsEtn, PtdSer is also found in inner leaflet of biomembranes. In contrast to PlsEtn, PtdSer is involved in regulation of neural receptors, modulation

of enzymic activities, apoptotic cell death, and neurotransmitter release (Garcia et al., 1998; Levi deStein et al., 1989).

6.4.1 DHA in Plasmalogens

Aging modulates dihydroxyacetone phosphate acyltransferase activity, the first enzyme of plasmalogen biosynthesis. Littermates from two generation of *n*–3-deficient rats fed with an equilibrated diet containing either α-linolenic acid alone or with two doses of DHA. After weaning, or 3, 9, or 21 months of diet, dihydroxyacetone phosphate acyltransferase activity and plasmalogen levels are markedly increased in rat brain at 3 month compared to age-matched brains from weaning. However, the enzymic activity is significantly decreased (30% and 40%) from 3 months to 9 and 21 months, respectively. Studies on determination of acyl compositions of PlsEtn, PtdEtn, and PtdSer in the frontal cortex and hippocampus from 2- and 18-month-old rats indicate that in 18-month-old rats, the fatty acid compositions of the above glycerophospholipids show an increase in monounsaturated fatty acid (18:1 *n*–9 and 20:1 *n*–9) and a decrease in PUFA (Favrelière et al., 2000). DHA is markedly decreased in hippocampus PtdEtn at 18 months. Hippocampus and frontal cortex undergo specific age-induced modifications in PlsEtn and PtdEtn acyl composition. It is proposed that decreased plasmalogen levels in aged rat brain may be not only caused by a reduced dihydroxyacetone phosphate acyltransferase activity but also due to increased activity of plasmalogen-selective phospholipase A_2 (André et al., 2005, 2006a,b). A deficiency of *n*–3 fatty acids in the diet may cause plasmalogen deficiency and abnormal signal transduction processes associated with synaptic membranes (Farooqui and Horrocks, 2001). Supplementation of fish oil-containing diet stabilizes synapse and normalizes signal transduction processes (Horrocks and Farooqui, 2004; Farooqui et al., 2008).

During brain development, plasmalogens play many roles (Fig. 6.1) (Lee, 1998; Nagan and Zoeller, 2001; Farooqui and Horrocks, 2001; Farooqui et al., 2008). Plasmalogens are major reservoir for ARA and DHA. Plasmalogens are not only involved in transport of ions across plasma membranes and membrane fusion but also provide protection to neural membranes against oxidative stress (Farooqui et al., 2008). They also participate in the efflux of cholesterol from cells (Mandel et al., 1998). Plasmalogens are also found in the nucleus where they are associated with cellular differentiation. The occurrence of plasmalogens in the synaptic cleft suggests that DHA-containing plasmalogens not only play an important role in synaptogenesis but may also be involved in vesicle formation during neurotransmitter release (Farooqui and Horrocks, 2001; Farooqui et al., 2008).

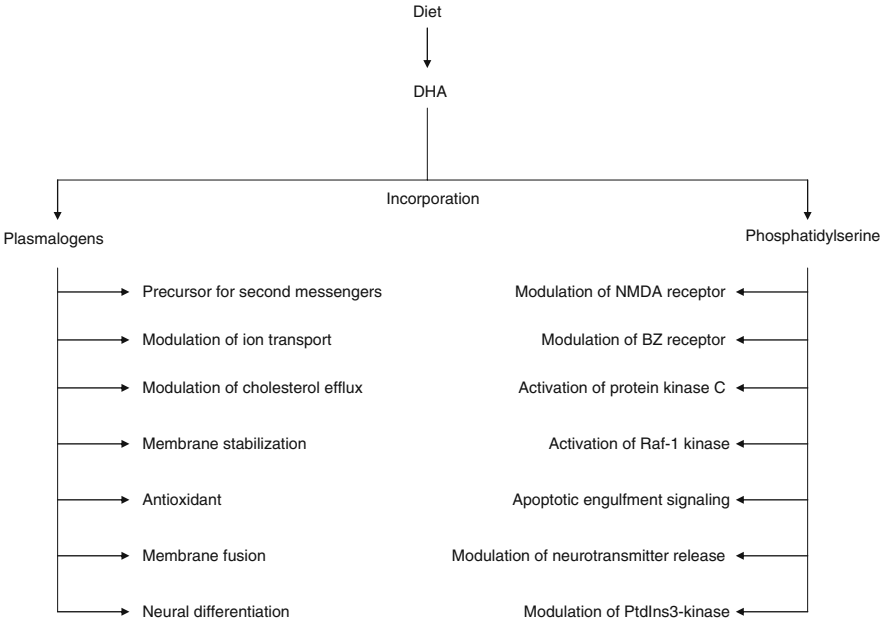

Fig. 6.1 Roles of ethanolamine plasmalogens and phosphatidylserine in the central nervous system

6.4.2 DHA in Phosphatidylserine

Neuronal membranes contain high levels of DHA-containing PtdSer (Garcia et al., 1998). Increase in PtdSer levels upon DHA enrichment is not a universal mechanism, but specific to neuronal cells (Guo et al., 2007). When cultured neural and non-neural cells are enriched with DHA, they show a marked enrichment of PtdSer metabolism. However, the increase of the total PtdSer levels only occurs in neural cells, whereas several PtdSer molecular species, such as 18:0, 18:1-PtdSer and 18:1, 18:1-PtdSer are significantly decreased in non-neuronal cells (Guo et al., 2007). DHA enrichment has no effect on mRNA levels of PtdSer synthase 1 (PSS1) and PtdSer synthase 2 (PSS2). Overexpression of genes encoding PSS1 or PSS2 has no affect on PtdSer level and DHA-mediated increase in PtdSer in neuronal cells. Based on these results, it is suggested that the increase in PtdSer is caused by DHA specifically in neuronal cells, and may represent a unique mechanism for expanding the PtdSer pool in mammalian brain (Guo et al., 2007).

PtdSer facilitates the translocation of protein kinases, such as PKC and Raf-1 kinase (Fig. 6.1). The translocation of Raf-1 kinase to plasma membrane is necessary for transducing growth factor-mediated signaling and neuronal survival (Ghosh et al., 1996; Yuryev and Wennogle, 1998; Akbar et al., 2005). In vivo reduction of DHA by dietary depletion of *n*–3 fatty acids decreases

hippocampal PtdSer and increases neuronal susceptibility to apoptosis in Neuro 2A cell cultures (Akbar et al., 2005). Although the replacement of DHA with DPA results in accumulation of DPA in neuronal membrane PtdSer during *n*–3 fatty acid deficiency compared to non-enriched control, the increase in DPA-containing PtdSer and translocation of Akt is significantly less than that observed with DHA enrichment. Like DHA, DPA enrichment also protects Neuro 2A cells against staurosporine-induced apoptosis, but to a lesser extent. Collective evidence suggests that DHA incorporation into PtdSer prevents staurosporine-mediated apoptosis by stimulating the PtdIns 3-kinase/Akt pathway and enhancing Raf-kinase translocation to the plasma membrane (Akbar et al., 2005; Kim et al., 2003). Studies on the effect of ethanol on PtdSer metabolism indicate that there is a direct correlation between the PtdSer levels and DHA content, which vary considerably with age, brain region, cell type, and subcellular organelles (Wen and Kim, 2004). Studies on the effect of ARA-, EPA-, and DHA-containing diacylglycerols (DAG) in transfected human Jurkat T cells indicate that three DAG molecular species bind to RasGRP, but only ARA- and DHA-containing DAG participate in the modulation of RasGRP-mediated activation of MAP kinases in Jurkat T cells (Madani et al., 2004). Collective evidence suggests that DHA-containing lipids produce different effects in neural and non-neural cells.

During the execution phase of apoptotic cell death, the asymmetric distribution of glycerophospholipid in lipid bilayer is lost due to the externalization of PtdSer from inner leaflet to the outer leaflet where it functions as a tag on the dying cell for recognition (eat me signal) and removal by phagocytosis (Fadok et al., 1992). Selective oxidation of PtdSer during apoptotic cell death precedes its externalization in plasma membrane, and is essential for the engulfment of apoptotic cells. It is not known whether PtdSer oxidation stimulates its externalization through its effects on aminophospholipid translocase activity or by enhanced PtdSer scrambling (Tvurina et al., 2004). In leukemia HL-60 cells and lymphoma Raji cells, both native PtdSer and oxidized PtdSer have similar affinity for aminophospholipid translocase, but oxidized PtdSer does not inhibit aminophospholipid translocase activity. It is proposed that oxidized PtdSer through its "non-enzymic scramblase" action contribute to PtdSer externalization (Tvurina et al., 2004). Experimentally, the externalization of PtdSer on a cell surface is visualized by fluorescent conjugate of annexin V (Zhang et al., 1997). In CNS, interactions between microglial cells and PtdSer-expressing apoptotic neurons mediate the activation of specific PtdSer receptor (PtdSerR), which are expressed both by resting and activated microglial cells. This process may be relevant to the removal of dead neurons without lysis (De Simone et al., 2004). PtdSer also binds with PtdSer-binding proteins, which are substrates of PKC (Gustincich et al., 1999). PtdSer also interacts with neuronal spectrin (fodrin), a cytoskeletal protein that has multiple binding sites (An et al., 2005). α-Spectrin and β-spectrin bind to PtdSer with high-affinity binding sites that are located within 8 of the 38 triple-helical structural repeats (An et al., 2004). Binding of spectrin dimers to PtdSer liposomes is inhibited by

single repeats containing PtdSer-binding site. PtdSer-binding sites in β-spectrin are grouped in close proximity to the sites of attachment both of ankyrin and of 4.1R, the proteins engaged in attachment of spectrin to the membrane. It is proposed that direct interaction of spectrin with PtdSer in the membrane may modulate its interactions with the proteins associated with formation of PtdSer-rich lipid domains, which may be involved in the red cell membrane functioning. PtdSer promotes neurotransmitter release and normalizes brain function in aging rats (Casamenti et al., 1991). It modulates glutamate and benzodiazepine receptors and influences synaptic efficiency (Cohen and Muller, 1992; Gagne et al., 1996; Buratta et al., 2004; Levi deStein et al., 1989). In platelets membrane, PtdSer upregulates factor Xa, resulting in modulation of prothrombin activation through active prothrombinase (Lentz, 2003). Interactions of PtdSer with coagulation factor Va induce conformational changes required for the activation of this protein. Other proteins that interact with PtdSer include Na^+,K^+-ATPase, synaptotagmin, nitric oxide synthase, diacylglycerol kinase, and neutral sphingomyelinase (Stekhoven et al., 1994; Hofmann et al., 2000). Accumulating evidence suggests that involvement of PtdSer is a key event in the control of blood coagulation.

Aging is accompanied by changes in fatty acid composition of neural membrane PtdSer and PtdEtn. The most striking alterations with respect to fatty acyl composition include a reduction in PUFAs (ARA and DHA) and an increase in monounsaturated fatty acids (oleic acid), mainly in ethanolamine and serine glycerophospholipids. Aging also produces alterations in the activity of the enzymes associated with phospholipid headgroup modification (Giusto et al., 2002; Ilincheta de Boschero et al., 2000; Tam and Innis, 2006). Serine incorporation into phosphatidylserine through base-exchange reactions and phosphatidylcholine synthesis through phosphatidylethanolamine methylation increases in the aged brain. Collective evidence suggests that age-associated fatty acid alterations in neural membrane composition may produce changes in synaptic efficiency of aged brain (Giusto et al., 2002; Salvador et al., 2002). Furthermore, maternal dietary *n*–3 fatty acid deprivation not only impairs fetal brain DHA accretion and PtdSer metabolism, but also alters PtdSer metabolism and release of lipid mediators and neurotransmitters that are important in brain function (Giusto et al., 2002; Tam and Innis, 2006).

6.5 Consequences of DHA Deficiency in Brain

The maintenance of optimal cognitive function is a central feature of healthy aging. Feeding an *n*–3-deficient diet for two generations produces differential DHA deficiency by 39–63% in the seven brain regions, including cerebellum, medulla, hypothalamus, striatum, hippocampus, cortex, and midbrain (Xiao et al., 2005). This depletion of DHA not only results in alterations in surface charge and physicochemical properties of neural membrane but also alters

activities of enzymes and induces abnormal receptor function. Collectively, these studies suggest that DHA deficiency may cause cognitive decline in the elderly (Fig. 6.2). Dietary DHA supplementation reverses age-related changes in synaptic function. Lipidomic analyses of glycerophospholipid species in cortical tissue of young (2–4 months) and aged (20–22 months), control- and DHA-treated (10 mg daily) rats following treatment for 8 weeks indicate that DHA supplementation normalizes the age-related decrease in unsaturation index, reduces the levels of ARA-containing glycerophospholipids in both young and aged animals, and facilitates the synthesis of new PtdSer and PtdIns species (Little et al., 2007). It is suggested that DHA produces alterations in the membrane lipid composition, which can consequently affect the production of proinflammatory mediators and signaling molecular species. Perinatal DHA deficiency selectively reduces myo-inositol levels in the adult rat prefrontal cortex, indicating that perinatal deficits in cortical DHA accrual significantly and selectively reduce myo-inositol concentrations and augment receptor-generated myo-inositol synthesis (McNamara et al., 2009). In addition, DHA provides neural membrane stability, modulates membrane fluidity, receptor function, and regulates activities of membrane-bound enzymes (Farooqui, 2009). These processes are essential for optimal functioning of neural membranes.

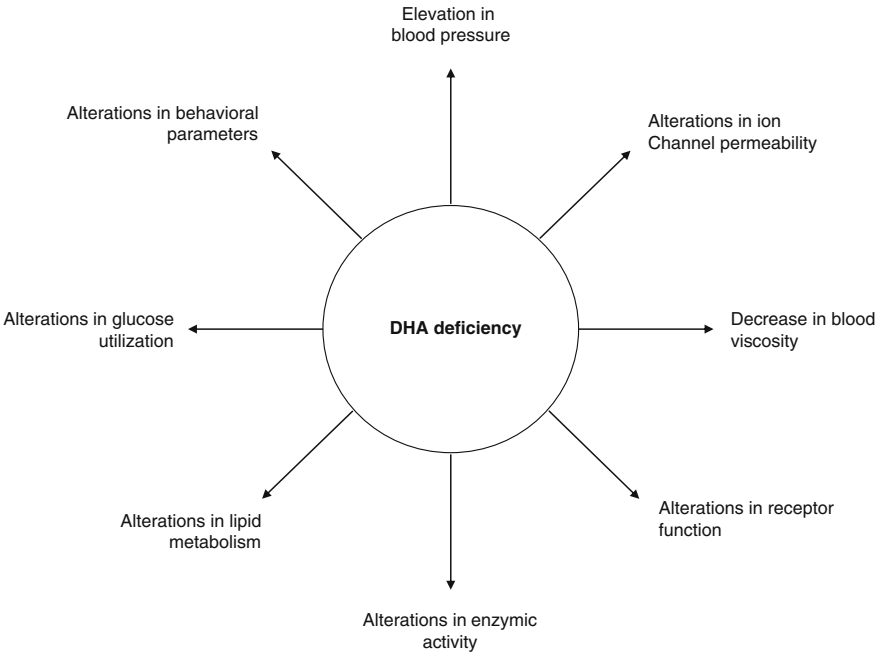

Fig. 6.2 Effect of DHA deprivation on neurochemical processes in the brain tissue

6.5.1 Effects of DHA Deficiency on Behavioral Parameters

As stated in Chapters 2 and 3, DHA is essential for normal growth and development of brain in animals and human. DHA deficiency produces a marked decrease (60–90%) in DHA content of brain (Ward et al., 1996). In brain, DHA deficiency results in impairment of learning and memory and cognitive behavior as judged by alterations in brightness discrimination learning test and Morris water maze task (Yoshida et al., 1997; Moriguchi et al., 2000, 2001). DHA contents and behavioral alterations can be restored by restituting a DHA-adequate diet. Similarly, the loss of brain DHA is associated with poorer performance in spatial tasks and an olfactory-cued reversal learning task. DHA-deficient animals made significantly more total errors in a 7-problem, 2-odor discrimination task compared to the *n*–3-adequate group. DHA contents of maternal diet also modulate auditory brainstem conduction times and the appearance of the auditory startle reflex in rat pups. Furthermore, the escape latency in the Morris water maze task test takes significantly longer time for the *n*–3-deficient rats compared to the *n*–3-adequate rats. No difference is observed in gross hippocampal morphology. DHA deficiency in the olfactory bulb, the piriform cortex, and the neocortex produces a mild olfactory learning impairment compared to control rats. Real-time PCR experiments reveal the expression of c-fos mRNA in all the three regions of the brain, whereas Gir and glucose transporter (GLUT1) mRNA are induced only in olfactory bulb and neocortex (Hichami et al., 2007). However, this increase is less marked in DHA-deficient rats. Taken together, these results indicate that the behavioral impairment in *n*–3-deficient rats is linked to DHA depletion in brain regions processing olfactory cues (Hichami et al., 2007). These studies indicate the importance of providing a source of *n*–3 fatty acids during mammalian growth and brain development (Salem et al., 2001), and indicate that rats with DHA deficiency in the central nervous system perform poorer in these tasks compared to rats with higher DHA levels, suggesting the deficits in presence of learning and memory and olfactory processing in DHA-deficient animals (Greiner et al., 1999).

6.5.2 Effects of DHA Deficiency on Glucose Utilization

DHA deficiency also induces alterations in regulation of energy metabolism in fronto-parietal cortex, hippocampus, and suprachiasmatic nucleus (Ximenes da Silva et al., 2002). DHA deficiency produces a 30% decrease in glucose uptake and a 20–40% decrease in cytochrome oxidase activity in the above brain regions. The DHA deficiency also causes changes in glucose transporters immunoreactivity, namely GLUT1 in endothelial cells and GLUT3 in neurons. In DHA-deficient rats, GLUT1-immunoreactivity

readily detectable in microvessels became sparse, whereas the number of GLUT3 immunoreactive neurons is increased. However, western blot analysis showed no significant difference in GLUT1 and GLUT3 protein levels between rats deficient in *n*–3 fatty acids and control rats. In addition, studies on GLUT1 expression in the cerebral cortex microvessels of rats fed different amounts of *n*–3 PUFA (low vs. adequate vs. high) indicate that endothelial GLUT1 immunoreactivity is significantly decreased (–23%) in the *n*–3 PUFA-deficient microvessels compared to control, whereas it increased (+35%) in the microvessels of rats fed the high *n*–3 PUFA diet. In addition, cytochalasin B-binding studies show that the maximum binding to GLUT1 (Bmax) is decreased in deficient rats. Supplementation of rat brain endothelial cells with DHA or EPA elevates the [^{3}H]-3-*O*-methylglucose uptake by 35% and 50%, respectively, while addition of exogenous ARA has no effect. It is proposed that DHA deficiency-mediated changes in glucose utilization and energy metabolism in endothelial cells of the blood–brain barrier are associated with glucose transporter, GLUT1 expression and activity involving posttranscriptional regulation of GLUT1 synthesis (Ximenes da Silva et al., 2002; Pifferi et al., 2007). Thus, low intake of DHA and alterations in glucose utilization are associated with several forms of cognitive decline in the elderly. Based on above studies, it can be speculated that DHA supports and maintains optimal brain function during aging.

6.5.3 Effects of DHA Deficiency on Lipid Metabolism

Although the molecular mechanisms associated with beneficial and harmful effects of DHA are not fully understood, many signal transduction processes associated with hippocampal region are upregulated or compromised. DHA deficiency not only decreases the size of neurons but also decreases the density of synaptic vesicles by 30% in the terminals of the CA1 region (Yoshida et al., 1997; Ahmad et al., 2002a,b). DHA deficiency produces differential effects on phospholipid molecular species composition in the rat hippocampus. Thus, DHA deficiency causes a reduction in DHA-containing glycerophospholipid molecular species in PtdCho, PtdEtn, PlsEtn, and PtdSer by 70–80% (Murthy et al., 2002). In general, DHA is replaced with DPA, but the replacement at the molecular species level does not always occur in a reciprocal manner, especially in PtdCho and PlsEtn. Although, the total glycerophospholipid contents are not affected by DHA deficiency, the relative amounts of PtdSer and PlsEtn are significantly decreased with a concomitant increase in PtdCho. It is proposed that decrease in DHA-containing glycerophospholipid species along with PtdSer and PlsEtn reduction and loss of synapse may represent key biochemical and morphological changes underlying losses in brain-hippocampal function associated with DHA deficiency (Murthy et al., 2002).

6.5.4 *Effects of DHA Deficiency on Receptor Function*

DHA deficiency affects several neurotransmitter receptors, including dopaminergic, cholinergic, serotonergic, and glutamatergic receptors (Table 6.1). DHA deficiency significantly alters mesocorticolimbic dopamine (DA) neurotransmission. Reverse transcription-real-time relative PCR studies on brains of rats fed for 3 weeks in utero and 2 weeks after birth on an *n*–3 fatty acid-deficient diet supplied to dams indicate that among various neurotransmeter receptors, dopamine D_1 and D_2 receptors are the most prominent receptors that are affected in the developing brain. Immunohistochemical staining of brain sections from 2-week-old pups shows a substantial enrichment of the D_2 receptor in discrete regions of the mesolimbic and mesocortical pathways, as well as in a large number of brain areas from the *n*–3 fatty acid-deficient pups (Ahmad et al., 2002a,b). The overwhelming expression of $D_1$and D_2 receptors may be attributed to a behavioral hypersensitivity mediated by the possible impairment of dopamine production during brain development (Kuperstein et al., 2005). Furthermore, maternal linolenic acid (LNA) dietary deficiency affects key dopamine-associated regulatory proteins in mesolimbic and mesocortical pathway. A marked decrease in tyrosine hydroxylase is accompanied by a downregulation of the vesicular monoamine transporter (VMAT-2) and a depletion of VMAT-associated vesicles in the hippocampus of *n*–3 fatty acid deficient offspring compared with adequately fed controls (Kuperstein et al., 2008). LNA deficiency has no effect on dopamine transporter, but increases in DAT/VMAT-2 ratio, which may enhance the risk of dopaminergic terminal damage. In addition to robust increase in dopamine receptor (D_1 and D_2) levels in cortex and striatum, LNA deficiency is also accompanied by enhancement in nuclear internalization of p65 NF-κB and microglial cell activation. These processes may cause reduction in the antioxidant capacity of developing brain and promote microglia activation and enhanced oxidative stress, resulting in an increase in the risk of certain dopamine-mediated neurological disorders (Kuperstein et al., 2008).

DHA deficiency also leads to impairment of central cholinergic neurotransmission system (Aids et al., 2003). The DHA deficiency results in alterations in neural membrane glycerophospholipid compositions in the frontal cortex and hippocampus, with a dramatic loss (62–77%) of DHA. However, changes in the cholinergic pathway occur in the hippocampus only and not in frontal cortex. There is a 72% increase in basal acetylcholine release in hippocampus of DHA-deficient rats than control rats. In contrast, the KCl-mediated release is decreased by 34%. The DHA-deprivation also produces a 10% reduction in muscarinic receptor binding. Acetylcholinesterase activity and the vesicular transporter in both brain regions are not affected. These studies indicate that DHA in cerebral glycerophospholipids increases the spontaneous release of acetylcholine in

the rat hippocampus and reduces its potassium chloride-evoked release. DHA deficiency modulates cholinergic neurotransmission through alterations in the neural membrane PUFA composition (Aids et al., 2003, 2005).

DHA also modulates serotonergic and glutamatergic neurotransmission (Garcia and Kim, 1997; Zimmer et al., 2000; Chalon, 2006). DHA is a component of PtdSer, a glycerophospholipid necessary for the maintenance of NMDA receptor function (Calon et al., 2005). DHA is also involved in regulation of glutamate transporters (GLT1, GLAST, and EAAC1) (Berry et al., 2005). DHA enhances GLT1 and EAAC1 transporter through a mechanism that involves extracellular Ca^{2+}, CaM kinase II, and PKC. In contrast, the inhibitory effect of DHA on GLAST neither requires extracellular Ca^{2+} nor involves CaM kinase II (Berry et al., 2005). In addition, it is well known that glutamate receptor subunits GluR2 and NR2B play a significant role in forebrain synaptic plasticity. Normal aging is associated not only with decrease in the brain DHA contents and neuroplasticity but also with decrease in the GluR2 and NR2B subunits. This decrease in neuroplasticity and GluR2 and NR2B subunits can be fully reversed by DHA supplementation (Dyall et al., 2007).

DHA deficiency in brain also affects amphetamine (AMPH)-induced locomotor activity and sensitization in DBA/2 J mice (McNamara et al., 2006). The development and expression of AMPH-induced sensitization is significantly increased in DHA-deficient mice. It is suggested that DHA deficiency and associated elevation in the AA:DHA ratio augments AMPH-induced sensitization in DBA/2 J mice, and this augmented response is associated with selective alterations in the mesolimbic dopamine pathway (McNamara et al., 2006).

The *n*–3 fatty acid deficiency produces changes in the synaptic levels of serotonin (5-hydroxytryptomine, 5-HT) under basal and fenfluramine-stimulated conditions in the hippocampus of awake 2-months-old rats (Kodas et al., 2004). Aging modulates the density of serotonergic receptors in brain tissue, and *n*–3 fatty acid deficiency increases serotonin 5-HT_2 receptor density by 18–46% in the frontal cortex during aging. Alterations in 5-HT levels can be reversed through the supplementation of *n*–3 fatty acids at birth or during the first 2 weeks of life though maternal milk. Collectively, these studies suggest that DHA modulates cholinergic, dopaminergic, serotonergic, and glutamatergic receptors, improving cognitive function and behavior by increasing neuroplasticity. DHA deficiency affects the storage and release of several neurotransmitters through the modulation of neural membrane composition and vesicle formation (Kodas et al., 2004).

Retinoid X Receptors (RXRs), a family of nuclear receptors, target and regulate multiple signaling pathways (Farooqui et al., 2004). The complexity of RXRs is derived from their ability to activate transcription as homodimers or as obligate heterodimeric partners of a multitude of other nuclear receptors. In addition, RXRs also affect gene expression in a ligand-dependent or - independent manner. Ligand binding is a critical component of RXR function. As stated in Chapter 5, DHA is a ligand for the RXR in brain (Lengqvist et al., 2004; de Urquiza et al., 2000). Collective evidence suggests that RXR modulates

differentiation, survival, nuclear signaling, and gene expression and serves as a fatty acid sensor in vivo.

6.5.5 *Effects of DHA Deficiency on Protein Function and Enzyme Activities*

DHA deficiency decreases the efficiency of visual signaling pathway and compromises the visual acuity. In brain, G-protein-coupled receptor (GPCR) signal transduction is a common signaling pathway, which is modulated by DHA acyl chains. In *n*–3 fatty acid-deficient rats, DHA is replaced with DPA, and this replacement correlates with desensitization of visual signaling in *n*–3 fatty acid-deficient rod outer segment, as evidenced by reduced rhodopsin activation, rhodopsin-transducin (G_t) coupling, cGMP phosphodiesterase activity, and slower production of metarhodopsin II (MII) and the MII-G_t complex relative to *n*–3 fatty acid-adequate rod outer segment (Niu et al., 2004). Rod outer segment membranes from *n*–3 fatty acid-deficient rats show a higher degree of glycerophospholipid acyl chain order relative to *n*–3 fatty acid-adequate rats. The *n*–3 fatty acid-deficient rats show reduced amplitude and delayed response in electroretinogram. Because G-protein-mediated signaling pathways are widespread in brain tissue, the effect of reduced GPCR signaling due to the loss of neural membrane DHA may provide an explanation for the suboptimal neural signaling observed in *n*–3 fatty acid deficiency (Niu et al., 2004).

Dietary DHA deprivation for 15 weeks produces different effects in rat liver and brain. DHA-deficient diet fed rat show an increase in mRNA and activity levels of Δ^5 and Δ^6 desaturases and elongases 2 and 5 in liver but not brain (Igarashi et al., 2007). Liver PPARα and SREBP-1 mRNA levels remain unchanged. In rats fed with DHA-adequate diet, activities of Δ^5 and Δ^6 desaturases and elongases 2 and 5 are generally higher in liver than in brain. These differences in chain elongation and desaturation enzyme expression explain why the liver has a greater capacity to synthesize DHA from circulating ALA than does the brain in animals on an adequate *n*–3 PUFA diet and why liver synthesis capacity is increased by dietary deprivation (Igarashi et al., 2007). These results indicate that liver *n*–3 PUFA metabolism determines DHA availability to the brain when DHA is absent from the diet. The *n*–3 fatty acid deficiency not only upregulates the expression of ARA-metabolizing enzymes, including cytosolic and secretory phospholipases A_2 and cyclooxygenase-2, but also reduce levels of brain-derived neurotrophic factor and cAMP response element-binding protein (Rapoport et al., 2007). It is proposed that reduction in growth factors and transcription factors renders brain more susceptible to neuropathological insults compared to brain of animals that consumed DHA-adequate diet.

The *n*–3 fatty acid depletion markedly regulates NMDA receptor in brain. The deficiency of *n*–3 fatty acid reduces the NR1 subunit in the hippocampus

and Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) activity in the cortex of Tg2576 mice, a transgenic mice overexpressing the human Alzheimer disease (AD) gene APPswe (Tg2576) (Calon et al., 2005). The effect of dietary *n*–3 fatty acid deficiency is more pronounced in Tg2576 mice compared to nontransgenic mice. Furthermore, the loss of the NR2B receptor subunit correlates with p85α phosphatidylinositol 3-kinase levels (Calon et al., 2005). The *n*–3 fatty acid deficiency dramatically increases levels of protein fragments, that can be minimal in nontransgenic mice, suggesting that *n*–3 fatty acid depletion controls caspase activation in the Tg2576 mouse model of AD. Dietary supplementation with DHA partly protects from NMDA receptor subunit loss and accumulation of fodrin and gelsolin fragments, but fully prevents CaMKII decrease. Thus, *n*–3 fatty acid depletion-mediated modulation of NMDA receptor and caspase/calpain activation in the cortex of an animal model of AD may provide new insights into how dietary *n*–3 fatty acids may influence cognition in elderly subjects and retard the risk of AD.

6.5.6 *Effects of DHA Deficiency on Growth Factors*

As stated earlier, accumulation of DHA into neural membrane glycerophospholipids is critical for optimal fetal brain development. This process is maximal during the period of rapid neurite outgrowth, neuritogenesis, which precedes the major growth phase, myelination. Neurite outgrowth and elongation involves the expansion of the plasma membrane and phospholipid synthesis. In PC12 cells, nerve growth factor (NGF)-mediated neurite outgrowth is modulated by DHA (Ikemoto et al., 1999). Dietary deficiency of *n*–3 fatty acids decreases NGF content in rat hippocampus (Ikemoto et al., 2000). It is well known that aging decreases NGF levels in the hippocampus, and no alterations in NGF content are observed in cerebral cortex, indicating that dietary *n*–3 fatty acids affect NGF levels differentially among various brain regions. Although the molecular mechanism associated with this process is not fully understood, TrkA/phosphatidylinositol 3-kinase/Akt (protein kinase B)/NF-κB cascade may play an important role. Deprivation of DHA in rats for 15 weeks results not only in a marked decrease in levels of DHA in frontal cortex but also causes a reduction in the expression of BDNF, cAMP response element-binding protein (CREB) transcription factor activity and p38 mitogen-activated protein kinase (MAPK) activity in frontal cortex (Rao et al., 2007). Activities of other CREB-activating protein kinases are not significantly affected. The addition of DHA to rat primary cortical astrocytes in vitro induces BDNF protein expression, and this BDNF expression can be prevented by a p38 MAPK inhibitor. It is proposed that DHA-mediated regulation of BDNF via a p38 MAPK-dependent mechanism may contribute to its therapeutic efficacy in brain diseases associated with disordered cell survival and neuroplasticity (Rao et al., 2007).

Growth cones are membranous structures associated with the distal end of growing axons. During neuronal differentiation, remarkable morphological changes take place in maturing neurons. During maturation, the axonal sprouting is followed by growth cone formation, axon elongation, pathfinding target selection, and transformation of growth cones into mature synaptic ending. These observations suggest that growth cones are predecessors of the synaptic membranes of nerve endings. The *n*–3 fatty acid restriction during gestation in rats has been reported to alter the composition of growth cone and neuronal cell body membrane fatty acids in newborns (Auestad and Innis, 2000). Growth cones from *n*–3 fatty acid-deficient female rats have lower DHA levels and a corresponding increase in docosapentaenoic acid levels, but *n*–3 deficiency has no effects on neuronal cell body fatty acid concentrations, indicating that accretion of DHA in growth cones but not neuronal cell bodies is affected by *n*–3 fatty acid restriction during gestation. Based on these studies, it is proposed that the addition of new membrane fatty acids to neurons during development occurs along the shaft of the axon or at the growth cone, rather than originating at the cell body (Auestad and Innis, 2000).

Successful axonal outgrowth in brain tissue is central to the process of nerve regeneration and brain repair. To date, much of information on axonal guidance and outgrowth comes from studies on neuritogenesis and patterning during development where distal growth cones constantly sample the local environment and respond to specific physical and trophic influences. During neurodevelopment growth, cone remodeling and reconstruction of cytoskeleton for establishing synaptic connections require DHA and neurotrophins such as BDNF (Martin and Bazan, 1992; Rao et al., 2007). These metabolites increase the synthesis of glycerophospholipids and contribute to axonal and dendritic outgrowth. Although the signaling cues underlying dendritic filopodial motility are mostly unknown, based on several studies it is proposed that BDNF-mediated phosphoinositide-3 kinase pathway may be associated with the movement of dendritic filopodia (Luikart et al., 2008). Collective evidence suggests that both DHA and BDNF are closely associated with neurite outgrowth and synaptogenesis. In adult brain, DHA is associated with neuronal dendrites, where it plays an important role in the extension and establishment of dendritic arboration, a process linked to memory formation.

6.5.7 Effects of DHA Deficiency on Ion Channels Permeability

It is well known that *n*–3 fatty acids are known to modulate ion channels and currents in neural and cardiac cells and protect from antiarrhythmia (Leaf, 2001; Leaf et al, 2003). Thus, DHA not only inhibits voltage-stimulated Na^+ channels in heart tissue but also modulates Na^+ and certain voltage-gated K^+ channels in hippocampal neurons and Chinese hamster ovary cells, respectively (Xiao and Li, 1999). Similarly, in MDA-MB-231 human breast cancer

cells, DHA suppresses cellular migration primarily via downregulation of voltage-gated Na^+ channel (neonatal Nav1.5) mRNA and functional protein expression (Isbilen et al., 2006). ALA, the precursor of DHA, also inhibits Kv1.5 channels in a time- and voltage-dependent manner, with an IC_{50}value of 3.7±0.3 μM (Guizy et al., 2008). Electrophysiology, protein biochemistry, and fluorescence anisotropy measurement studies indicate that ALA at 2.5 μM reduces the Kv1.5 current, shifts the midpoint of the activation curve by –8.8±4.3 mV, accelerates the activation kinetics of Kv1.5 due to a negative shift in its voltage dependency, and slows its deactivation process. Similarly, DHA and EPA also reduce the steady-state levels of Kv1.5 protein. Based on protein chemistry and electophysiological studies, it is proposed that ALA directly blocks atria-specific Kv1.5 channels without modifying their expression or the bilayer order (Guizy et al., 2008). The *n*–3 fatty acid deficiency may result in abnormal ion channel permeability and irreversible effect ion homeostasis.

6.5.8 *Effects of DHA Deficiency on Blood Pressure*

Hypertension (high blood pressure) is a major risk factor for cardiovascular and cerebrovascular diseases. DHA has antihypertensive effects. As stated above, this fatty acid is deposited into neural membranes at a high rate during the perinatal period, and perinatal environment is important for the normal development of blood pressure control. Studies on the importance of perinatal *n*–3 fatty acid supply in the control of blood pressure in adult Sprague-Dawley rats indicate that inadequate levels of DHA in the perinatal period are associated with permanent elevations in blood pressure in later life (Armitage et al., 2003). Vasorelaxant actions of DHA in spontaneously hypertensive rats (SHR) aorta are due to the synthesis of eicosanoids that activate ATP-sensitive K^+ channels. At lower concentrations, DHA-mediated relaxation is attributed to modulation of intracellular Ca^{2+} release and L-type Ca^{2+} channels in vascular smooth muscle cells (Engler and Engler, 2000). The vasorelaxant properties of DHA may contribute, in part, to the blood pressure-lowering effect of dietary fish oil in spontaneously hypertensive rat model. The molecular mechanism associated with DHA deficiency-mediated alterations in blood pressure is not understood. However, long-term DHA deficiency-mediated oxidative stress may contribute to elevated blood pressure through permanent imbalance between docosanoids and eicosanoids levels.

6.6 Conclusion

DHA is an essential fatty acid that plays important roles in supporting the growth of developing brain, and stabilizes and optimizes normal functions of aged brain. In brain, DHA selectively esterifies at the *sn*-2 position of glycerol in

PtdEtn, PlsEtn, and PtdSer. DHA deficiency is associated with impairments in cognitive and behavioral performance as well as disturbances in visual, olfactory functions in animals. Considerable information is available on enhanced intellectual development in breastfed children and reports linking DHA deficiency with neurodevelopmental disorders suggesting physiological importance of DHA in visual and neural systems in humans. Esterified DHA is involved in neuronal signaling, neurogenesis, neurotransmission, and protection against oxidative stress. These functions relate to the roles of DHA within the hydrophobic core of neural membranes. DHA also modulates physicochemical properties of the lipid bilayer. Non-esterified DHA modulates gene expression and ion channel activities. Thus, DHA is necessary for optimal neurotransmission to support cognitive function in the brain and optimal visual transduction and functioning. A deficiency of *n*–3 fatty acids in the perinatal period results in low nerve growth factor levels, changes in dopamine and serotonin production, alterations in learning behavioral, and permanent elevations in blood pressure. These changes can be reversed by supplementing DHA after weaning when the levels of competing ARA in the diet and brain lipids are limited.

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Chapter 7
Status and Potential Therapeutic Importance of *n*–3 Fatty Acids in Neurodegenerative Disease

7.1 Introduction

Neurodegenerative diseases are a complex heterogeneous group of diseases associated with site-specific premature and slow death of certain neuronal populations in brain and spinal cord tissues (Graeber and Moran, 2002). For example, in Alzheimer disease (AD) neuronal degeneration occurs in the nucleus basalis, whereas in Parkinson disease (PD) neurons in the substantia nigra die. The most severely affected neurons in Huntington disease (HD) are striatal medium spiny neurons. The neuronal population that is lost in neurodegenerative diseases modulates functions such as controlling movements, processing sensory information, and making decisions. The most important risk factors for neurodegenerative diseases are old age, positive family history, unhealthy lifestyle, and exposure to toxic environment. It is suggested that during normal aging, the ability of the brain to modify its own structural organization and functioning becomes week and liable resulting in loss of some cognitive function (Farooqui and Farooqui, 2009), but neurodegenerative diseases are accompanied by dramatic impairment in ability to modulate structural organization and functioning of the brain tissue. Other risk factors such as genetic defects, abnormalities of antioxidant enzymes, excitotoxicity, cytoskeletal abnormalities, autoimmunity, mineral deficiencies, oxidative stress, metabolic toxicity, blood–brain barrier dysfunction, and hypertension may also contribute to the pathogenesis of neurodegenerative diseases (Rao and Balachandran, 2002; Farooqui, 2009). In addition, a common denominator among various neurodegenerative diseases is neuroinflammation (Farooqui et al., 2007a; Farooqui and Horrocks, 2007; Farooqui, 2009). Microglia, which are the resident macrophages in brain, secrete a barrage of factors (IL-1, TNFα, NO, PGE_2, and superoxide) that at high concentration not only are toxic to neurons but also propagate neuronal injury. The inflammatory reaction is an attempt by the brain to eliminate the challenge imposed on the brain tissue, clear the system of the dead and damaged neurons, and rescue the normal functioning of this vital organ (Farooqui et al., 2007a).

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Although each neurodegenerative disease has a separate etiology with distinct morphological and clinical characteristics, the progressive cell loss in specific neuronal populations in a specific region is the prominent pathological hallmark of each neurodegenerative disease. For the most part, the nature, time course, and molecular causes of neuronal cell death in neurodegenerative diseases remain unknown, but age-associated decline in cellular antioxidant defenses and resultant accumulation of lipid, protein, and DNA damage in central nervous system along with genetic and environmental factors have been mechanistically implicated in the etiology and pathogenesis of neurodegenerative diseases (Farooqui, 2009) (Fig. 7.1). In addition, loss of synapses plays an important role in the instigation and progression of neuronal loss in neurodegenerative diseases. Attempts are underway to protect and stabilize synapses for slowing the progression of neurodegenerative diseases (Wishart et al., 2006).

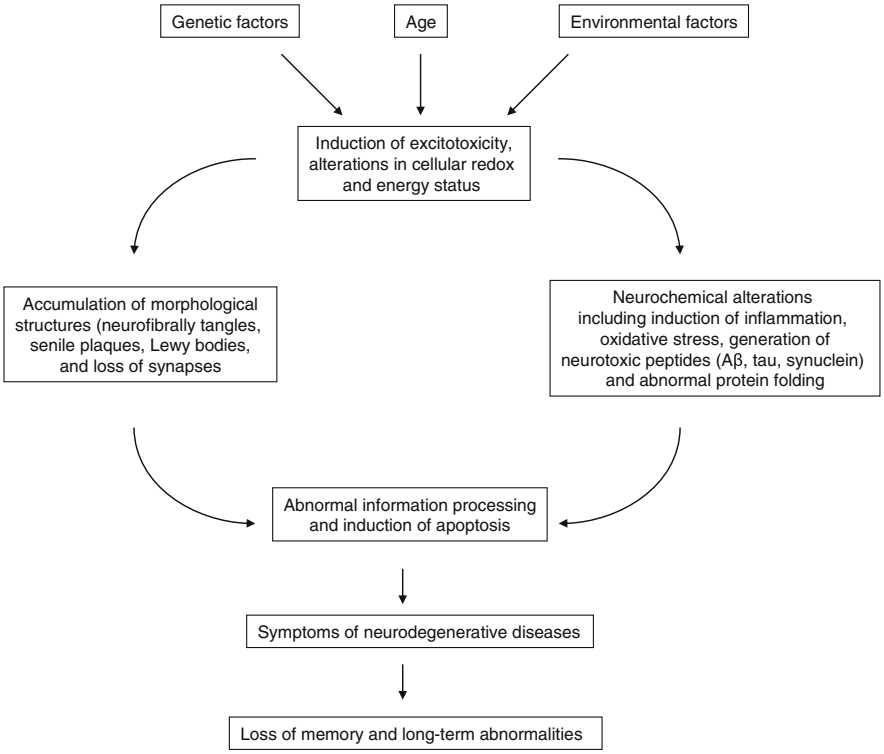

Fig. 7.1 Risk factors and neurochemical change associated with pathogenesis of neurodegenerative diseases

Neurons possess a high metabolic activity, and are especially vulnerable to the long-term effects of continuous exposure to endogenous ROS, supporting the view that ROS production-mediated brain damage may play a major role in the onset and progression of neurodegenerative disorders (Farooqui and

Farooqui, 2009). Neurodegenerative disorders are also involved in the accumulation of ubiquitinated proteins in neuronal inclusions, along with signs of inflammation. In these disorders, the abnormal protein aggregates may themselves trigger the expression of inflammatory mediator-generating enzymes, such as COX-2 and LOX, indicating that impairment of the ubiquitin/proteasome pathway may contribute to this neurodegenerative process (Farooqui and Horrocks, 2007; Farooqui, 2009). Thus, in addition to the generation of ROS (oxidative stress), pathophysiology of neurodegenerative diseases may also share many common terminal neurochemical processes, such as inflammation, excitotoxicity, and accumulation of intracellular or extracellular protein aggregates (Farooqui and Horrocks, 2007; Forman et al., 2004).

Excitotoxicity, inflammation, and oxidative stress are interrelated processes (Table 7.1), which may contribute to neural cell demise independently or synergistically. It is well known that during aging an upregulation of interplay among exicitotoxicity, oxidative stress, and neuroinflammation is present throughout the normal elderly brain (Facheris et al., 2004; Farooqui and Horrocks, 2007), but in neurodegenerative diseases this interplay turns on specific genes that affect only a specific neuronal population in a particular regions where neuronal degeneration occurs (Dwyer et al., 2005; Migliore et al., 2005). This proposal is supported by the hypothesis that the nature of neuron–neuron connections as well as interactions between neurons and glial cells is essential for determining the selective neuronal vulnerability of neurons in neurodegenerative diseases (Wilde et al., 1997; Farooqui et al., 2007a,b). It remains controversial whether exicitotoxicity, oxidative stress, and neuroinflammation are the cause or consequence of neurodegeneration (Andersen, 2004; Juranek and Bezek, 2005; Farooqui, 2009). There is a considerable support to the

Table 7.1 Status of fatty acids, eicosanoids, 4-hydroxynonenal, excitotoxicity, oxidative stress, and neuroinflammation in neurodegenerative diseases

Neurochemical parameter	AD	PD	ALS	HD
Glycerophospholipid Metabolism	Altered	Altered	Altered	Altered
Free Fatty Acid Composition	Altered	–	–	Altered
Eicosanoids	Increased	Increased	Increased	Increased
Lipid peroxidation	Increased	Increased	Increased	Increased
4-Hydroxynonenal	Increased	Increased	Increased	Increased
Excitotoxicity	Increased	Increased	Increased	Increased
Oxidative stress	Increased	Increased	Increased	Increased
Neuroinflammation	Increased	Increased	Increased	Increased
Neurodegeneration	Increased	Increased	Increased	–

Information summarized from the following sources (Farooqui and Horrocks, 1994, 2006; Farooqui et al., 2006; Farooqui and Farooqui, 2009).

proposal that oxidative stress initially occurs in the disease-specific, site-restricted sources such as amyloid β in the cerebral cortex of AD brain, α-synuclein in the brain stem of PD brain, and glutamate receptor-coupled Ca^{2+} channel in the motor system of ALS spinal cord. Subsequent events in the neurons common to these diseases are glutamate-induced neurotoxicity and increased cytosolic Ca^{2+} levels, resulting in activation of Ca^{2+}-dependent enzymes including NADPH oxidase, cytosolic phospholipase A_2, xanthine oxidase, and neuronal nitric oxide synthase (NOS) (Farooqui and Horrocks, 2007; Sun et al., 2007; Shibata and Kobayashi, 2008). Even if exicitotoxicity, oxidative stress, and neuroinflammation may not be the primary triggering processes that initiate the pathogenetic cascade of neurodegenerative diseases, it is becoming increasingly evident that they may promote and maintain factors that aid the progression of AD, PD, ALS, and HD (Farooqui and Horrocks, 2007; Farooqui and Farooqui, 2009). In addition, lifestyle, environmental factors, and age-associated metabolic alterations may also contribute to the pathogenesis of above neurodegenerative diseases. At present, very little information is available on the rate of neurodegeneration and clinical expression of neurodegenerative diseases with age. Neurons are more susceptible to excitotoxicity, oxidative stress, and inflammatory injury than glial cells. In brain, glutamate, ROS, and RNS not only contribute to neuronal injury by modulating the expression of inflammatory and stress-sensitive genes (genes for cytokines) (Farooqui and Horrocks, 2006), but also by activating mechanisms that result in a glia cell-mediated inflammation associated with secondary neuronal damage (Block and Hong, 2005; Farooqui and Horrocks, 2007). These activated glial cells are histopathological hallmarks of acute neural trauma and neurodegenerative diseases (Farooqui et al., 2007a,b). Even though direct contact of activated glia with neurons per se may not necessarily be toxic, the immune mediators released by activated glial cells are endogenous neurotoxins (e.g., nitric oxide and ROS, proinflammatory cytokines and chemokines) that facilitate neuronal death. In aged brain, the number of microglial cells is increased, and they transform themselves to proinflammatory state, making brain tissue ready for greater inflammatory response to inflammogens (Shie and Ling, 2007). These alterations are accompanied by compromised capacity to remove oxidative products, allowing mutual perpetuation of neuroinflammation and oxidative stress (Shie and Ling, 2007).

Within the cell, mitochondria and NADPH oxidase are a major source of oxidative stress. However, additional intracellular sources of ROS and reactive nitrogen species (RNS) exist (Sun et al., 2007; Farooqui, 2009) as well as extracellular sources such as those resulting from exposure to neurotoxins. Constituent endogenous cellular defense mechanisms responsive to the challenge of ROS and RNS represent only one crossroad whereby environment can influence genetic predisposition. Production of

ROS and/or RNS resulting from age-mediated mitochondrial dysfunction along with chronic neuroinflammation may turn on specific genes in a specific area of the brain, and contribute to neuronal death in various neurodegenerative diseases (Fig. 7.2) (Farooqui, 2009; Farooqui and Farooqui, 2009).

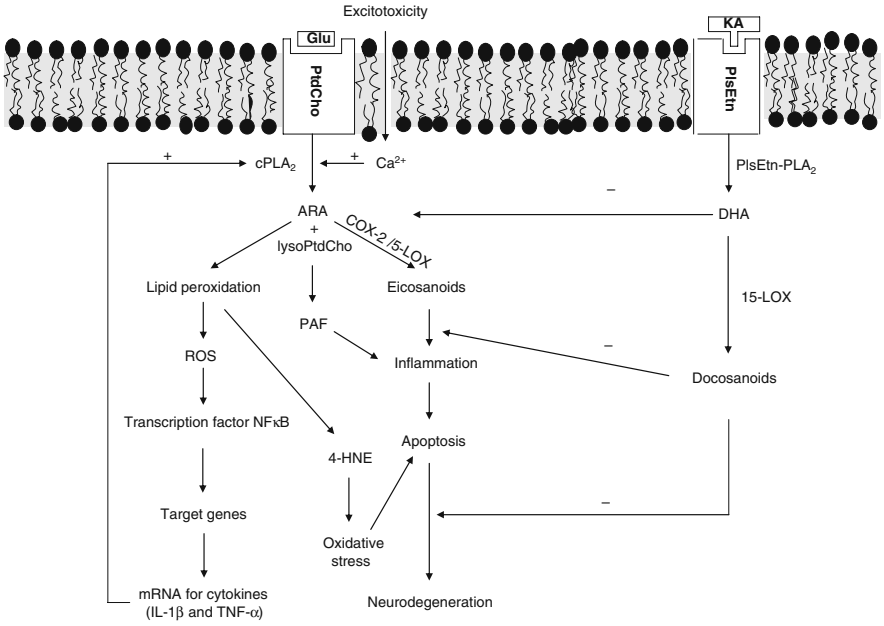

Fig. 7.2 Oxidation of arachidonic and docosahexaenoic acids by cyclooxygenases and lipoxygenases and interplay between arachidonic acid and docosahexaenoic acid-derived lipid mediators. Glutamate (Glu); Kainate (KA); cytosolic phospholipase A_2 ($cPLA_2$); plasmalogen-selective phospholipase A_2 ($PlsEtn\text{-}PLA_2$); phosphatidylcholine (PtdCho); lyso-phosphatidylcholine (lyso-PtdCho); ethanolamine plasmalogen (PlsEtn); platelet-activating factor (PAF); arachidonic acid (ARA); docosahexaenoic acid (DHA); cyclooxygenase-2 (COX-2); 15-lipoxygenase (15-LOX); lysophosphatidylcholine (lyso-PtdCho); 4-hydroxynonenal (4-HNE); reactive oxygen species (ROS); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6; and nuclear factor κB-response element (NF-κB)

Free radical-mediated oxidative stress is balanced by the body's endogenous antioxidant systems with an input from vitamins that one ingests from exogenous sources. Thus, interplay between free radicals and antioxidants are important for maintaining healthy aging and age-related neurodegenerative diseases. A cocktail of antiexcitotoxic, antioxidant, anitiinflammatory compounds that can correct the fundamental excitotoxicity and oxidant/antioxidant imbalance in patients with neurodegenerative diseases may be useful for the treatment of neurodegenerative diseases

(Gilgun-Sherki et al., 2006; Wang et al., 2006). The molecular mechanism involved in the neuroprotective effects of antiinflammatory and antioxidant agents not only depends upon the antioxidant activity of neurons but also on the downregulation of NF-κB activity (Shen et al., 2003), suppression of genes induction by proinflammatory cytokines, and stabilization of synapses (Gilgun-Sherki et al., 2006; Wang et al., 2006; Wishart et al., 2006). The effectiveness of a mixture of antiinflammatory and antioxidant agents in protecting against neurodegenerative diseases depends on their ability to cross the blood–brain barrier, their potential in terms of subcellular distribution in mitochondria, plasma membrane, and cytoplasm, and their multifunctional capacity as well as their synergistic actions (Gilgun-Sherki et al., 2006; Tan et al., 2003). Consideration of these factors in a cocktail along with agents that activate the Nrf2-ARE pathway and increase the production of ATP in degenerating neurons can improve the therapeutic outcome of neurodegenerative diseases (de Vries et al., 2008). A clearer appreciation of the potential therapeutic ability of agents that activate the Nrf2-ARE pathway along with antiinflammatory and antioxidant cocktail would emerge only when in vivo importance of interactions among excitotoxicity, neuroinflammation, and oxidative stress is realized and is fully understood at the molecular level (Farooqui and Horrocks, 2007; Farooqui et al., 2007a,b).

7.2 Apoptotic Cell Death in Neurodegenerative Diseases

Multiple evidences indicate that in experimental models chronic neurodegenerative disease and in AD brain tissue, the underlying neurodegenerative mechanism involves morphological and biochemical features of apoptosis (Jellinger, 2001; Farooqui et al., 2004a; Farooqui, 2009). At the cellular level, neuronal apoptosis in AD is initiated by synergistic actions of excitotoxicity and oxidative stress and disruption of cellular calcium homeostasis. The molecular mechanisms of the neurochemical cascades of apoptosis are beginning to be understood (Farooqui, 2009). These mechanisms involve upstream effectors such as Par-4, p53, and pro-apoptotic Bcl-2 family members that induce mitochondrial dysfunction and facilitate the release of proapoptotic proteins, such as cytochrome c or apoptosis inducing factor (AIF), and subsequent caspase-dependent and caspase-independent pathways, which finally produce the degradation of proteins and nuclear DNA (Fig. 7.3) (Jellinger, 2001; Farooqui, 2009). The regulation of apoptotic cascades is a complex process that involves transcriptional control as well as posttranscriptional protein modifications, such as protease-mediated cleavage, ubiquitination or poly(ADP-ribosylation). In addition, regulation of protein phosphorylation by kinases and phosphatases contribute to mechanisms that control apoptosis in brain tissue. Some studies have focused on the antiapoptotic

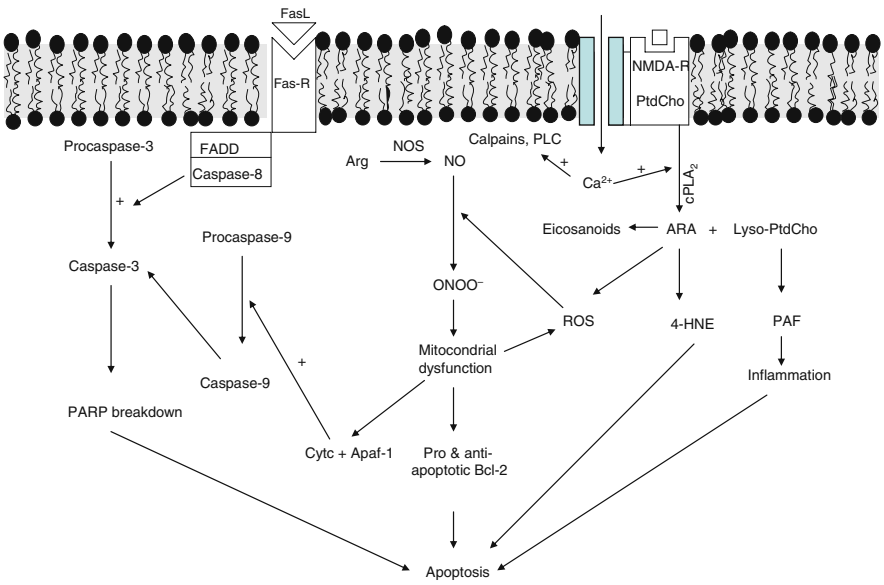

Fig. 7.3 Involvement of Fas and NMDA receptors in apoptotic cell death. Fas ligand (FasL); Fas receptor (Fas-R); *N*-methyl-D-aspartate receptor (NMDA-R); phosphatidylcholine (PtdCho); phospholipase A_2 (PLA_2); arginine (Arg); nitric oxide synthase (NOS); nitric oxide (NO); peroxynitrite ($ONOO^-$); reactive oxygen species (ROS); arachidonic acid (ARA); lyso-phosphatidylcholine (lyso-PtdCho); platelet activating factor (PAF); 4-hydroxynonenal (4-HNE); cytochrome c (Cytc); apoptosome complex with apoptosis-activating factor-1 (Apaf-1); and poly(ADP)ribose polymerase (PARP)

effects of insulin-like growth factor-I (IGF-I). IGF-1 and other insulin-related growth factors are widely expressed in the developing and adult nervous system, and positively modulate a number of processes during neural development, as well as in adult neuronal and glial physiology. These factors also show neuroprotective effects following neurotraumatic and neurodegenerative neural damage (Varela-Nieto et al., 2003).

7.3 Factors Influencing the Onset of Neurodegenerative Diseases

As stated above, the most important risk factors for neurodegenerative diseases are old age and a positive family history. The onset of neurodegenerative diseases is often subtle, and usually occurs in mid to late life. Their progression depends not only on genetic but also on environmental factors and lifestyle (Graeber and Moran, 2002). These diseases lead to progressive cognitive and motor disabilities with devastating consequences to their patients. It is becoming increasingly evident that neurodegenerative diseases are multifactorial disorders caused by a

complex interaction of genetic and environmental factors. Among neurodegenerative diseases, Guam ALS and PD are ideal for studying the relative contribution of diet–gene–environment interactions in the etiology of disease process because these diseases occur in geographical clustered homogenous groups of people (Lynch et al., 2008). The disappearance of ALS from Guam over the past 30 years is due to rapid changes in the ecology, socioeconomy, and Westernization of the lifestyle (Chen, 1995). This slow but steady decline is believed to be the consequences of radical changes from food collection to wage-based lifestyle and elimination of cycad-enriched diet and other exogenous factors (environmental trace metals).

7.3.1 Genetic and Environmental Factors

Genetic and environmental factors modulate the onset and frequency of neurodegenerative diseases (Coppede et al., 2006). Though familial form of neurodegenerative diseases occur rarely, the frequency of occurrence of sporadic forms of neurodegenerative diseases is quite high. Candidate genes involved in the pathogenesis of familial and sporadic neurodegenerative diseases modulate functioning of cholinergic, dopaminergic, and glutamatergic neurons. Some of these genes interact with environmental factors and increase the risk of neurodegenerative diseases. For example, P450 2D6 gene may increase the risk of PD among persons exposed to pesticides. Other genes for PD include SNCA, Parkin, UCHL1, NR4A2, DJ1, PINK1, and LRRK2. Familial PD is caused by mutations in Parkin, PINK-1, or DJ-1 genes. These mutations are closely associated with increased oxidative stress. Pathogenesis of sporadic PD may also involve exposure to other chemicals (metals) that may cause an increase in oxidative stress (Nunomura et al., 2007). In addition to metal and pesticide exposure, early life events such as infections, stress, and poor nutrition can also contribute to the pathogenesis of PD.

Three "causative" AD genes for early-onset familial AD and one "susceptibility" gene that affects risk and age of onset of AD in familial and sporadic late-onset AD have been identified (Levy-Lahad et al., 1998; Priller et al., 2007). The three causative genes are the amyloid precursor protein (APP gene) located on chromosome 21, the presenilin-1 gene located on chromosome 14, and the presenilin-2 gene located on chromosome 1. The third susceptibility gene is the apolipoprotein E (APOE) gene, which is located on chromosome 19 (Levy-Lahad et al., 1998). Mutation in genes causing familial AD (amyloid β protein precursor, presenilin-1, or presenilin-2 gene) results in intensification of oxidative stress. In addition, several known genetic (e.g., Apolipoprotein Eε4 variant) and environmental (e.g., metals or pesticides exposure) risk factors of sporadic AD also involve oxidative stress (Nunomura et al., 2007). Mutations in presenilin proteins cause the most aggressive form of familial AD. These mutations lead to altered intramembranous cleavage of the β-amyloid

precursor protein by the protease called γ-secretase. γ-Secretase is a multiprotein complex composed of presenilin, nicastrin (NCT), APH-1, and PEN-2. These subunits are expressed predominantly in neurons and to some extent in axons. Their distributions and levels of expression are not affected by mutant presenilin-1. In a presenilin-1/amyloid precursor protein double knock-in mouse, γ-secretase subunits are associated with plaques. Immunocytochemical localization of γ-secretase subunits shows that this enzyme is predominantly localized in lipid rafts and protein isoprenylation influencing the activity of γ-secretase complex in association with lipid rafts. In familial AD, presenilin 1 (PS1) mutations results not only in enhanced calcium responses and increased sensitivity of cells to undergo apoptosis but also upregulates γ-secretase activity (Selkoe, 2001; Siman and Salidas, 2004; Urano et al., 2005; Popescu et al., 2004; Cedazo-Minguez et al., 2002; Priller et al., 2007). APOE ε4 gene is another important risk factor for AD. The presence of one copy of the APOE ε4 gene increases the risk of AD two to three times and among subjects with two copies the risk is increased by 12–15 times compared to those without the ε4 allele (Petot and Friedland, 2004). Involvement of APOE ε4 gene links lipid metabolism to the pathogenesis of AD. APOE is associated with lipid transport in the blood, brain, and cerebrospinal fluid.

Recent epidemiological studies have focused on the possible role of environmental risk factors present during early life or aging. Early life events such as infections, stress, poor nutrition, and environmental factors, e.g., chemical and pesticide exposure, may initiate and "set the stage" for neurodegenerative disease to appear late in adult life by modulating above mentioned genes and genetic factors (Logroscino, 2005; Miller and O'Callaghan, 2008). In addition, adiposity has also been reported to contribute positively to the pathogenesis of both PD and AD. Since early-life events contribute to the development of obesity, linkages may exist between early determinants of obesity and the subsequent development of these chronic neurodegenerative diseases (Logroscino, 2005; Miller and O'Callaghan, 2008).

7.3.2 Lifestyle and Neurodegenerative Diseases

It is increasingly recognized that lifestyle factors, such as physical exercise or diet (see below), influence brain plasticity. Regular physical exercise ameliorates age-related neuronal loss and produce positive effect on neurodegenerative diseases (Trejo et al., 2002). In the brain, exercise produces both acute and long-term changes that can be interpreted as beneficial, such as increased levels of various neurotrophic factors or enhanced cognition. Although the signals and molecular mechanisms associated with exercise-induced changes in the brain are not yet well understood, it is proposed that physical exercise mediates signals through increased metabolic activity-induced neuroadaptive and neuroprotective changes in brain function (Trejo et al., 2002). Regular exercise

enhances executive functions of cognition and some types of learning, including motor learning in the spinal cord. These adaptations in the brain tissue may have beneficial effects not only on depression but also on neurodegenerative diseases (Dishman et al., 2006). Chronic voluntary physical excercise also attenuates neural cell responses to stress in brain circuits that regulate peripheral sympathetic activity, indicating that exercise may reduce hypertension, decrease chances of heart failure, and decrease elevated sympathetic nervous system activity.

Exercise upregulates brain-derived neurotrophic factor (BDNF), a molecule important for synaptic plasticity and learning and memory (Vaynman et al., 2004; Vaynman and Gomez-Pinilla, 2006). Studies with a specific antibody (TrkB-IgG) that selectively binds to BDNF indicate that inhibition of BDNF action blocks the beneficial effects of exercise on cognitive function (Vaynman et al., 2004). Inhibition of BDNF expression also retards the effect of exercise on downstream systems modulated by BDNF, and is important for synaptic plasticity mediated by CREB and synapsin I. There is exercise-induced relationship between CREB and BDNF expression and cognitive function. Thus, fast learner and the best memory recall rats show the highest expression of BDNF along with increased CREB mRNA levels. These findings suggest a functional role for CREB under the control of BDNF in mediating the exercise-induced enhancement in learning and memory. Synapsin I, a neuron-specific phosphoprotein associated with neurotransmitter release, may also contribute to this BDNF-mediated mechanism (Vaynman et al., 2004; Vaynman and Gomez-Pinilla, 2006). In addition, exercise also produces changes in the brain that are essential for optimal brain function. These changes are mediated by insulin-like growth factor I (IGF-I), a 79 amino acids containing circulating hormone with three intramolecular disulfide bridges (mol. mass 7649 kDa) that induces physical exercise-mediated potent neurotrophic activities (Carro et al., 2001). Interactions of IGF-1 with its receptor (IGF-1R) results in tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and subsequent activation of PtdIns 3-kinase, PtdIns-dependent kinase, and PKB/AKT (Okajima and Harada, 2008). These neurotropic activities are blocked by IGF-I antibody and IGF-I receptor antagonists. Subcutaneous injections of anti-IGF-I antibody to exercising animals block exercise-mediated brain uptake of IGF-I and abrogates the neuroprotective effects of exercise in domoic acid and 3-acetylpyridine-mediated neurotoxicity. The antibody-treated animals show sedentary animal-like damage to the brain tissue. These results support the view that exercise prevents and protects from brain damage through increased uptake of circulating IGF-I by the brain. Similarly, exogenous administration of IGF-I has also been shown to restore motor function in rats with neurotoxin-induced cerebellar deafferentation, and infusion of an IGF-I receptor antagonist into the lateral ventricle blocks gradual recovery of limb coordination that spontaneously occurs after partial deafferentation of the olivo-cerebellar circuitry (Fernandez et al., 1999). The molecular mechanism of IGF-I may include normalization of several markers of neural cell function in the

cerebellum, including calbindin, glutamate receptor 1 (GluR1), γ-aminobutyric acid (GABA), and glutamate, which are downregulated in 3-acetylpyridine (3AP)-mediated deafferentation (Fernandez et al., 1999). IGF-I also facilitates functional reinnervation of the cerebellar cortex by inferior olive (IO) axons. In the IO, IGF-I significantly downregulates increased expression of bax in neurons and bcl-X in astrocytes following 3AP toxicity. In contrast, IGF-I blocks the decrease in poly-sialic-acid neural cell adhesion molecule (NCAM) and GAP-43 expression mediated by 3AP in IO cells. IGF-I also significantly increases the number of neurons expressing bcl-2 in brainstem areas surrounding the IO (Fernandez et al., 1999). Collectively, these studies indicate that subcutaneous IGF-I therapy promotes functional recovery of the olivo-cerebellar pathway by acting at two sites within this circuitry: (i) by modulating death- and plasticity-related proteins in IO neurons; and (ii) by impinging on homeostatic mechanisms leading to normalization of cell function in the cerebellum (Fernandez et al., 1999).

Blocking of hippocampal IGF-I receptors with a specific antibody against the IGF-I receptor has no effect on the ability of exercise to enhance learning acquisition, but blocks the effect of exercise on augmenting recall (Ding et al., 2006). Blocking the IGF-I receptor significantly reverses the exercise-mediated increase in the levels of BDNF mRNA and protein and pro-BDNF protein, suggesting that the effects of IGF-I may be partially mediated by modulation of BDNF synthesis from its precursors. Molecular analysis indicates that exercise significantly upregulates proteins downstream to BDNF activation important for synaptic function such as synapsin I and phosphorylated calcium/calmodulin protein kinase II and phosphorylated mitogen-activated protein kinase II (Ding et al., 2006). Blocking the IGF-I receptor retards these exercise-induced increases. These results provide information on the molecular mechanisms by which IGF-I modulate the BDNF system to mediate exercise-induced synaptic and cognitive plasticity. BDNF not only facilitates long-term potentiation, an electrophysiological correlate of learning and memory, but also increases the activities of free radical scavenging enzymes and hence protect neurons against oxidative stress (Pelleymounter et al., 1996).

Alterations in serum levels of insulin, IGF-I, and their binding proteins (IGFBPs) have been reported to occur in AD, ALS, and cerebellar ataxia (CA), ataxia-telangiectasia (AT), and Charcot-Marie-Tooth 1A disease (Busiguina et al., 2000; Steen et al., 2005). Since these neurodegenerative diseases have very different etiology, it is proposed that alterations in IGF-I is probably due to metabolic and endocrine derangements due to lack of exercise. Collective evidence suggests that regular exercise mediates its neuroprotective effects through IGF-I supporting the view that IGF-I can be used as a therapeutical aid in chronic brain disease (Carro et al., 2001; Dietrich et al., 2007).

Exercise also upregulates the expression of the mitochondrial uncoupling protein 2, an energy-balancing factor concerned with ATP production and free radical management (Vaynman et al., 2006), supporting the view that in brain tissue physical exercise promotes a fundamental mechanism by which key

elements of energy metabolism may modulate the substrates of hippocampal synaptic plasticity.

7.3.3 Diet and Neurodegenerative Diseases

A diet high in fat induces inflammation and oxidative stress in cardiovascular and cerebrovascular systems. The increase in plasma fatty acids due to high-fat diet results in the activated redox cycling of the copper–albumin complex and excessive lipid peroxidation, and accumulation of advanced glycation end products (AGEs) and subsequent activation of the receptor for AGEs (RAGE) may also represent important mediators of cardiac and neural injuries following exposure to a high-fat diet (Ghosh et al., 2007; Yamato et al., 2007). High-fat diet also results in significant upregulation of gp91(phox) subunit of NADPH oxidase and downregulation of superoxide dismutase isoforms, glutathione peroxidase, and heme oxygenase-2 in various body tissues (Roberts et al., 2006). This is accompanied not only by an increase in plasma malondialdehyde levels and impaired vasodilatory response to acetylcholine. These observations strongly support the presence of oxidative stress and endothelial dysfunction in rats consuming high-fat diet (Roberts et al., 2006).

Diet enriched in antioxidant and antiinflammatory agents (curcumin, green tea, and ferulic acid) is reported to lower the risk of developing neurodegenerative diseases. Dietary supplementation of colored fruit and vegetable extracts decreases the age-enhanced vulnerability to oxidative stress and inflammation. In addition, polyphenolic compounds found in red wine and fruits such as blueberries also exert their beneficial effects through signal transduction and neuronal communication, delaying dementia (Lau et al., 2007; Joseph et al., 2007). The consumption of extra-virgin olive oil, which contains micronutrients and polyphenolic antioxidants including tyrosol [2-(4-hydroxyphenyl)ethanol], hydroxytyrosol, oleuropein, and oleocanthal, retards the development of neurodegenerative diseases (Lopez-Miranda et al., 2007). It is suggested that greater adherence to an olive oil-containing Mediterranean diet results in a significant improvement in health status, as seen by a significant reduction in overall mortality (13%) in PD and AD patients (Sofi et al., 2008).

Excessive calorie intake increases the risk of chronic visceral and neurodegenerative diseases. Reducing energy intake by controlled caloric restriction (CR) or intermittent fasting increases lifespan and protects brain against neurodegenerative diseases due to increase cellular stress resistance (Lee et al., 2000; Mattson, 2008) (Fig. 7.4). CR lowers plasma insulin levels and mediates greater sensitivity to insulin; lowers body temperatures; and reduces cholesterol, triglycerides, and blood pressure. It also elevates HDL and slows age-related decline in circulating levels of dehydroepiandrosterone sulfate. CR induces profound effects on brain function and vulnerability to injury and disease (Fontan-Lozano et al., 2008). It stimulates the production of new

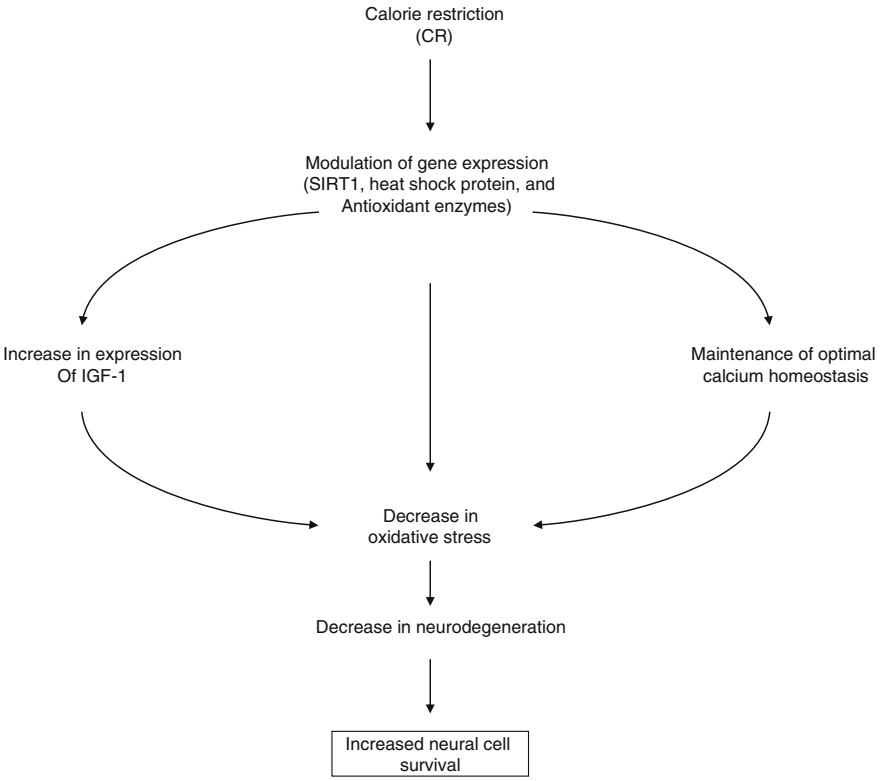

Fig. 7.4 Effects of calorie restriction on neurodegeneration

neurons from stem cells (neurogenesis) and can enhance synaptic plasticity, which not only enhances cognitive function but also increases the ability of the brain to resist aging. These CR-mediated effects are produced by the synthesis of cellular stress response-stimulating proteins (neurotrophic factors, neurotransmitter receptors, protein chaperones, and mitochondrial biosynthesis regulators) that enhance neuronal plasticity and resist oxidative and metabolic insults (Lee et al., 2000; Fontan-Lozano et al., 2008). Decreased levels of IGF-1 during aging may contribute to age-related decline in neural function.

CR-mediated increase in IGF-1 level not only increases lifespan in nematodes, fruit, and flies, but also prolongs life in mice through IGF-1-mediated signaling. Modulation of aging by IGF-I may involve reduction in insulin signaling, enhancement of sensitivity to insulin, reduction in generation of ROS, improvement in antioxidant defenses resulting in reduced oxidative damage. Detailed investigation using microarray and RT-PCR studies of gene expression indicate that effects of the "longevity assurance genes" (Prop1 df or Ghr-/-) and CR overlap, but they are not identical. Subjecting Ames dwarf mice to 30% CR starting at 2 months of age produces significant increase in their

average and maximal lifespans (Bartke et al., 2008). In contrast, identical CR regimen produces either no or very little effect (depending on gender) on longevity of GH receptor GH-binding protein knockout (GHRKO) mice. It is proposed that this difference in response is related to the fact that CR improves insulin sensitivity in Ames dwarfs but does not further increase the extreme insulin sensitivity of GHRKO mice (Bartke et al., 2008). To discover the effect of CR-mediated extension of longevity, attempts are made to study the expression of insulin- and IGF1-related genes in the liver, skeletal muscle, and heart of normal and GHRKO mice. Results indicate that reduction in Akt phosphorylation and PPAR β/δ expression in the liver, downregulation in JNK1 phosphorylation, and upregulation of PGC1α expression in the muscles, and elevation in expression of IGF1 and insulin receptor in the heart are either related to mechanisms of CR action on longevity or represent potential biomarkers of delayed aging (Bartke et al., 2003). Collective evidence suggests that decrease in IGF1 action and increase in insulin sensitivity along with blockade of growth hormone (GH) may be associated with life extension and an apparent slowing of the aging process. Thus, the discovery of pharmacological modulators of insulin/IGF-1 signaling pathway mimetic may affect the lifespan extending mutations through longevity genes. It is proposed that antidiabetic biguanides may be promising candidates for both the lifespan extension and the prevention of cancer (Anisimov, 2003).

Hormesis is an adaptive response where a toxic substance acts as a stimulant at low levels, but produces inhibitory effects at high doses. This biphasic response either directly or indirectly induces compensatory biological processes following an initial disruption in homeostasis. Hormesis involves several interrelated cellular signaling molecules including gases like oxygen, carbon monoxide, and nitric oxide, neurotransmitters like glutamate, metal ions like calcium, and cytokines like tumor necrosis factor. In each case, low levels of these signaling molecules are beneficial and protect against neurodegenerative disease, but their high doses induce neurodegeneration (Mattson, 2008). Hormesis is mediated by genes and enzymes, such as kinases and deacetylases, sirtuin-FOXO pathway, and transcription factors such as Nrf-2 and NF-κB. In addition, hormesis upregulates the expression of cytoprotective and restorative proteins including growth factors, antioxidant enzymes, and protein chaperones (Mattson, 2008; Son et al., 2008; Farooqui and Farooqui, 2009). Another group of neuroprotectants (phytochemicals) (Liu et al., 2008) exhibit biphasic dose responses on cells at low doses and activate signaling pathways resulting in increased expression of genes that encode cytoprotective proteins including antioxidant enzymes, protein chaperones, growth factors, and mitochondrial proteins (Mattson, 2008; Son et al., 2008). Examples include activation of the nuclear factor-E2-related factor-2 (Nrf2)-antioxidant response element (Nrf-2-ARE) pathway by sulforaphane (an anticancer and antimicrobial compound) and curcumin (principal component of tumeric); activation of transient receptor potential (TRP) ion channels by allicin (major component of garlic) and capsaicin (the active component of chili peppers); and activation of

sirtuin-1 by resveratrol. A better understanding of hormesis at the cellular and molecular levels may provide clues for prevention and treatment of neurodegenerative diseases (Mattson, 2008; Son et al., 2008).

In addition, to manage and survive neurodegenerative injury brain induces endogenous responses. These responses are called as longevity assurance processes. Genes that regulate longevity assurance processes are called as vitagenes (HSP70 and HSP32). They detect and control diverse forms of stress. In particular, HSP32, also known as heme oxygenase-1 (HO-1), has received considerable attention, as it has been recently shown that HO-1 induction may represent a protective system potentially active against oxidative stress-mediated brain injury by generating the vasoactive molecule carbon monoxide and the potent antioxidant bilirubin (Calabrese et al., 2004). Increasing evidence suggests that the HO-1 gene is redox-regulated, and its expression appears closely related to conditions of oxidative and nitrosative stress (Calabrese et al., 2004). Several lines of evidence indicate that increased rate of ROS and NRS production and decreased efficiency of proteolysis contribute to age-related elevation in the level of oxidative stress and brain damage. It is proposed that maintenance of the activity of vitagenes may possibly delay the aging process and decrease the occurrence of age-related diseases with resulting prolongation of a healthy lifespan (Calabrese et al., 2004).

7.4 Importance of *n*–3 Fatty Acid in Diet

As stated in Chapter 6, DHA deficiency is associated with impairments in cognitive and behavioral performance as well as disturbances in visual and olfactory functions in aged individuals. Introduction of vegetable oils (corn, sunflower seeds, safflower seeds, cottonseed, and soybeans) for cooking and processed food, which contain large amounts of *n*–6 fatty acids, has resulted in an enormous increase in the consumption of *n*–6 fatty acids in Western diet. The ratio of *n*–6 to *n*–3 fatty acids in present day diet is approximately 20:1 (Simopoulos, 2004; Simopoulos, 2002). In contrast, the traditional diet on which humans have lived all their lives and genetically programmed contained a ratio of 1:1. The present Western diet not only has high *n*–6 contents but also has low levels of antioxidants and micronutrients compared to traditional diet on which humans have evolved. Thus, the high intake of *n*–6 fatty acids has shifted the physiological state of human body to one that is proinflammatory, prothrombotic, and proaggregatory as characterized by increase in blood viscosity, vasospasm, and vasoconstriction and reduction in bleeding time. Consumption of *n*–6 fatty acids in vegetable oils not only elevates proinflammatory eicosanoids but also upregulates the expression of proinflammatory cytokines such as IL-1, IL-6, and TNF-α. In addition, the introduction of hydrogenated oils (margarine) has resulted in an increase in serum cholesterol due to the presence of *trans* fatty acids. In contrast, consumption of *n*–3

fatty acid-containing diet produces antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory effects (Simopoulos, 2004, 2002, 2006; Cordain et al., 2005; Farooqui et al., 2007a,b). These beneficial effects of *n*–3 fatty acids retard brain damage in animal models of a number of neurodegenerative diseases, such as AD and ALS, as well as chronic visceral diseases, such as heart disease and diabetes. Collective evidence suggests that *n*–3 fatty acid-enriched diet has beneficial effects on lipoprotein metabolism, improves endothelial and platelet function, and exerts cardioprotective, immunosuppressive, and neuroprotective effects (Simopoulos, 2006). At the molecular level, *n*–3 fatty acids suppress the expression of TNF-α by inhibiting NF-κB activation and preventing the phosphorylation of IκB-α. Thus, a lower AA:DHA ratio suppresses neurodegenerative diseases.

The importance of *n*–6/*n*–3 fatty acid ratio has been recently questioned on the basis of two cardiovascular trials. It is reported that the amounts of ALA and LA in the diet is more important than the ratio of these fatty acids (Gerster, 1998; Brenna, 2002). Their amounts in diet determine ALA conversion into *n*–3 fatty acids (Griffin et al., 2006; Goyens et al., 2006; Harris, 2007, 2008; Griffin, 2008) (see Chapter 10). In humans, the conversion of ALA into DHA through elongation and desaturation of ALA is very slow (less than 0.5%) compared to other mammalian species. It still remains an open question of how does LA and ALA influence the conversion of ALA, especially under pathophysiological conditions? Thus, more studies are needed on this important topic in normal and pathological cardiovascular and cerebrovascular conditions.

7.4.1 Docosahexaenoic Acid in Alzheimer Disease

AD is the most common neurodegenerative disease that causes dementia in elderly population. The risk of AD doubles every 5 years after age 65. AD is characterized by two pathological hallmarks: the intracellular neurofibrillary tangles (NFT), which accumulate in neuronal perikarya, and the extracellular amyloid deposits in the senile plaques (SP) (Maccioni et al., 2001; Octave, 2005). The NFT are aggregates of hyperphosphorylated microtubule τ protein. The formation of NFT is thought to be associated with a collapse of the microtubule network, disturbances of axoplasmic transports, synapse loss, neuritic atrophy, and neuronal death. Senile plaques have amyloid fibrils, which contain the β-amyloid (Aβ) peptides-Aβ40 and Aβ42 derived from the amyloid precursor protein (APP), a transmembrane glycoprotein expressed in all body tissues. Although the exact biological role of APP is not known, it is proposed that APP regulates trophic function, induction of apoptosis, neurite outgrowth formation, cell adhesion, and neural cell migration (Table 7.2). Abnormality in cognitive function in AD is known to be correlated with the NFT, but the loss of presynaptic terminal is not related with the formation of neuritic plaques (Russo et al., 2005).

Table 7.2 Functions of amyloid precursor protein in brain

Function	References
Modulation of trophic function	Russo et al. (2005)
Modulation of neural cell adhesion	Russo et al. (2005)
Modulation of neuritic outgrowth	Russo et al. (2005)
Modulation of neural cell migration	Russo et al. (2005)
Modulation of synaptic plasticity and neural cell excitability	Turner et al. (2003)
Modulation of synaptogenesis and learning and memory	Gralle and Ferreira (2007)
Induction of apoptosis	Venezia et al. (2004)

Activated microglia, overexpressing interleukin-1 (IL-1), and activated astrocytes, overexpressing S100β, are associated with the formation and evolution of tau2-immunoreactive neuritic plaques in AD. Abnormalities on the action of α-, β- and γ- secretase activity on APP are closely involved in the pathogenesis and progression of both familial and sporadic forms of AD (Maccioni et al., 2001; Octave, 2005). Thus, AD is characterized by progressive atrophy and loss of neurons producing cognitive deficits, confusion and dementia, and death. The underlying cause of sporadic AD is unknown, but a number of environmental and genetic factors may contribute to the pathogenesis of AD. Aging and alterations in diet have been recently implicated in the pathogenesis of AD (Farooqui and Farooqui, 2009). High intake of *n*–6 fatty acids and cholesterol in diet makes human body susceptible to oxidative stress and inflammation due to the increased production of ROS, eicosanoids, and hydroxycholesterol.

Determination of DHA levels in plasma and hippocampus of AD patients show that there is a significant decrease in DHA level in AD compared to age-matched control (Conquer et al., 2000; Tully et al., 2003; Söderberg et al., 1991; Schaefer et al., 2006) (Fig. 7.5). This decrease correlates with upregulation of PlsEtn-PLA_2 (Farooqui et al., 2006) and a significant reduction in plasmalogen levels in AD patient (Söderberg et al., 1990, 1991; Wells et al., 1995; Guan et al., 1999; Han et al., 2001). Together these studies indicate that deficiency of DHA and plasmalogens is reflected in the loss of synapses and cognitive impairment in AD (Wishart et al., 2006). Although the loss of synapse along with neuroinflammation occur very early in the disease, it is not clear whether particular synapses are lost selectively, gradually, or abruptly. In contrast, other studies indicate that there was no significant association between risk of AD and *n*–3 fatty acids intake (Maclean et al., 2005; Plourde et al., 2007). Thus, more studies are required on these important topics.

Epidemiological studies indicate that increase in fish consumption or supplementation of DHA in diet reduces the risk of AD (Kalmijn et al., 2004). This suggestion is strongly supported by studies on consumption of several experimental diets in transgenic APPswe/PS1dE9 mice for 3–4 months. Supplementation of diets with varying *n*–6/*n*–3-ratio, saturated and polyunsaturated fatty acid and cholesterol contents to transgenic APPswe/PS1dE9 mice at a young adult age

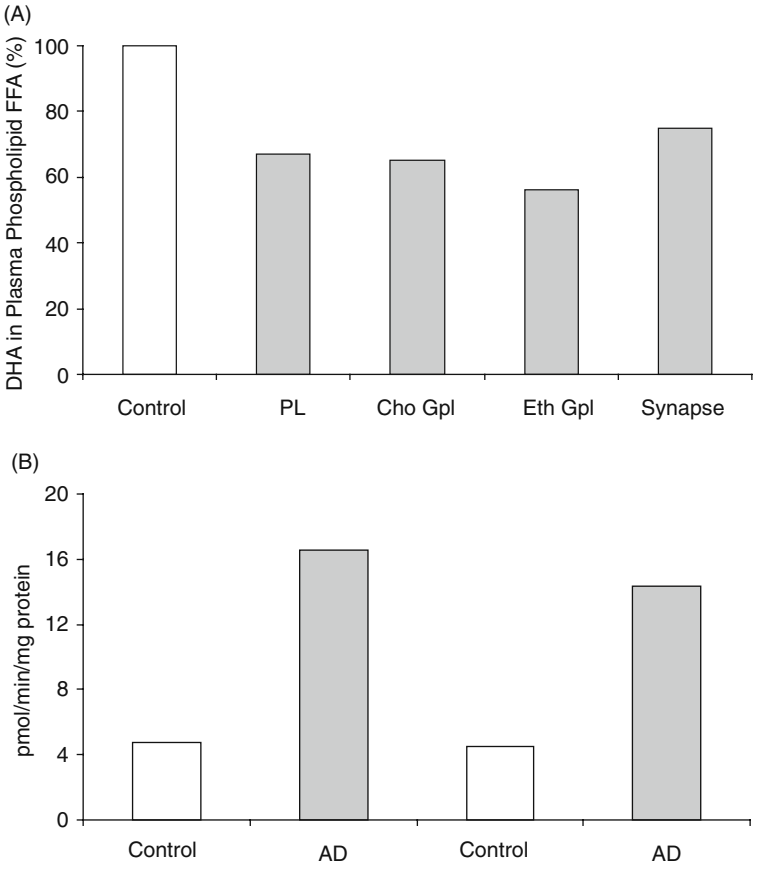

Fig. 7.5 Relationship between decrease in levels of DHA in total plasma glycerophospholipids (PL), choline glycerophospholipids (choline Gpl); ethanolamine glycerophospholipids (ethanolamine Gpl) and number of synapse in Alzheimer disease (**A**). Upregulation of PlsEtn-PLA_2 in nucleus basalis from 2 AD patients (**B**). Upregulation of PlsEtn-PLA_2 results in decrease in ethanolamine Gpl, and this decrease is reflected in decrease in number of synapse in brains of AD patients. Data modified from earlier studies (Conquer et al., 2000, Farooqui et al., 2006; Wishart et al., 2006)

(6 months) indicates that diet with 40% saturated fatty acids and 1% of cholesterol (similar to western diet) upregulates, while diets enriched in DHA downregulates Aβ levels compared to regular (soy oil-based) diet. DHA-enriched diet also reduces the number of activated microglia in hippocampus and induces exploratory activity of transgenic mice, but has no effect on spatial learning in the water maze (Oksman et al., 2006). Although the molecular basis of neuroprotective effects of DHA is illusive, several possibilities have been proposed. DHA may affect amyloid precursor protein processing by inhibiting α- and β-secretase activities (de Wilde et al., 2003). Thus, DHA supplement may prevent β-amyloid deposition. Fish oil (27% DHA content) supplementation in the diet of 2-year-old rats for 1 month and analysis of fatty acid and molecular species composition of the major phospholipid

species along with gene expression indicates that in hippocampus the expression of 23 genes is altered in response to fish oil feeding (Puskas et al., 2003). Furthermore, RT-PCR studies indicate that the transcription of transthyretin (TTR) is increased by 10-fold. Since TTR is an amyloid β-protein scavenger, increase in its expression may block amyloid aggregate formation. It is proposed that the beneficial effects of fish oil may be similar to other agents, such as nicotine and Ginkgo biloba extract, which induce TTR expression (Puskas et al., 2003). In neuronal cultures, addition of Aβ peptide not only induces the release of ARA, but also increases the production of eicosanoids, suggesting that *n*–6 fatty acid-derived eicosanoids may contribute to oxidative stress and neuroinflammation in AD. DHA incorporation in neural membranes blocks the production of proinflammatory eicosanoids. This suggestion is supported by recent studies that indicate that the enrichment of DHA in diet of 3xTg-AD mouse, an animal model of AD, reduces the intraneuronal accumulation of both Aβ and τ. In contrast, combining DHA with ARA and DPA decreases the efficacy of DHA over a 12-month period (Green et al., 2007). Reduction in soluble Aβ may be related to a decrease in steady-state levels of presenilin 1. Furthermore, the enrichment of DPA in the diet reduces levels of early-stage phospho-τ epitopes, which correlates with downregulation of tau kinase (Greenet al., 2007). Hyperphosphorylation of τ protein is one of the critical steps in the formation of neurofibrillary tangles. Hyperphosphorylated τ protein causes impairment in learning and memory. This impairment may be due to an imbalance in regulation in protein kinases and protein phosphatases in the affected neurons (Sun et al., 2003). Injections of calyculin A, a potent and specific inhibitor of protein phosphatase (PP) 2A and PP1, into rat hippocampus bilaterally cause AD-like deficiency in dephosphorylation system resulting not only in decline in memory retention ability in Morris water maze test but also through hyperphosphorylation of τ at Ser396/Ser404 (PHF-1) and Ser-262/Ser-356 (12E8). This suggests that hyperphosphorylation of τ at PHF-1 and 12E8 sites may be a crucial step in mediating alterations in spatial memory formation in AD and its animal model, and dysfunction of protein phosphatases in the affected neurons may be a possible causative factor to AD development (Sun et al., 2003; Chen, 2005).

In rat model of AD, dietary intake of DHA significantly reduces the levels of Aβ(1-40), cholesterol, and saturated fatty acids (Hashimoto et al., 2006; Hashimoto et al., 2008). Fluorescence and electron micrography studies indicate that the formation of Aβ fibrils can be attenuated by their incubation with DHA. DHA treatment also decreases the intensity of thioflavin fluorescence in preformed-fibril Aβ peptides, showing the anti-amyloidogenic effects of DHA. Detailed investigations on fibril formation indicate that DHA reduces the levels of oligomeric amyloid species in a concentration-dependent manner, supporting the view that dietary DHA-mediated suppression of in vivo Aβ (1-40) aggregation occurs through the inhibitory effect of DHA on oligomeric amyloid species (Hashimoto et al., 2008).

In another animal model of AD (Tg2576), safflower oil produces a deficiency of *n*–3 fatty acids. This deficiency results in reduction in the expression of *N*-methyl-D-aspartate (NMDA) receptor subunits, NR2A and NR2B, in the cortex and hippocampus. *n*–3 fatty acid depletion has no affect on the

presynaptic markers, synaptophysin and synaptosomal-associated protein 25 (SNAP-25) (Calon et al., 2005). *n*–3 fatty acid deficiency also reduces the expression of NR1 subunit in the hippocampus and Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) activity in the cortex of Tg2576 mice. The effects of *n*–3 fatty acid depletion are greatly amplified in Tg2576 mice compared to nontransgenic mice (Calon et al., 2005). Loss of the NR2B receptor subunit cannot be explained by changes in mRNA expression. However, it may be correlated with p85α phosphatidylinositol 3-kinase activity. The depletion of *n*–3 fatty acid markedly enhances levels of protein fragments, corresponding to caspase/calpain-cleaved fodrin and gelsolin in Tg2576 mice. This effect is minimal in nontransgenic mice, suggesting that *n*–3 fatty acid deficiency may promote caspase activation in the Tg2576 mouse (Calon et al., 2004, 2005).

Enrichment of DHA in diet partly protects not only from NMDA receptor subunit loss and accumulation of fodrin and gelsolin fragments but also fully prevents CaMKII decrease. Image analysis of brain sections with an antibody against Aβ demonstrate that overall plaque burden is significantly reduced (40.3%), with the largest reductions (40–50%) in the hippocampus and parietal cortex (Lim et al., 2005). Based on these findings, it is proposed that DHA modulates APP processing by decreasing both α- and β-APP C-terminal fragment products and full-length APP. Furthermore, β-secretase activity, apolipoprotein E, and transthyretin gene expression are not affected by DHA-enriched diet. Further investigations in transgenic models of AD indicate that activity of extracellular signal-related kinase (ERK) is first increased, and then decreased. The reason for base line alterations in ERK signaling is not fully understood. However, studies on the effect of Aβ immunoneutralization on cannulation injury-mediated ERK activation in familial AD transgene APPsw indicate that the trauma-mediated activation of ERK observed in Tg^{-1} mice can be dramatically attenuated in Tg^{+} at 12 and 22 months of age. Intracerebroventricular anti-Aβ infusion not only enhances phosphorylation of ERK and cAMP-response element-binding protein (CREB) but also modulates its downstream target, the NMDA receptor subunits (Ma et al., 2007b).

LR11 (ApoE receptor, sorLa), a neuronal sorting protein that reduces amyloid precursor protein (APP) trafficking to secretases which generate Aβ, is markedly downregulated in patients with sporadic AD (Ma et al., 2007a; Rogaeva et al., 2007). LR11 colocalizes with APP and regulates its trafficking in endocytic compartments responsible for APP processing and Aβ generation. Accumulating evidence suggests that the multifunctional receptor LA11 acts as an endocytic receptor for APP, promoting localization of APP in the Golgi apparatus, thus inhibiting APP cleavage (Spoelgen et al., 2006; Jaeger and Pietrzik, 2008). LR11 is regulated by DHA in primary rat neurons, aged non-Tg mice, an aged DHA-depleted APPsw mouse model of AD, and in a human neuronal line, suggesting that DHA upregulates the expression of LR11 in multiple systems. In vivo elevation of LR11 is also observed in fish oil-fed young rats with insulin resistance. These results suggest that DHA-mediated

induction in LR11 does not require DHA-depleting diets and is not age dependent. Reduction in LR11 stimulates Aβ production and may be a significant genetic cause of late-onset AD etiology (Cole and Frautschy, 2006; Ma et al., 2007a). Collective evidence suggests that supplementation of DHA exerts many beneficial effects against pathological signs of AD, including Aβ accumulation, cognitive impairment, synaptic marker loss, and hyperphosphorylation of tau. Enrichment of *n*–3 fatty acids in AD patients results in increased plasma concentrations of DHA and EPA, which reduces release of IL-1β, IL-6, and granulocyte colony-stimulating factor from peripheral blood mononuclear cells (Vedin et al., 2008). These studies should be extended and more parameters related to delaying and preventing of AD in patients should be included. Recent studies have also indicated that in hippocampal slices AMPA receptor-mediated excitotoxicity can be reduced by DHA, but not by EPA (Ménard et al., 2008). Studies on biotinylation of AMPA receptor indicate that the neuroprotective actions of DHA may be due to downregulation of AMPA receptors in hippocampal membranes (Ménard et al., 2008).

Multiple mechanisms may be involved in neuroprotective effects of DHA (Florent et al., 2006). These mechanisms not only include antioxidant, antiinflammatory, and antiexcitotoxic effects of DHA, but also involve inhibition of ARA metabolism by DHA and its metabolites. Thus, chronic pre-administration of DHA prevents β-amyloid-induced impairment of an avoidance ability-related memory function in a rat model of AD (Hashimoto et al., 2005), and protects mice from synaptic loss and dendritic pathology in another model of AD (Calon et al., 2004). DHA and its metabolite, neuroprotectin D_1 (NPD_1), prevent apoptosis and β-amyloid secretion from aging brain cells (Lukiw et al., 2005; Bazan, 2005). The integrity of retinal pigment epithelial cells is critical for photoreceptor cell survival and vision. In retina, the synthesis of NPD_1 redirects cellular fate toward successful preservation of retinal pigment epithelial (RPE)--photoreceptor cell integrity. Generation of NPD_1 from DHA appears to redirect cellular fate toward successful preservation of retinal pigment epithelial (RPE)-photoreceptor cell integrity and brain cell aging. The Bcl-2 pro- and antiapoptotic proteins, neurotrophins, and NPD_1 lie along a cell fate-regulatory pathway whose component members are highly interactive, and have potential to function cooperatively in cell survival (Mukherjee et al., 2007). It is stated that antiapoptotic protein, Bcl-2, neurotrophins, and NPD_1 interact with each other and potentiate cell survival. The hippocampal CA1 region from AD patients shows a major reduction in NPD_1 (Mukherjee et al., 2007). It is proposed that NPD_1-mediated neuroprotective signaling may counteract eicosanoid-mediated proinflammatory cell-damaging events triggered by multiple converging cytokine and Aβ. In brain and retina, NPD_1 synthesis is upregulated by pigment epithelium-derived factor (Lukiw and Bazan, 2008). This docosanoid has been reported to possess important determinant and regulatory interactions with the molecular-genetic mechanisms affecting β-amyloid precursor protein and Aβ peptide neurobiology. Collective evidence suggests that DHA deficiency contributes to decrease in nerve growth factor, inflammatory signaling, apoptosis, and neuronal

dysfunction in AD (Ikemoto et al., 2000; Lukiw and Bazan, 2008). Agents and diet that stimulate NPD_1 synthesis may be useful for delaying neurodegenerative diseases (Bazan, 2005; Serhan, 2005).

Docosanoids retard inflammation and oxidative stress through several mechanisms, including upregulation of γ-glutamyl-cysteinyl ligase and glutathione reductase activities, inhibition of p65 subunit transcription factor NF-κB, preventing cytokine secretion, blocking the synthesis of eicosanoids, blocking Toll-like receptor-mediated activation of macrophages, and reducing leukocyte trafficking (Farooqui and Horrocks, 2006, 2007). It is also proposed that DHA enrichment in Neuro 2A cells prevents staurosporine-mediated apoptotic cell death in a PtdSer- and PtdIns3-Kinase-dependent manner (Akbar and Kim, 2002). Collectively, these studies suggest that DHA is beneficial in preventing learning deficiencies in animal AD models (Lukiw et al., 2005; Freund-Levi et al., 2006). Similarly, DHA protects against other types of dementia (Lim et al., 2005).

A decrease in DHA levels also occurs in serum of AD patients (Conquer et al., 2000; Tully et al., 2003; Lim et al., 2005; Calon et al., 2005; Calon and Cole, 2007; Schaefer et al., 2006). This may either be caused by low dietary intake of DHA or increased oxidation of PUFA. The consumption of *n*–3 fatty acids reduces the risk of AD (Morris et al., 2003). In one study, adult human subjects show not only increase in intelligence but also improvement in visual acquity following supplementation with oil containing DHA (15%) and EPA (3%). Non-demented subjects also show significant improvements in intelligence and visual acquity (Suzuki et al., 2001). Similarly, in cell culture system, enrichment of DHA in plasma membranes protects neurons from soluble Aβ oligomer-mediated apoptotic cell death (Florent et al., 2006). Pretreatment with DHA significantly increases neuronal survival by preventing cytoskeleton perturbations, caspase activation, and apoptosis, as well as by promoting ERK-related survival pathways (Florent et al., 2006; Ma et al., 2007b). As stated above, DHA-enriched diet decreases the number of activated microglia in hippocampus, and increases exploratory activity of transgenic mice, without affecting their spatial learning in the water maze (Oksman et al., 2006).

7.4.2 Docosahexaenoic Acid in Parkinson Disease

The gradual and selective degeneration of dopaminergic neurons in the substantia nigra pars compacta becomes vulnerable to oxidative stress due to monoamine oxidase-mediated dopamine metabolism and hydrogen peroxide generation. These neurons regulate body movement. Degeneration of these neurons in PD results in resting tremor, rigidity, bradykinesia, postural instability, and gait disturbance in the patients with PD. Furthermore, increasing studies indicate that deficits in mitochondrial function, oxidative and nitrosative stress, the accumulation of aberrant or misfolded proteins, and ubiquitin-

proteasome system dysfunction may also represent the principal molecular pathways that are closely associated with the pathogenesis of sporadic and familial forms of PD. In addition, dominant mutations in the gene for α-synuclein, a small presynaptic protein (14 kDa), which is found in both cytosolic and membrane-bound form, can cause PD. α-Synuclein is a highly conserved protein, which is concentrated in nerve terminal. The physiological role of α-synuclein is poorly understood, but recent studies support the view that this protein may be associated with synaptic vesicle recycling and transport and modulation of dopamine release. In addition, α-synuclein promotes neurodegeneration. The molecular mechanism associated with α-synuclein-mediated neurodegeneration is debatable, but increasing evidence indicates that α-synuclein becomes toxic to vulnerable neurons as a result of its tendency to aggregate. α-Synuclein is the major component of Lewy bodies, which are the neuropathological hallmarks of PD (Moore et al., 2005). Studies on the effect of α-synuclein on lipid oxidation in membranes containing glycerophospholipids with unsaturated fatty acids indicate that monomeric α-synuclein efficiently blocks lipid oxidation, whereas fibrillar α-synuclein has no such effect. The inhibition of unsaturated lipid oxidation by α-synuclein requires its binding to the lipid membrane. The antioxidant function of α-synuclein can be attributed to its facile oxidation via the formation of methionine sulfoxide (Zhu et al., 2006). These findings support the view that the inhibition of lipid oxidation by α-synuclein may be a physiological function of the protein. The overexpression of wild-type human α-synuclein decreases viability and increases the levels of degenerative changes during differentiation of neuroblastoma cells that are reduced by selenomethionine treatment (Kumar et al., 2005), supporting the view that one mechanism of α-synuclein action may involve increased oxidative stress. In addition, α-synuclein interacts with aromatic amino acid decarboxylase (AADC) in the striatum where it significantly inhibits phosphorylation and enzyme activity of AADC, suggesting that its interaction with AADC plays a role in dopamine homeostasis (Tehranian et al., 2006).

Parkin, an E_3 ubiquitin-protein ligase, which is associated with the degradation of cellular proteins by the proteasomal pathway, has been recently shown to protect cells against α-synuclein toxicity (Baptista et al., 2004; Burke, 2004). Deletions or point mutations in the gene for parkin also cause an autosomal recessive, early-onset form of PD. Although the molecular mechanism associated with perkin-mediated neurodegeneration in PD is not fully understood. However, the loss of parkin function may alter the breakdown of some unfolded proteins by proteosomes and induce the accumulation of their substrates, which may cause the degeneration of dopaminergic neurons (Cookson, 2003). Furthermore, parkin gene generates a large number of protein isoforms through the alternative splicing process, and to date, 12 parkin transcript variants have been detected (Kitada et al., 1998, 2000). These parkin isoforms encode for proteins with different amino acid composition, post-translational modifications and functional domains. Parkin variants have a specific brain and cellular distribution and different function. It is possible that mutations and

interactions between α-synuclein and parkin genes may play a role in the pathophysiology of idiopathic PD.

Stereotaxic injections of lipopolysaccharide into substantia nigra of M7KO and M83KO mice produce neuroinflammation and dopaminergic neuronal death, with the accumulation of insoluble aggregated α-synuclein as cytoplasmic inclusions in nigral neurons (Gao et al., 2008). Furthermore, nitrated/oxidized α-synuclein is detected in these inclusions, and abatement of microglia-derived nitric oxide and superoxide provides significant neuroprotection in neuron-glia cultures from M7KO mice, suggesting that nitric oxide and superoxide released by activated microglia may be mediators that link inflammation and abnormal α-synuclein metabolism with neurodegeneration in PD (Gao et al., 2008). The overexpression of α-synuclein may not be specific for PD; other demyelination conditions, such as MS and experimental autoimmune encephalomyelitis (EAE), also show upregulation of α-synuclein in neurons and glial cell (Papadopoulos et al., 2006). Although the increased expression of α-synuclein is detected as a granular cytoplasmic labeling rather than inclusion bodies, these results do suggest that immune-mediated inflammatory demyelination and neuronal cell death in MS and EAE may share some common features with PD (Papadopoulos et al., 2006).

Very little information is available on neural membrane fatty acid composition of PD patients (Farooqui and Horrocks, 1998). α-synuclein binds glycerophospholipids and has a role in brain fatty acid metabolism. Studies on determination of masses of individual glycerophospholipid classes and neutral lipid in brains from wild-type and α-synuclein$^{-/-}$ mice indicate that total brain glycerophospholipid mass is not altered, but cardiolipin and phosphatidylglycerol masses are decreased by 16% and 27%, respectively. In addition, no changes are reported in plasmalogen or polyphosphoinositide masses. In ethanolamine glycerophospholipids and phosphatidylserine, DHA is decreased 7%, while palmitic acid is increased 1.1-fold and 1.4-fold, respectively (Barcelo-Coblijn et al., 2007). The exact mechanism of α-synuclein-mediated alterations in fatty acid metabolism is not fully understood. However, it is proposed that α-synuclein facilitates incorporation of fatty acid in glycerophospholipids by presenting fatty acids to microsomal acyl-CoA synthetase (Barcelo-coblijn et al., 2007; Golovko et al., 2006). The significance of these observations in relation to pathogenesis of PD and neurodegeneration remains unknown. However, in α-synuclein gene-ablated mice a significant decrease in the incorporation rate of ARA into brain glycerophospholipid pools occurs due to downregulation in recycling of ARA through the endoplasmic reticulum-localized long-chain acyl-CoA synthetases (Golovko et al., 2008). This suggests that α-synuclein may be associated with inflammatory process. α-Synuclein is also important for brain and astrocyte cholesterol metabolism, where its deletion results in an elevation of cholesterol and cholesteryl esters. This increase may be due to the interaction of α-synuclein with membrane-bound enzymes involved in lipid metabolism such as long-chain acyl-CoA synthetases. Collectively, these studies suggest that α-synuclein is critical in modulating brain

eicosanoid synthesis and microglial activities. In the absence of α-synuclein, microglia are basally activated and demonstrate increased proinflammatory cytokine secretion (Golovko et al., 2008).

Activities of PLA_2, COX-2, and rate of lipid peroxidation are increased in *N*-methyl,4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) induced model of PD and PLA_2, COX inhibitors, and some antioxidants protect neural cells from MPTP-mediated neural cell injury (Yoshinaga et al., 2000; Teismann et al., 2003; Farooqui et al., 2006). Gathering evidence suggests that increased dopamine-mediated oxidative stress in nigrostriatal tract, impairment of energy metabolism, and neuroinflammation may contribute to the progressive loss of dopaminergic nigral neurons (Beal, 1998; Jenner and Olanow, 2006; Farooqui and Horrocks, 2007).

Levodopa, the chemical precursor of dopamine, is commonly used for the treatment of PD. The effectiveness of levodopa therapy declines after years of treatment, and the long-term treatment of PD should be carefully managed. Long-term levodapa therapy results in dyskinesias (LIDs). Due to interrelationship and interdependence of risk factors in clinical populations, it is difficult to independently examine factors that may affect the development of LIDs (Schneider et al., 2003). In MPTP-induced Parkinsonism, the rate of symptom progression, symptom severity, and response and duration of levodopa therapy in macaque monkeys indicate that levodopa therapy induces the development of LIDs. Short-term MPTP exposure produces rapid symptom onset, and short symptom duration prior to initiation of levodopa therapy causes dyskinesia between 11 and 24 days of daily levodopa administration. In contrast, monkeys with long-term MPTP exposure, slow symptom progression, and/or long symptom duration prior to initiation of levodopa therapy develop dyskinesia no sooner than 146 days of chronic levodopa administration, indicating that these monkeys are more resistant to developing LIDs. These studies suggest distinct differences in the propensity to develop LIDs in monkeys with different rates of symptom progression or symptom durations prior to levodopa (Schneider et al., 2003). DHA administration decreases LIDs in both paradigms without alteration of the anti-parkinsonian effect of levodopa, indicating that DHA can decrease the severity or delay the development of LIDs in a nonhuman primate model of PD (Samadi et al., 2006). Determination of brain fatty acid profile in cerebral cortex of MPTP model of monkeys indicate that levodopa increases ARA content, but reduces DHA concentration and total *n*–3:*n*–6 fatty acids ratio compared to drug-naive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animals (Julien et al., 2006). Interestingly, PD patients who experienced motor complications to levodopa have higher ARA concentrations in the cortex compared to controls and to levodopa-treated PD patients devoid of motor complications. Collective evidence suggests that levodopa treatment produces changes in brain fatty acid concentrations not only in a nonhuman primate model of Parkinsonism but also in PD patients (Julien et al., 2006).

In general, nutrients or drugs mediate alterations in behavior by modulating synaptic transmission not only by changing the quantities of particular

neurotransmitters present within synaptic clefts but also by acting directly on neurotransmitter receptors or signal transduction molecules. Administration of uridine-5′-monophosphate (UMP) and DHA in rats increases synaptic membranes and dendritic spines. Studies on *d*-amphetamine-induced rotational behavior and dopaminergic markers in rats following partial unilateral 6-hydroxydopamine (6-OHDA)-induced striatal lesions indicate that rats receiving UMP and DHA show decrease in ipsilateral rotations by 57% and significant increase in striatal dopamine, tyrosine hydroxylase (TH) activity, TH protein, and synapsin-1 on the lesioned side compared to non-lesioned side (control), indicating that uridine and DHA administration may partially restore dopaminergic neurotransmission in this model of PD (Cansey et al., 2008). Similarly, treatment of control or a high-*n*–3 fatty acid-fed mice diet (2 to 12 month old) with MPTP indicates that high-*n*–3 fatty acid-fed mice are completely protected from MPTP-mediated neurotoxicity (Bousquet et al., 2008). Thus, high-*n*–3 PUFA dietary consumption not only prevents the MPTP-induced decrease of tyrosine hydroxylase (TH)-labeled nigral cells but also downregulates Nurr1 mRNA and dopamine transporter mRNA levels in the substantia nigra. Although *n*–3 PUFA dietary treatment produce no effect on striatal dopaminergic terminals, the high-*n*–3 PUFA diet protects against the MPTP-induced decrease in dopamine and its metabolite, dihydroxyphenylacetic acid, in the striatum, indicating that a high-*n*–3 PUFA dietary intake produces neuroprotective effects in an animal model of Parkinsonism (Bousquet et al., 2008). This suggestion is supported by recent studies on the intake of dietary fatty acids (de Lau et al., 2005). It is suggested that high intake of unsaturated fatty acids may protect against PD.

7.4.3 Docosahexaenoic Acid in Amyotropic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a clinically severe and fatal neurodegenerative disease characterized by a loss of both upper and lower motor neurons, resulting in progressive muscle loss and paralysis. While the exact cause of neuronal death in ALS remains unknown, it is proposed that multiple pathophysiological mechanisms trigger motor neuron cell death. These mechanisms include oxidative stress, mitochondrial impairment, protein aggregation, glutamate cytotoxicity, transcription dysfunction, inflammation, and apoptotic cell death. Thus, ALS is a rare but fatal motoneuron disorder caused by the selective loss of both upper and lower motor neurons in the brain and spinal cord, with consequent progressive paralysis and death from respiratory complications on average in 3 to 5 years from disease onset. The disease is accompanied by progressive muscle weakness, atrophy, and spasticity. The molecular mechanism associated with the pathogenesis of ALS remains elusive, but synergistic action of excitotoxicity, oxidative stress, and neuroinflammation

may play a major role in neurodegeneration in ALS (Table 7.1) (Shaw and Ince, 1997; Rao and Weiss, 2004; Farooqui et al., 2008).

ALS occurs in sporadic and familial forms. The pathogenesis of neuronal degeneration in both sporadic and familial ALS associated with mutations in superoxide dismutase may involve oxidative stress. Mitochondrial dysfunction (alterations in respiratory complexes I and III) is an early event associated with degeneration of motor neuron not only in transgenic mice that overexpress mutant superoxide dismutase (SOD)1 gene but also in human ALS patients (Almer et al., 2001; Liu et al., 2002). In addition, protein oxidative damage and specific changes in fatty acid levels, specifically of *n*–3 series (as DHA), also occur in ALS (Ilieva et al., 2007). The sporadic form of ALS is characterized by a prominent neuroinflammatory component, upregulation of COX-2 mRNA, and oxidative stress (Drachman and Rothstein, 2000; Yasojima et al., 2001; Drachman et al., 2002). Abnormalities in glutamate and its receptors, as well as disordered function of voltage-dependent Ca^{2+} channels and superoxide dismutase, have been shown to occur in ALS patients. Furthermore, the activity of PKC, a Ca^{2+}, phospholipid-dependent enzyme, is also substantially increased in tissue from ALS patients, suggesting that alterations in intracellular free Ca^{2+} may be closely associated with potential pathogenic mechanisms involved in ALS (Farooqui et al., 2008).

Recent studies on retinoid (RXR) and peroxisome proliferator-activated receptorγ (PPARγ receptor) in spinal cord of rat model of ALS and autoimmune diseases indicate that these receptors are associated with inflammatory reactions (Jokic et al., 2007; Diab et al., 2004). These receptors belong to a superfamily of nuclear receptors that are linked to the upregulation of isoforms of $PLA_{2,}$ distribution, and release of ARA and DHA in brain tissue (Farooqui et al., 2004b, 2006; Farooqui and Horrocks, 2007). A direct link between motor neuron disease and retinoic receptor (RARα) has been reported to occur in rats fed on a retinoid-deficient diet. In the spinal cord of these rats, a decrease in number of RARα is similar and proportional to decrease in RARα in spinal cord of ALS patient (Corcoran et al., 2002). Gene expression studies in ALS indicate that gene encoding for the cellular retinal-binding protein is markedly upregulated in ALS spinal cord (Malaspina and de Belleroche, 2004; Malaspina and Turkheimer, 2007).

Treatment of primary sensory neuronal cultures from aged animals with ARA, EPA, and DHA results in increased neurite outgrowth. *n*–3 fatty acids, in particular DHA, show a remarkable effect on neurite outgrowth. The amplitude of *n*–3 fatty acid effect is comparable to nerve growth factor and all-*trans*-retinoic acid adult and aged animals (Robson et al., 2008). Based on these observations, it is proposed that DHA and EPA can be used for the treatment of ALS in animal models. Recent advances in the area of stem cells and growth factors encourage optimism regarding brain regeneration. A combination of DHA, glycerophosphocholine, acetyl-L-carnitine, and PtdSer may protect neural cells from neurodegeneration in ALS (Kidd, 2005). Clinical trials for the treatment of ALS with *n*–3 fatty acids

have not been performed in humans, and are now urgently needed to judge the efficacy of *n*–3 fatty acids

7.4.4 Docosahexaenoic Acid in Huntington Disease

Huntington disease (HD) is a rare and progressive movement disorder caused by an autosomal dominant mutation in a gene located on chromosome 4 (Cepeda et al., 2001). The genetic defect underlying HD has been identified as an unstable CAG trinucleotide repeat in exon 1 of the HD gene, which encodes for a polyglutamine expansion near the N′-terminal end of a large protein called huntingtin. Insoluble aggregates containing huntingtin occur in cytosol and nuclei of HD patients, transgenic animal, and cell culture models of HD. The molecular mechanism involved in aggregate formation is not fully understood. However, it is proposed that interactions of huntingtin with other proteins may promote its own polymerization to form insoluble aggregates. These aggregates have been shown to trigger neurodegeneration (Scherzinger et al., 1997). The intraneuronal aggregates of huntingtin may produce neurodegeneration by modulating gene transcription, protein interactions, protein transport inside the nucleus and cytoplasm, as well as vesicular transport (Bonilla, 2000). At the molecular level, abnormal huntingtin binds to CREB protein, which hampers its ability to turn on transcription of other genes by blocking acetyltransferase activity CREB protein. Thus, aggregation and accumulation of huntingtin may result in a dramatic loss of striatal medium-sized spiny GABAergic projection neurons (MSNs) and gliosis (reactive astrocytosis) in the striatum and cerebral cortex (Cepeda et al., 2001). EPA has been used for the treatment of HD. Randomized, placebo-controlled, double-blind studies show that EPA has beneficial effects in HD patients (Puri, 2005; Das and Vaddadi, 2004; Murck and Manku, 2007). Although the molecular mechanism associated with beneficial effects of EPA is not known, it is suggested that fatty acids may prevent or block polyQ aggregation, inhibit histone deacetylation, activate the ubiquitin-proteasome system, and restore mitochondrial dysfunction (Das and Vaddadi, 2004; Murck and Manku, 2007).

7.5 Interactions Among Excitotoxicity, Oxidative Stress, and Neuroinflammation in Neurodegenerative Diseases

Excitotoxicity, oxidative stress, and neuroinflammation play an important role in the pathophysiology of neurodegenerative diseases (Farooqui and Horrocks, 2007) (Fig. 7.6). Cell culture and animal model studies indicate that *n*–3 fatty acids and their metabolites inhibit excitotoxicity, oxidative stress, and neuroinflammatory reactions. Although the clinical evidence that *n*–3 fatty acids prevent or slow the course of neurodegenerative diseases is weak and

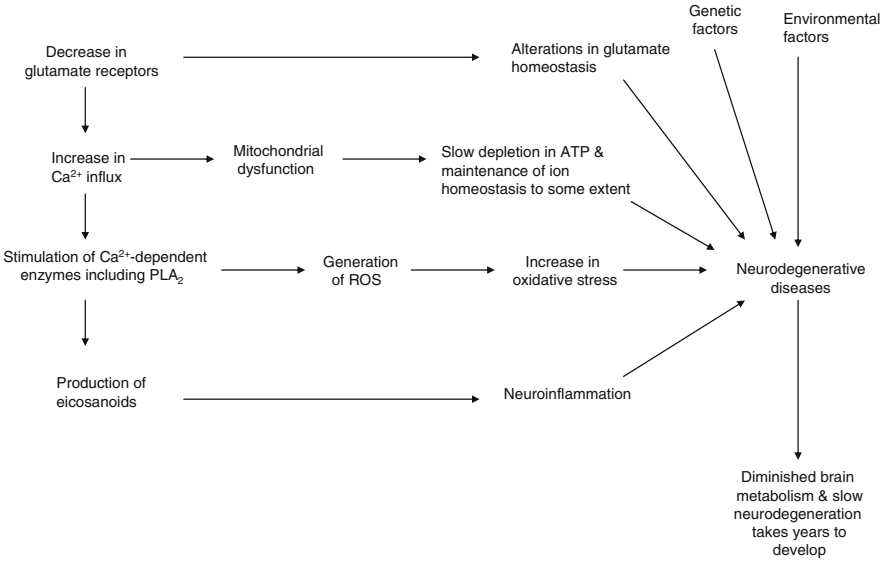

Fig. 7.6 Synergistic effects of genetic, metabolic, and environmental factors in pathogenesis of neurodegenerative diseases

controversial, recently there have been several developments that support the antioxidant, antiexcitotoxic, and antiinflammatory effects of *n*–3 fatty acids (Farooqui et al., 2007b, 2008).

In neurodegenerative disease such as AD, there is not an excessive release of glutamate, but a marked reduction in the expression of NR2A and NR2B subunit mRNA in the hippocampus and entorhinal cortex in brain from AD patients (Geddes et al., 1992; Bi and Sze, 2002). Similarly, differential expression of metabotropic glutamate receptors (mGluRs) has also been reported to occur in neocortex and hippocampal regions compared to age-matched controls AD (Lee et al., 2004). These alterations may induce changes in glutamate homeostasis in AD, causing a major disturbance in Ca^{2+} homeostasis (Mattson, 2002). Abnormal Ca^{2+} homeostasis modulates alterations in the activities of PKC (Shimohama et al., 1990) and phospholipases A_2 (Farooqui et al., 2003a,b). Collective evidence suggests that anomalous glutamatergic activity and changes in Ca^{2+} homeostasis are associated with alterations in postsynaptic receptor function and downstream defects that not only leads to neuronal injury and death but also to cognitive deficits associated with dementia (Wenk et al., 2006).

As stated above, there is a marked increase in mGluR2 expression in AD compared to brain from age-matched controls. Agonists for group II mGluRs activate ERK. This enzyme is chronically stimulated in vulnerable neurons of AD. ERK phosphorylates tau protein, so the upregulation of mGluR2 in vulnerable neurons may represent the upstream mediator of abnormal tau

phosphorylation in AD. Immunocytochemical studies show considerable overlap between mGluR2 and neurofibrillary alterations (Lee et al., 2004). This decrease may cause alterations in long-term potentiation, a process closely associated with learning and memory formation. Furthermore, the loss of pyramidal neurons and their synapses in AD along with presynaptic (and postsynaptic) glutamatergic hypoactivity may represent a "double blow" to the brain tissue as the activity of glutamatergic neurons is heavily influenced by the cholinergic systems, which have been reported to be dysfunctional in AD (Francis, 2003). Clinically, changes in glutamatergic and cholinergic dysfunction strongly correlate with cognitive decline in AD. The mechanism by which glutamatergic (and cholinergic) cells die may be related to several factors including alterations in calcium influx and stimulation of calcium-dependent enzymes. In AD and other neurodegenerative diseases, oxygen, nutrients, and ATP are available to the nerve cell, and ion homeostasis is maintained to a limited extent, neural cells may take longer time period (years) to degenerate. In contrast, in acute neural trauma glutamate release and glutamate-mediated neurodegeneration may be rapid not only because of the sudden lack of oxygen and a quick drop in ATP but also due to rapid alterations in ion homeostasis (Farooqui and Horrocks, 2007). In cortical cultures, Aβ treatment stimulates $cPLA_2$ activity in a dose-dependent manner, and this stimulation can be blocked by $cPLA_2$ antisense oligonucleotides (ODN). These results support the view that $cPLA_2$-mediated arachidonic acid cascade may generate eicosanoids, and ROS contribute to the pathogenesis of AD by inducing neuroinflammation and oxidative stress (Kriem et al., 2005; Farooqui and Horrocks, 2007). ROS not only contribute to neuronal injury by intensifying the expression of inflammatory and stress-sensitive genes, including genes for cytokines and chemokines (Farooqui and Horrocks, 2006), but also by activating mechanisms that result in a glia cell-mediated secondary neuronal damage (Block and Hong, 2005; Farooqui et al., 2007a,b). These activated glial cells and neuroinflammation are histopathological hallmarks of neurodegenerative diseases, and may contribute to neurodegenerative processes (Farooqui and Horrocks, 2007). A direct contact of activated glia with neurons per se is not necessarily for neurodegenerative process to occur, but the release of mediators, such as nitric oxide, eicosanoids, ROS, and pro-inflammatory cytokines and chemokines, by activated glial cells may initiate the neurodegenerative process. There is a growing appreciation of the role of cytokine-mediated inflammatory processes in AD, PD, ALS, HD, and vascular dementia. In neurodegenerative diseases, harmful effects of altered glutamate homeostasis and oxidative stress along with inflammatory reactions may cause impairment in neuroplasticity, loss of synapse, and alterations in dendritic morphology in the hippocampus and nucleus basalis area of human brain. By altering the hippocampal plasticity, these processes may not only influence LTP induction and spatial memory formation but also alter debdritic morphology (Kim and Yoon, 1998). It is proposed that coordinated interplay among excitotoxicity, oxidative stress, and neuroinflammation in aged humans and animals may cause abnormalities in

motor and cognitive performance. An enhanced rate of interplay among the above processes may be associated with the increased vulnerability of neurons in neurodegenerative diseases. This interplay along with energy status of neurons, genetic and environmental factors may be common mechanism of brain damage in various neurodegenerative diseases (Farooqui and Horrocks, 1994, 2007).

The most intriguing current and future impacts on public health may come from a greater intake of *n*–3 fatty acids (Moyad, 2005; Farooqui et al., 2007b). These fatty acids may retard or delay a variety of diverse chronic diseases, such as neurodegenerative diseases, cancer, diabetes, and arthritis. In cell culture and animal models of neurodegenerative diseases, *n*–3 fatty acids produce neuroprotective effects by downregulating of NF-κB activity (Farooqui and Horrocks, 2007), and suppressing genes induced by proinflammatory cytokines and other mediators released by glial cells. Recent studies indicate that resolvins and neuroprotectins, which are oxidized products of DHA, produce beneficial effects. These metabolites antagonize the effects of eicosanoids, modulate leukocyte trafficking, and downregulate the expression of cytokines in glial cells and modulate interactions among neurons, astrocytes, oligodendrocytes, microglia, and cells of the microvasculature (Serhan, 2005; Bazan, 2005, 2006, 2007). In addition, in cell culture NPD_1 downregulates oxidative stress and apoptotic DNA damage. NPD_1 also upregulates the anti-apoptotic Bcl-2 proteins, Bcl-2 and bclxL, and decreases the expression of the pro-apoptotic proteins, Bax and Bad. It also blocks IL-1-mediated expression of COX-2 (Bazan, 2005). Based on above studies, it is proposed that generation of resolvins and NPD_1 is an internal neuroprotective mechanism for preventing brain tissue damage (Serhan, 2005; Bazan, 2005; Lukiw et al., 2005).

n–3 fatty acids may also act by slowing the progression of oxidative stress-mediated neurodegenerative diseases though the involvement of nuclear factor E2-related factor 2 (Nrf2), which translocates from the cytoplasm into the nucleus and transactivates expression of genes with antioxidant activity. In AD, Lewy body variant of AD (LBVAD) and, in PD, Nrf2 are predominantly located in cytoplasm of hippocampal neurons and are not a major component of β-amyloid plaques or neurofibrillary tangles (Ramsey et al., 2007). In contrast, in normal brain hippocampi, Nrf2 is expressed in both the nucleus and the cytoplasm, with predominant expression in the nucleus. Immunoblotting studies reveal that there is a significant decrease in nuclear Nrf2 levels in AD cases. In PD Nrf2 is predominantly present in the nucleus of nigral neurons but cytoplasmic in substantia nigra of normal, AD, and LBVAD cases. Based on these results, it is proposed that Nrf2-mediated transcription is not induced in neurons in AD despite the presence of oxidative stress (Ramsey et al., 2007). In contrast in PD, nuclear localization of Nrf2 is strongly induced, but this response may be insufficient to protect neurons from degeneration, and inclusion of *n*–3 fatty acids may promote endogenous antioxidant protection.

Another mechanism of *n*–3 fatty acid-mediated neuroprotection may involve their effect on complement system, which is used by the brain tissue for

eliminating aggregated and toxic proteins associated with neurological disorders (Bonifati and Kishore, 2007). An exaggerated activation of the complement system may produce deleterious effect through the activation of microglia, secretion of many proinflammatory cytokines, and generation of oxidative products. As stated above, *n*–3 fatty acids downregulate not only the expression of proinflammatory cytokines but also inhibit activities of COX and LOX, the enzymes responsible for the production of prostaglandins, leukotrienes, and thromboxanes.

Transcription factors play a very important role in induction and maintenance of oxidative stress and neuroinflammation in neurodegeration. This makes neurons sensitive to disruptions in transcription factor trafficking. There occurs a continuous nucleocytoplasmic shuttling that is regulated by binding to anchoring proteins, which mask nuclear localization/export signals and/or target the factor for degradation. Two functional groups of karyopherins, importins and exportins, mediate RanGTPase-dependent transport through the nuclear pore (Chu et al., 2007). Several studies indicate that an aberrant cytoplasmic localization of transcription factors and their regulatory kinases in degenerating neurons associated with AD, PD, and ALS. The molecular mechanisms involved in aberrant nucleocytoplasmic shuttling remain elusive. However, impaired nuclear import, enhanced export, suppression of degradation, and sequestration in protein aggregates or organelles may play an important role in this process (Chu et al., 2007). Detrimental consequences of an aberrant nucleocytoplasmic shuttling of transcription factor include suppression of neuroprotective transcription mediated by cAMP- and electrophile/antioxidant-response elements. *n*–3 fatty acids upregulate above processes, and dietary intake of long-chain *n*–3 fatty acids may have beneficial effects on neurodegereative diseases (Kang and Weylandt, 2008; Singer et al., 2008; Farooqui, 2009).

7.6 Conclusion

Neurodegenerative diseases are a heterogeneous group of diseases such as AD, PD, ALS, and HD. These diseases occur in sporadic and familial forms. Genetic and environmental factors along with lifestyle play an important role in modulating the pathogenesis of neurodegenerative diseases. Physical exercise, in the form of voluntary wheel running, induces gene expression changes in the brain. Animals that exercise show an increase in expression of BDNF, a molecule that increases neuronal survival, enhances learning, and protects against cognitive decline. Although the molecular mechanism involved in pathogenesis of neurodegenerative diseases remains unknown, oxidative stress, excitotoxicity, and neuroinflammation are closely associated with pathogenesis and progression of neurodegenerative diseases (Farooqui and Horrocks, 2007). The generation of ROS and RNS produces oxidative and/or nitrosative damage to

cellular proteins, lipids, and DNA. During aging, oxidative stress increases due to an aberrant generation of ROS/RNS and a gradual decline in cellular antioxidant defense mechanisms. In neurodegenerative diseases, neurodegeneration is initiated by microglia activation and mediated by oxidative stress and excitotoxicity. In AD, levels of DHA and DHA-containing glycerophospholipids are markedly decreased and restoration of DHA in neural membranes results in restoration of neural membrane functions. Dietary DHA incorporates into neuronal membranes and restores neuronal functions. DHA regulates synaptogenesis and maintains synaptic integrity. Synapse density is reduced in during aging. DHA and newly identified DHA-derived messenger, NPD_1, protect synapses and decrease the number of activated microglia in the hippocampal system. It is suggested that the degree of saturation of fatty acids and the position of the first double bond in essential fatty acids are the most critical factors in determining the effect of dietary fats on the risk of AD, with unsaturated fats and *n*–3 double bonds conferring protection and an overabundance of saturated fats or *n*–6 double bonds increasing the risk (Cooper, 2003).

Chronic preadministration of DHA prevents β-amyloid-induced impairment of an avoidance ability-related memory function in a rat model of AD and protects mice from synaptic loss and dendritic pathology in another model of AD. Similarly, DHA also has beneficial effects in PD and HD. Gathering evidence suggests that DHA is not a drug, but exerts its beneficial effects through multiple mechanisms, including (a) regulation of the expression of potentially neuroprotective genes, (b) activation of antiinflammatory pathways, and (c) modulation of functional properties of the synaptic membranes. In addition, DHA acts as an antiexcitotoxic agent and antioxidant. It induces antioxidant defenses by enhancing cerebral activities of catalase, glutathione peroxidase, and levels of glutathione. DHA also exerts neuroprotective effects by modulating the secretion of cytokines and inhibiting neuroinflammation and oxidative stress. DHA-derived metabolites (docosatrienes and resolvins) promote resolution and protect neural cells from apoptotic cell death. This suggests that the generation of resolvins and docosatrienes may be an internal neuroprotective mechanism for preventing brain damage.

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Chapter 8
Status and Potential Therapeutic Importance of *n*–3 Fatty Acids in Acute Metabolic Trauma and Neurotraumatic Disorders

8.1 Introduction

Normal functioning of brain requires a very high metabolic rate, which is obtained by an uninterrupted supply of both glucose and oxygen. Glucose and oxygen are needed by brain for the production of ATP, which maintains the high metabolic rate. High metabolic rate is required for maintaining the appropriate ionic gradients across the neural membranes (low intracellular Na^+, high K^+, and very low cytosolic Ca^{2+}) and optimal cellular redox potentials (Farooqui and Horrocks, 2007). Interruption in glucose and oxygen supply may result in compromised ATP generation, loss of ion homeostasis, mitochondrial dysfunction, production of ROS, such as superoxide and hydroxyl anion, and RNS, such as NO and $ONOO^-$, and changes in redox status of neural cells. These processes also contribute to cerebral edema, which is the primary cause of patient mortality after stroke. The initial response to a transient insufficiency of energy is depolarization, which causes Na^+ influx into axons. Prolonged depletion of ATP produces a massive influx of Ca^{2+} that facilitates neural cell death (Farooqui and Horrocks, 2007). Requirement of high oxygen consumption and availability of high oxygen levels along with relatively inefficient enzymic antioxidant defense also make brain tissue vulnerable to generation of ROS and ROS-mediated oxidative damage. At the injury site, all vascular cells (endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts) produce ROS primarily via cell membrane-bound NADPH oxidase. In addition, oxidation of biogenic amines by monoamine oxidases generates hydrogen peroxide (H_2O_2), which in the presence of copper generates hydroxyl radicals ($^{\cdot}OH$). Thus, CNS and PNS are vulnerable to oxygen deficiency and high oxygen levels. Neurons are particularly vulnerable to oxidative damage not only because of alterations in mitochondrial membrane potential (Atlante et al., 2000) but also due to inactivation of glutamine synthetase. This decreases glutamate uptake by glial cells and increases glutamate availability at the synapse, producing excitotoxicity (Farooqui et al., 2008). Neuronal membrane peroxidative injury is produced by reactions between ROS and unsaturated lipids in plasma membrane, leading to chemical cross-linking. This depletion of unsaturated fatty acids in neural membranes is

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associated with an alteration in membrane fluidity changing in activity of membrane-bound enzymes, ion channels, and receptors (Farooqui and Horrocks, 2007). ROS also attack proteins and DNA bases causing damage through hydroxylation, ring opening, and fragmentation. This reaction generates 8-hydroxy-2′-deoxyguanosine (8-OHdGua) (Jenkinson et al., 1999). In addition, the presence of peroxidized glycerophospholipids in neural membranes induces a membrane-packing defect, making the *sn*-2 ester bond at glycerol moiety more accessible to the action of calcium-independent PLA_2. In fact, glycerophospholipid hydroperoxides are a better substrate for PLA_2 than are the native glycerophospholipids. Glycerophospholipid hydroperoxides inhibit the reacylation of lyso-glycerophospholipids in neuronal membranes (Zaleska and Wilson, 1989). This inhibition may constitute another important mechanism whereby peroxidative processes contribute to irreversible neuronal injury and death.

8.2 Similarities and Differences Between Ischemic and Traumatic Neural Injuries

Ischemic and traumatic injuries to brain and spinal cord fall within the scope of acute neural trauma. These injuries arise from very different kinds of initial insults to brain and spinal cord tissues. Traumatic injuries are the leading cause of death and disability in young adults, and the incidence in older patients is increasing throughout the world. Ischemic injury is a metabolic insult caused by severe reduction or blockade in cerebral blood flow. This blockade not only decreases oxygen and glucose delivery to brain tissue but also results in the build-up of potentially toxic products in brain. Since glycogen storage capacity of neurons is low, there is a rapid reduction in ATP synthesis, producing a marked impairment in ion homeostasis, release of glutamate from neurons, decrease in glutamate uptake by glial cells (Fig. 8.1). These processes set the stage for the induction of excitotoxicity and upregulation in free radical production resulting in neuronal injury and cell death (Table 8.1) (Siesjö et al., 2000; Liu et al., 2004; Farooqui and Horrocks, 1994). Ischemic injury also involves the loss of synapses and damage to presynaptic nerve terminal. Neurons undergoing severe ischemic injury degenerate rapidly (minutes to hours) through necrotic cell death at the core of ischemic injury, whereas penumbral region neurons undergo delayed neuronal death by apoptosis (Farooqui et al., 2004; Farooqui, 2009). Apoptosis is characterized not only by nuclear chromatin condensation, DNA fragmentation, cell shrinkage, and bleb and apoptotic body formation, but also by alterations in mitochondrial membrane permeability due to the induction of p53, Bax, and Par-4 (Beattie et al., 2000; Mattson, 2003; Farooqui et al., 2004). Plasma membrane and other subcellular organelles, such as mitochondria and endoplasmic reticulum, remain active during

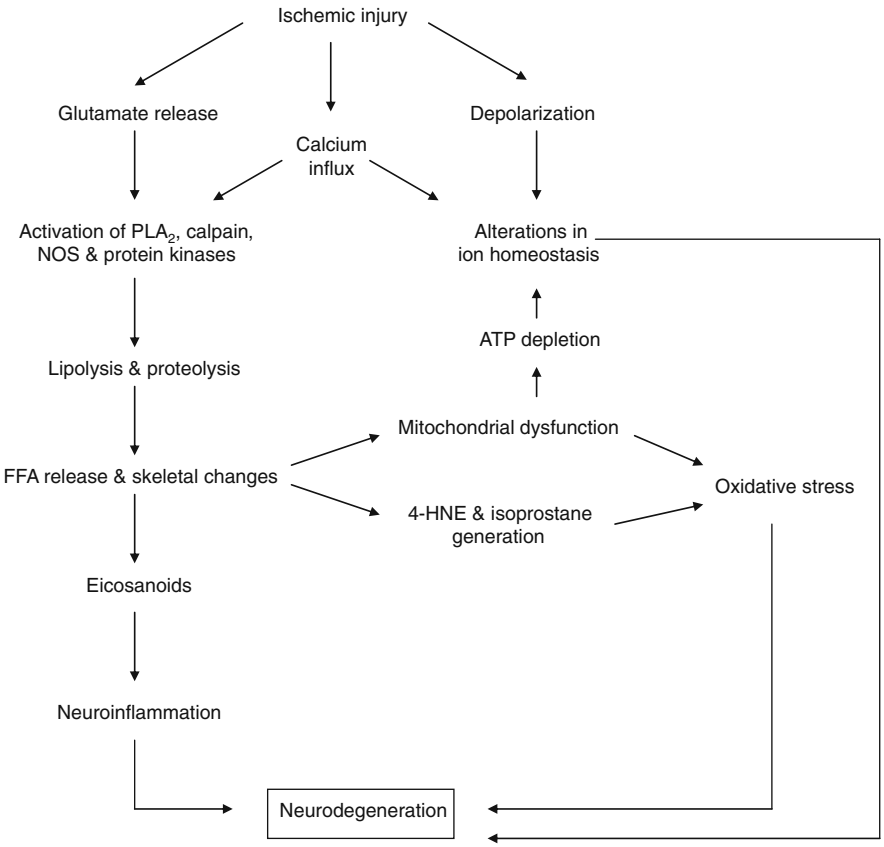

Fig. 8.1 Diagram showing neurochemical changes in ischemic injury. Phospholipase A_2 (PLA_2); nitric oxide synthase (NOS); free fatty acids (FFA); and 4-hydroxynonenal (4-HNE)

Table 8.1 Status of fatty acids, eicosanoids, 4-hydroxynonenal, excitotoxicity, oxidative stress, and neuroinflammation in acute neural trauma

Neurochemical parameter	Ischemia	Spinal cord injury	Head Injury	Epilepsy
Glycerophospholipid Metabolism	Altered	Altered	Altered	Altered
Free Fatty Acid Composition	Increased	Increased	Increased	Increased
Eicosanoids	Increased	Increased	Increased	Increased
Lipid peroxidation	Increased	Increased	Increased	Increased
4-Hydroxynonenal	Increased	Increased	Increased	Increased
Excitotoxicity	Involved	Involved	Involved	Involved
Oxidative stress	Increased	Increased	Increased	Increased
Neuroinflammation	Increased	Increased	Increased	Increased
Neurodegeneration	Increased	Increased	Increased	Increased

Information summarized from the following sources (Farooqui et al., 1994, 2007a; Farooqui and Horrocks, 2006; Farooqui and Farooqui, 2009).

apoptosis. In contrast, necrosis is characterized by the permeabilization of plasma membrane, rapid decrease in ATP, sudden loss of ion homeostasis, glutathione depletion, and activation of lysosomal enzymes, resulting in a passive cell death through lysis (Nicotera and Lipton, 1999; Farooqui et al., 2004). Common participants in necrotic cell death irrespective of the stimulus are calcium and ROS. During necrosis, elevated cytosolic calcium levels produce mitochondrial calcium overload, abnormalities in bioenergetics, and activation of proteases and phospholipases. As stated earlier, ROS initiates damage to lipids, proteins, and DNA that consequently results in ion balance deregulation and loss of membrane integrity. Membrane destabilization during necrosis is also mediated by other factors such as activation of PLA_2 and calpains. Furthermore, necrosis releases cellular contents with immunomodulatory factors that lead to recognition and engulfment by phagocytes and the subsequent immunological response (Farooqui et al., 2007a). Apoptosis and necrosis are interrelated mechanisms with considerable overlap between biochemical events. Apoptosis and necrosis not only require well-organized signaling cascade but also involve extensive cross-talk between several biochemical and molecular events at different cellular levels (Farooqui, 2009).

Glial cell response to ischemic injury is extremely complex. On one side, astrocytes protect neurons from excitotoxicity, and on the other side, astrocytes contribute to the extracellular glutamate increase during severe ischemic injury (Dronne et al., 2007). Thus, under conditions of mild ischemic situation, astrocytes take up glutamate via the glutamate transporter and potassium via the $Na^+/K^+/Cl^-$ cotransporter, which limits glutamate levels and increase potassium in the extracellular space. In contrast, under severe ischemic conditions, astrocytes are unable to maintain potassium homeostasis and contribute to the excitotoxicity by expelling glutamate out of the cells via the reversed glutamate transporter (Dronne et al., 2007). Oligodendroglial cells are highly vulnerable to glutamate-mediated ischemic injury. Competitive inhibition of cystine uptake and accumulation of intracellular peroxides along with chromatin fragmentation and condensation are also associated with ischemia-reperfusion injury-mediated oligodendroglial cell death (Farooqui and Horrocks, 2007).

Microglial cells respond to ischemic injury by transforming themselves into activated form. They not only change their shape into "ameboid" morphology but also release matrix metalloproteinases, ROS, RNS, and other proinflammatory cytokines (Farooqui and Horrocks, 2007) followed by neutrophil entry after the onset and monocyte infiltration later at the injury site. Microglial cells contain a wide range of receptors that allow them to identify and internalize numerous pathogens. In brain tissue, NF-κB and MAPK, p38, are associated with proinflammatory cytokine production, generation of ROS, production of eicosanoids, and neurodegeneration

following acute metabolic injury (Vexier et al., 2006; Barone and Kilgore, 2006; Sun et al., 2007).

In contrast to metabolic injury in ischemia, traumatic injury is caused by the mechanical impact and shear forces (McIntosh et al., 1998; Fiskum et al., 1999; Bramlett and Dietrich, 2004). The traumatic injury to head and spinal cord consists of two broadly defined components: a primary component, attributable to the mechanical insult itself, and a secondary component that consist of a series of systemic and local neurochemical and pathophysiological changes that occur in brain and spinal cord after the initial traumatic insult (Bramlett and Dietrich, 2004; Klussmann and Martin-Villalba, 2005). The primary injury causes a rapid deformation of brain and spinal cord tissues, leading to rupture of neural cell membranes, release of intracellular contents, and disruption of blood flow and breakdown of the blood–brain barrier. In contrast, secondary injury to brain and spinal cord is characterized by alterations in glial cell metabolism including activation of microglia and astroglia and demyelination involving oligodendroglia (Beattie et al., 2000; Farooqui et al., 2004). Neurochemically, secondary injury is characterized by excitotoxicity, a process by which high levels of glutamate excite neurons and bring about their demise and oxidative stress, cytotoxic consequences produced by oxygen free radicals (Farooqui et al., 2004, 2008; Farooqui, 2009; Farooqui and Horrocks, 2009) (Fig. 8.2). Induction and participation of cytokines and the complement system during traumatic and ischemic injuries propagate and maintain inflammatory reactions and responses with the involvement of PLA_2, COX, and p38 MAPK-mediated signaling. Like ischemic injury, in head and spinal cord injuries neurons die rapidly (hours to days) at the injury core by necrotic cell death, whereas in the surrounding area neurons undergo apoptotic cell death (several days to months) (McIntosh et al., 1998; Farooqui et al., 2004). Furthermore, cerebral ischemic injury as well as head and spinal cord injuries trigger similar autoprotective mechanisms including the induction of heat-shock proteins, antiinflammatory cytokines, and production of endogenous antioxidants (Leker and Shohami, 2002). Genetic and gender factors also play important role in pathophysiology of metabolic and traumatic injuries. However, the fact that these injuries arise from different types of primary insults result in diverse cellular vulnerability patterns as well as a spectrum of injury processes. Blunt spinal cord and head injuries result in shear forces that produce primary membrane damage to neuronal cell bodies, white matter structures and vascular beds as well as secondary injury mechanisms (Bramlett and Dietrich, 2004; Farooqui and Horrocks, 2009). Severe cerebral ischemic insults produce metabolic stress, ionic perturbations, and a complex cascade of biochemical and molecular events ultimately resulting in neuronal death (Farooqui and Horrocks, 2007). This suggests that similar therapeutic strategies can be used for the treatment of ischemic and traumatic brain injuries.

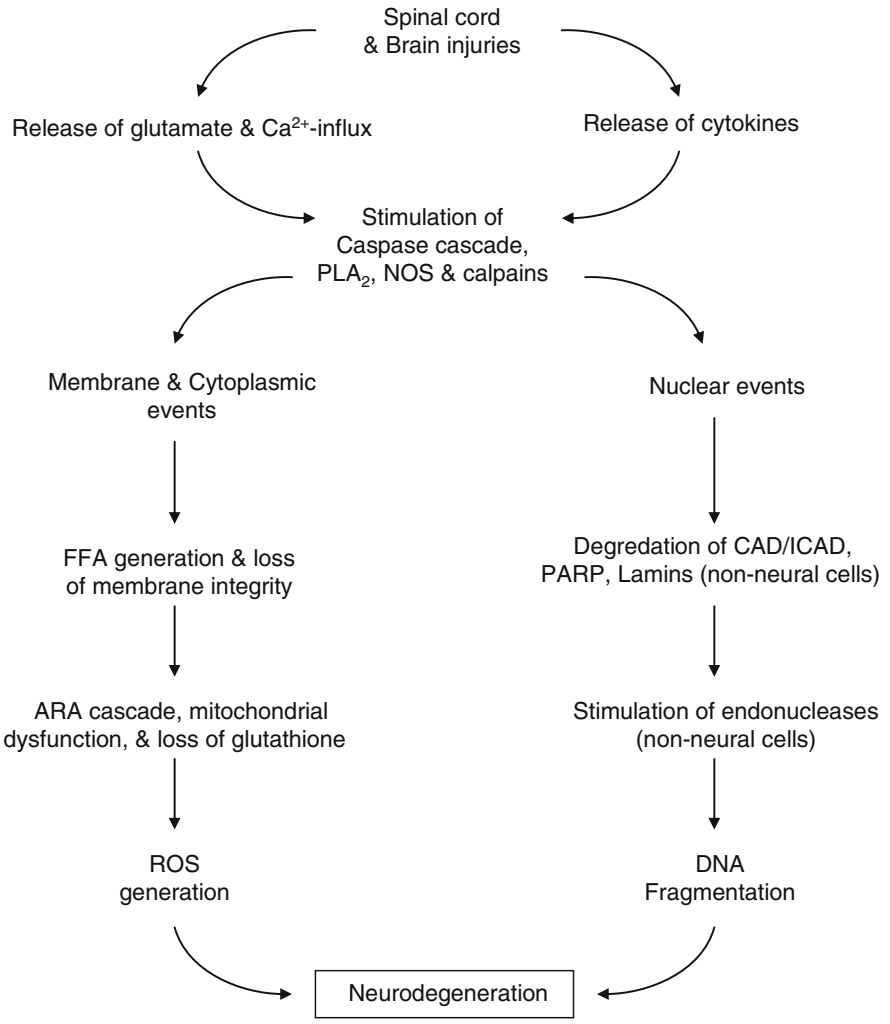

Fig. 8.2 Diagram showing neurochemical changes in traumatic injury to brain and spinal cord. Phospholipase A_2 (PLA_2); nitric oxide synthase (NOS); free fatty acids (FFA); arachidonic acid (ARA); reactive oxygen species (ROS*);*poly (ADP-ribose) polymerase (PARP); caspase-activated DNase (CAD); and inhibitor of CAD (ICAD)

8.3 Glycerophospholipids and Fatty Acids Alterations in Ischemic Injury

Under normal conditions, the release of ARA is coupled with the stimulation of glutamate, dopamine, muscarinic, serotonin, P_2-purinergic, cytokine, growth factor receptors along with the activation of various isoforms of PLA_2 in brain

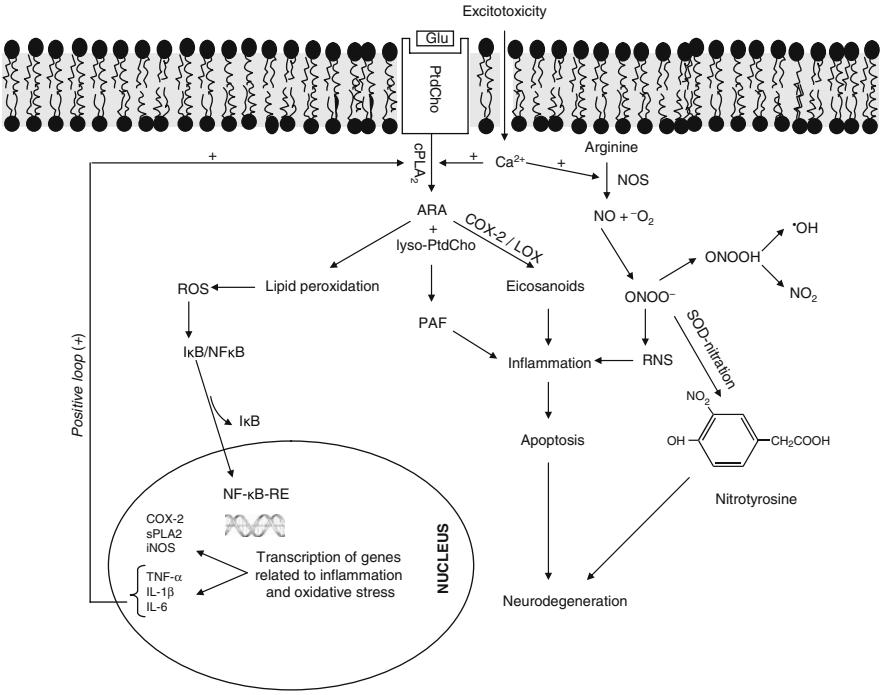

Fig. 8.3 Glutamate-mediated degradation of neuronal membrane glycerophospholipids and generation of reactive nitrogen species during excitotoxicity. Glutamate (Glu); phosphatidylcholine (PtdCho); lysophosphatidylcholine (lyso-PtdCho); cytosolic phospholipase A_2 ($cPLA_2$); secretory phospholipase A_2 ($sPLA_2$); arachidonic acid (ARA); platelet-activating factor (PAF); cyclooxygenase-2 (COX-2); lipoxygenase (LOX); nitric oxide synthase (NOS); peroxynitrite (ONOO-); reactive nitrogen species (RNS); reactive oxygen species (ROS); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6; inhibitory form of nuclear factor kappa B (IκB/NF-κB); nuclear factor κB-response element (NF-κB-RE); and inhibitory subunit of NF-κB (IκB)

(Dumuis et al., 1988; Farooqui et al., 2006). The release of ARA either acts as a second messenger itself or is metabolized to eicosanoids (Fig. 8.3). Lysophospholipid, the other product of PLA_2-catalyzed reaction, is either rapidly reacylated to restore normal neural membrane phospholipid composition or acetylated to platelet-activating factor. In contrast, ischemic injury produces overstimulation of isoforms of phospholipases A_2 and C, marked alterations in neural membrane glycerophospholipids with a massive release of free fatty acids and accumulation of lysoglycerophospholipids in brain (Table 8.1). This is called as the Bazan effect (Bazan, 1970; Edgar et al., 1982; Rordorf et al., 1991; Farooqui et al., 1994; Sun and Foudin, 1984; Sapirstein and Bonventre, 2000; Adibhatla et al., 2003; Sun et al., 2004; Farooqui et al., 2006). In addition, excessive glutamate-receptor stimulation produces the activation of nitric oxide synthase resulting in the generation of NO, which combines with superoxide to

form peroxynitrite. Peroxynitrite not only reacts with sulfhydryl groups but also hydroxylates the aromatic rings of amino acid residues (Beckman et al., 1992). Peroxynitrite reduces mitochondrial respiration, inhibits membrane pumps, depletes cellular glutathione, and damages DNA by activating poly (ADP-ribose) synthase (Pryor and Squadrito, 1995). All these processes may contribute to inflammation in ischemic injury (Fig. 8.3).

How does the accumulation of free fatty acids (Bazan effect) results in neuronal cell death in ischemic injury is still unknown? However, following points should be carefully considered. (a) Prolonged stimulation of isoforms of PLA_2 and PLC may cause the loss of essential glycerophospholipids with the accumulation of ARA along with increased levels of lysophospholipids, which may have a detergent-like effect on neuronal membranes. (b) Sustained generation of free ARA may cause uncoupling of oxidative phosphorylation resulting in mitochondrial dysfunction (Farooqui et al., 2006). ARA also produces mitochondrial swelling and induces changes in membrane permeability by regulating ion-channels. ARA modulates gene expression and can turn on genes that promote neuronal cell death. ARA also inhibits glutamate uptake mediated by excitatory amino acid transporters. (c) Platelet-activating factor, which is synthesized by acetylation of lysophospholipids, can mediate the activation of leukocytes and inflammation on the endothelial cell surface. (d) The accumulation of ARA triggers an uncontrolled "arachidonic acid cascade." This sets the stage for increased production of high levels of eicosanoids and ROS through the upregulation of COX, LOX, and EPOX (Phillis et al., 2006). ROS stimulates NF-κB, which is present in the cytoplasm in an inhibitory form (attached to its inhibitory protein, I-κB) (Fig. 8.3). Upon stimulation, IκB is rapidly phosphorylated, ubiquinated, and then degraded by proteasomes, resulting in the release and translocation of active NF-κB to the nucleus where it mediates the transcription of more than 150 genes including many genes implicated in inflammation, oxidative stress, and immune responses. These genes code for enzymes, intracellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), E-selectin, cytokines, and matrix metalloproteinases (MMP) (Block and Hong, 2005; Farooqui et al., 2007a,b). The peroxidation of ARA produces 4-HNE that impairs the activity of key metabolic enzymes and transporters, including Na^+, K^+-ATPase, glucose 6-phosphate dehydrogenase, the glutamate transporter in astrocytes, and the glucose transporter (Mark et al., 1997). As stated above, the reaction between ROS and proteins or unsaturated lipids leads to a chemical cross-linking of membrane proteins and lipids and a reduction in membrane unsaturation. This depletion of unsaturated membrane lipids is associated with decreased membrane fluidity and decreases in the activity of membrane-bound enzymes, ion-channels, and receptors (Farooqui and Horrocks, 2007). (e) Finally, an uncontrolled sustained increase in calcium influx through increased glycerophospholipid degradation can lead to increased membrane permeability and stimulation of many enzymes associated with lipolysis, proteolysis, and disaggregation of microtubules with a disruption of cytoskeletal and membrane

structures. These processes together with compromised energy metabolism and alterations in the activity of glutathione cycle may be responsible for neuronal injury (Farooqui and Horrocks, 1994, 2007). The most compelling evidence for the involvement of $cPLA_2$ in neurodegeneration comes from recent reports indicating that mice with targeted deletion of the gene encoding $cPLA_2$ show reduced infarct size, less edema, and fewer neurological defects after cerebral ischemic injury (Bonventre et al., 1997). Studies on brain glycerophospholipid metabolism in $cPLA_2$-knockout mice indicate that there is no net change in unesterified ARA. However, a 50% reduction in esterified ARA in phosphatidylcholine supports the involvement of $cPLA_2$ (Rosenberger et al., 2003). $cPLA_2$-knockout mice also show a 62% reduction in the rate of formation of prostaglandin E_2, supporting the view that there is a coupling between $cPLA_2$ and cyclooxygenase activities (Murakami et al., 1997; Bosetti and Weerasinghe, 2003; Phillis et al., 2006). Primary neural cell cultures prepared from $cPLA_2$-deficient mice also generate significantly smaller amounts of prostaglandins and leukotrienes (Uozumi and Shimizu, 2002). This suggests that the $cPLA_2$-deletion contributes to a decrease in arachidonic acid supply to COX-2, and a resultant decrease in synthesis of prostaglandins. Collective evidence suggests that the Bazan effect along with ATP depletion results in alterations in ion homeostasis, and loss of cellular redox may be responsible for neural cell death in ischemic injury.

Ischemic injury is accompanied by post-ischemic neurogenesis, a process that has been identified as a compensatory mechanism to repair the damaged brain after stroke (Guan et al., 2003; Smith, 2003; Carmichael, 2008). In the adult rodent brain, neurogenesis from neural stem/progenitor cells continues in two regions: the subgranular zone in the dentate gyrus and the subventricular zone lining the lateral ventricles. The generated neuroblasts migrate to their appropriate location and differentiate to mature granule cells and olfactory bulb interneurons, respectively. Following ischemic injury, neuroblasts generated in the subventricular zone migrate also into areas which are not normally neurogenic, such as striatum and cerebral cortex (Ekdahl et al., 2009). Recent studies indicate that microglia under traumatic situations support the different steps in neurogenesis, progenitor proliferation, survival, migration, and differentiation. Several factors are released following ischemic insult by microglial cells of the injured tissue. These factors mediate proliferation, differentiation, and migration of neural stem cells (Ohab and Carmichael, 2008; Kalluri and Dempsey, 2008). Some of them are stimulatory while others are inhibitory for neurogenesis. A major stimulatory factor is insulin-like growth factor-I (IGF-1). It induces proliferation in the presence of fibroblast growth factor-2 (FGF-2), and promotes differentiation in the absence of FGF-2 (Kalluri and Dempsey, 2008). Transforming growth factor-β induces the differentiation of neurons while inhibiting the proliferation of neural stem cells. IGF-1 is mostly produced by the liver, but has been shown to modulate normal brain function as well as brain response to injury. IGF-1 levels are modulated by exercise. Serum IGF-I levels are modified in stroke (Guan et al., 2003; Smith, 2003). It is

becoming increasingly evident that IGF-I modulates the BDNF system to mediate the synaptic and cognitive plasticity. BDNF not only facilitates long-term potentiation, an electrophysiological correlate of learning and memory, but also increases the activities of free radical-scavenging enzymes and hence protect neurons against oxidative stress (Pelleymounter et al., 1996).

8.4 Effect of *n*– 3 Fatty Acids on Ischemic Injury

As stated in Chapter 7, introduction of vegetable oils in early 1950s has resulted in an enormous increase in the consumption of *n*–6 fatty acids in American diet. Consumption of higher amounts of *n*–6 fatty acids not only elevates production of eicosanoids but also upregulates the expression of proinflammatory cytokines, such as IL-1, IL-6, and TNF- α. Production of these cytokines results in hyperneuroinflammation and oxidative stress. In contrast, *n*–3 fatty acids consumption produces antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, vasodilatory, and immunosuppressive effects (Simopoulos, 2006; Farooqui et al., 2007b, 2008). These beneficial effects of *n*–3 fatty acids block ischemic injury-mediated brain damage.

n–3 fatty acids have neuroprotective effects against ischemic brain damage in rats (Okada et al., 1996; Terano et al., 1999; Cao et al., 2005, 2006, 2007; Strokin et al., 2003, 2006; Bas et al., 2007; Ozen et al., 2008). DHA administration not only facilitates cerebral blood flow but also inhibits PLA_2, COX, and LOX activities, and reduces levels of brain post-ischemic prostaglandins, thromboxanes, and leukotrienes. In mammalian retina, DHA prevents sensory retina from ischemic-reperfusion cell damage (Murayama et al., 2002). Although the molecular mechanism associated with neuroprotective effects is not fully understood, DHA may produce its effects in several ways. DHA acts as an antioxidant (Hossain et al., 1998). DHA induces antioxidant defenses by enhancing cerebral activities of catalase, glutathione peroxidase, and levels of glutathione (Hossain et al., 1999; Choi-Kwon et al., 2004; Ozen et al., 2008). Treatment of ischemic rats with DHA is accompanied by decreases in blood–brain barrier disruption, brain edema, and elevation of antioxidative capacity, as evidenced by a reduction in malondialdehyde level, and increase in superoxide dismutase activity and glutathione level along with increase in phosphorylation of ERK and elevation in Bcl-2 expression following the chronic daily administration for 3 days (Pan et al., 2008; Ozen et al., 2008). DHA also exerts its beneficial effect by inhibiting production of inflammatory cytokines and antagonizing the metabolism of arachidonic acid and its downstream metabolites. It downregulates NF-κB via a PPAR-γ-dependent pathway, suggesting that *n*–3 fatty acid-mediated activation of PPAR-γ may be another neuroprotective mechanism. Other neuroprotective mechanism may involve inhibition of oxidative stress-mediated neurodegeneration by DHA-derived, NPD_1, which downregulates eicosanoid production following

ischemic/reperfusion injury (Fig. 8.3). NPD_1 also upregulates the anti-apoptotic Bcl-2 proteins, Bcl-2 and bclxL, and decreases the expression of the proapoptotic proteins, Bax and Bad (Bazan, 2005). NPD_1 blocks reperfusion-induced leukocyte infiltration, proinflammatory signaling, and infarct size. NPD_1 not only inhibits cytokine-mediated COX-2 expression but also promotes homeostatic regulation of the integrity of neural cells particularly during oxidative stress, and this protective signaling may be relevant to neural cell survival following ischemic injury (Bazan, 2009). These processes strengthen the survival mechanisms through ERK-mediated and/or Bcl-2-mediated prosurvival cascade. Resolvins, another lipid mediator of *n*–3 fatty acid metabolism, also have neuroprotective effects. Neuroprotectins and resolvins are collectively known as docosanoids. They attenuate neutrophil migration and tissue injury in ischemia-reperfusion injury. Their production is elevated in the later stages of an inflammatory response, at which time they enhance the removal of neutrophils. The generation of docosanoids may be an internal neuroprotective mechanism for preventing neural damage (Serhan, 2005a,b; Bazan, 2005, 2006; Bazan et al., 2005; Lukiw et al., 2005).

Dietary EPA and DHA slow the progression of ischemic injury. Although the molecular mechanism associated with mitigation of progression is not known, NF-E2-related factor-2 (Nrf2), a Cap-N-Collar family of basic region-leucine zipper transcription factor that plays a major role in transcriptional activation of antioxidant response element (ARE)-driven genes, has been closely associated with mitigation of progression (Gao et al., 2007). Nrf2-regulated gene expression is involved in detoxification of ROS. The oxidized *n*–3 fatty acids have been shown to react directly with the negative regulator of Nrf2, Keap1, initiating Keap1 dissociation with Cullin3, thereby inducing Nrf2-directed gene expression (Gao et al., 2007). Analyses of oxidized EPA by lipidomics indicate the presence of novel cyclopentenone-containing molecules termed J3-isoprostanes, which mediate the induction of Nrf2-directed gene expression. Nrf2 pathway is activated by endogenous 15d-PGJ_2 during inflammation. 15d-PGJ_2 is a specific potent ligand for the peroxisome proliferators activator receptor-γ. It is proposed that formation of J-ring containing compounds from *n*–3 fatty acid oxidation in vivo can reach concentrations high enough to induce Nrf2-based cellular defense systems (Gao et al., 2007). In addition, dietary *n*–3 fatty acids may provide neuroprotection from ischemic injury by upregulating the expression of IGF-1, which interacts with vascular endothelium to activate nitric oxide synthase, thereby promoting vascular health. IGF-I may also promote the structural integrity of cerebral arteries, thereby offering protection from further injury (McCarty, 2003). The upregulation of IGF-1 promotes longlasting functional recovery after cerebral infarction. The improved functional performance is paralleled by enhanced neovascularization and neurogenesis (Zhu et al., 2008). Furthermore, IGF-1 may also act antagonizing the interferon-γ mediated IL-1β expression in microglial cell activation, and this process may contribute to neuroprotection (Maher et al., 2006).

Dietary supplementation of ARA and DHA also improves the spatial memory and hippocampal long-term potentiation in rodents and cognitive function of humans. Although the molecular mechanism associated with long-term potentiation and cognitive function is not fully understood, it is proposed that G-protein-coupled receptor 40 (GRP40) may play an important role in these processes. GRP40 receptor mediates the amplification of long-chain fatty acid effect on glucose-induced insulin secretion (Yamashima et al., 2008). Studies on expression of GPR40, in the neurogenic niche of the adult monkey hippocampus under normal and postischemic conditions, indicate that GPR40 immunoreactivity is present in neural progenitors, immature neurons, astrocytes, and endothelial cells of the subgranular zone (SGZ) of the dentate gyrus (DG), a well-known neurogenic niche within the adult brain (Ma et al., 2008). There is a significant increase in GPR40 protein in the second week following global cerebral ischemia as compared with the control. This is compatible with the post-ischemic increment of GPR40-positive cells in the SGZ. It is proposed that DHA and ARA by acting as endogenous ligands may facilitate adult hippocampal neurogenesis in primates via GPR40 receptors, and there is a putative link among PUFA, GPR40, and hippocampal newborn neurons. Collective evidence suggests that PUFA–GPR40 interaction may be crucial for adult neurogenesis and/or memory formation. (Ma et al., 2008).

8.5 Glycerophospholipids, Fatty Acid, BDNF, and cAMP in Spinal Cord Injury

Neurochemical alterations in injured spinal cord include a rise in intracellular calcium, activation of phospholipases and lipases (Anderson et al., 1985), and hydrolysis of glycerophospholipids, which results in production of free fatty acids, diacylglycerols, eicosanoids, and lipid peroxides (Demediuk et al., 1985). In the spinal cord, plasmalogens account for about one-third of the total glycerophospholipids. During the first minute of spinal cord injury, 10% of the ethanolamine plasmalogen (PlsEtn) is hydrolyzed with an overall loss of 18% found at 30 min after injury (Horrocks et al., 1985). Similar observations are made in rabbit spinal cord following injury (Lukácová et al., 1996). The loss of PlsEtn after compression and ischemic injuries is due to the stimulation of various isoforms of PLA_2 caused by shear stress (Taylor, 1988; Liu et al., 2006; Liu et al., 2007; Titsworth et al., 2008). This stimulation of PLA_2 isoforms may contribute to increase in Ca^{2+} influx, impairment in mitochondrial function, and subsequently generation of ROS. Increase in Ca^{2+} not only produces uncoupling of mitochondrial electron transport, but also activates many Ca^{2+}-dependent enzymes including calpains, nitric oxide synthases, protein kinases, protein phosphatases, and matrix metalloproteinases (Fig. 8.2) (Pavel et al., 2001; Ray et al., 2003; Arundine and Tymianski, 2004; Xiong et al., 2007). In spinal cord injury, increase in intracellular Ca^{2+} also activates the

cysteine protease, calpain, leading to the degradation of several cellular targets including cytoskeletal protein (α-spectrin) (Xiong et al., 2007). Western blot analysis of α-spectrin breakdown products reveal that calpain-mediated degradation of α-spectrin generates 145-kDa fragments one hour following spinal cord trauma although the peak increase in 145-kDa fragment does not occur until 72 h post-injury. The activation of calpain may be linked to peroxynitrite-mediated secondary oxidative impairment of calcium homeostasis. In addition to phospholipases, nitric oxide synthase, and calpains, the stimulation of Ca^{2+}-dependent protein kinases, protein phosphatases, and matrix metalloproteinases, along with a rapid depletion in ATP level, generation of high levels of ROS, alterations in ion homeostasis, and cellular redox, and induction of inflammation may contribute to irreversible damage and neurodegeneration in spinal cord injury (Denecker et al., 2001; Svensson and Yaksh, 2002). Necrotic cell death normally occurs at the core of injury site whereas apoptotic cell death occurs several hours or days after injury in the surrounding area. Collectively, these studies indicate that inflammation and oxidative stress are major components of secondary injury in spinal cord trauma. In addition to above changes, spinal cord injury is also accompanied by the upregulation in expression of BDNF mRNA (Ikeda et al., 2001), which reaches maximum levels 24 h after the spinal cord trauma. Expression of BDNF mRNA comes back to the levels of sham-operated control animals within 3 days of the injury. In situ hybridization studies indicate that BDNF is widely expressed among the different cell types in the spinal cord, including motor and sensory neurons, and in glia cells (astrocytes and oligodendrocyte). BDNF is also expressed in putative macrophages and/or microglia, but not until day 7 following the spinal cord injury. It is proposed that BDNF is synthesized in both neurons and astrocytes during the acute response to spinal cord injury to perform a neuroprotective role in earlier phases. This is followed by a later phase of expression in which the expression of BDNF occurs in macrophages and/or microglia, apparently for neural cell restoration and survival (Ikeda et al., 2001). Initial trauma to spinal cord also downregulates CuZn superoxide dismutase (CuZnSOD) within 24 h, but continuous infusion of BDNF blocks the acute downregulation of CuZnSOD expression (Ikeda et al., 2002). In situ hybridization studies show that CuZnSOD is expressed in both neurons and glia (Ikeda et al., 2002). Similarly, myelin basic protein (MBP) expression is greatly downregulated after injury, but BDNF or progesterone administration facilitates the recovery of MBP expression nearly to a control level after 2 weeks. Furthermore, BDNF or progesterone administration is also accompanied by behavioral recovery (Ikeda et al., 2002; De Nicola et al., 2006). The increased expression of BDNF or progesterone-mediated neurochemical changes correlate with increase in immunoreactivity for the BDNF receptor TrkB and for phosphorylated CREB in motor neurons. Other molecular parameters, such as choline acetyltransferase and Na,K-ATPase, also respond to BDNF or progesterone treatment positively suggesting that

BDNF and progesterone treatments work through similar mechanisms. These results suggest BDNF or progesterone usefulness in human clinical applications. The attenuation of CuZnSOD downregulation may be related to a protective effect of BDNF or progesterone, and the promotion of MBP upregulation may be related to a longlasting restorative effect of these mediators in a paracrine or autocrine fashion (Ikeda et al., 2001, 2002; De Nicola et al., 2006).

8.6 Effect of *n*–3 Fatty Acids on Spinal Cord Injury

Studies on the effect of PUFA after lateral spinal cord injury rats indicate that injections of α-linolenic acid (ALA) and DHA 30 min after injury not only result in significant improvement in locomotor performance and neuroprotection but also reduce lesion size, inhibit apoptosis, and increase neuronal and oligodendrocyte survival (Lang-Lazdunski et al., 2003; King et al., 2006; Michael-Titus, 2007). The molecular mechanism associated with neuroprotective effects of *n*–3 fatty acids in spinal cord injury is not fully understood. However, based on reduction or oxidation of proteins and RNA/DNA and inhibition of lipid peroxidation, it is suggested that the neuroprotective effect of *n*–3 fatty acids may be associated not only with antioxidant activity of these fatty acids but also with generation of resolvins and neuroprotectins (Fig. 8.3) (King et al., 2006; Michael-Titus, 2007; Huang et al., 2007). In addition, neuroprotective effect may also involve the interactions among TWIK-related K^+ channel (TREK), TWEK-related K^+ channel (TRAAK), and *n*–3 fatty acids (Lauritzen et al., 2000). *n*–3 fatty acids may also act by downregulating NF-κB and proapoptotic protein, Bax immunoreactivity, and preventing apoptotic and necrotic neuronal death (Lang-Lazdunski et al., 2003). Furthermore, ALA may be converted to EPA, which is oxidized by COX and LOX into antiinflammatory series-3 prostaglandins. Series-3 prostaglandins also inhibit oxidation of ARA into proinflammatory eicisanoids. In contrast, ARA injections in rats produce a significantly worse outcome of injured animals than controls. Parallel studies in a facial nerve injury model in mice also show pro-regenerative effects of chronic dietary administration of DHA after nerve lesion. Furthermore, in SH-SY5Y cells, EPA plus BDNF exert beneficial effects on cell survival through modulating neurotrophin receptor expression (Kou et al., 2008). These studies suggest that there is a striking difference in efficacy of *n*–3 and *n*–6 fatty acids on the outcome of spinal cord and facial nerve injuries, with *n*–3 fatty acids being neuroprotective and *n*–6 fatty acids having damaging effects (King et al., 2006; Michael-Titus, 2007; Huang et al., 2007). Thus, treatment with *n*–3 fatty acids may represent promising therapeutic agents for the management of spinal cord injury.

8.7 Glycerophospholipid and Fatty Acids Alterations in Traumatic Brain Injury

Traumatic brain injury (TBI) is caused by a blow or jolt to the head or a penetrating head injury that disrupts the normal function of the brain. Like spinal cord injury, neurochemical changes in TBI include glutamate-mediated increased degradation of neural membrane glycerophospholipids, activation of $cPLA_2$ and PLC/DAG-lipase pathway, and accumulation of ARA, diacylglycerols, and generation of eicosanoids (Fig. 8.2) (McIntosh et al., 1998; Schuhmann et al., 2003; Shohami et al., 1987, 1989; Wei et al., 1982; Dhillon et al., 1994, 1999; Homayoun et al., 1997, 2000). These neurochemical changes occur in traumatic as well as fluid percussion (FPI) models of brain injury. Impact TBI induces marked elevations in free fatty acids (threefold) and diacylglycerols (twofold) in the injured cortex and to a lesser extent in the contralateral cortex compared to sham-operated rats. The stimulation of PLA_2 and PLC results in increased fatty acid release, elevation in free radicals and eicosanoids generation, impaired mitochondrial function, and generation of ROS. These processes may be closely associated with neurodegeneration. Other neurochemical changes in TBI include release of cytokines, activation of caspases, loss of glutathione, glial cell reactions involving both activated microglia and astroglia, and demyelination involving oligodendroglia (McIntosh et al., 1998). Astrocyte-mediated brain edema and increase in intracranial pressure are major consequences of FPI. Exposure of cultured rat astrocytes to 5 atm of pressure produces significant cell swelling at 1 to 24 h following FPI with maximal swelling at 3 h. Neurochemical changes that contribute to astrocytic swelling include oxidative stress, mitochondrial permeability transition (mPT), and MAPKs (extracellular signal-regulated kinase 1/2, c-Jun-N-terminal kinase, and p38-MAPK). Oxidative stress and MAPKs activate NF-κB, a transcription factor that activates many genes, including inducible nitric oxide synthase (iNOS), secretory phospholipase A_2 ($sPLA_2$), and COX-2. iNOS generates NO, which is known to cause astrocyte swelling. In TBI and FPI, these neurochemical changes are reduced by antioxidants, *N*-nitro-L-arginine methyl ester, the mPT inhibitor cyclosporin A, and inhibitors of MAPKs block trauma-induced astrocyte swelling (Jayakumar et al., 2008). Both apoptotic as well as necrotic cell death occur in TBI and FPI (Wingrave et al., 2003). In humans, free fatty acid levels in cerebrospinal fluid can be used as a predictive marker of outcome following traumatic brain injury (Pilitsis et al., 2003).

8.8 Effect of *n*– 3 Fatty Acids on Traumatic Brain Injury

It is well known that adult neurons, unlike embryonic neurons, normally do not regenerate their axons after injury because of the presence of myelin-associated inhibitory molecules, astrogliosis and formation of glial scar, and a deficiency

of growth-promoting substances, such as growth factors (Filbin, 2003; Silver and Miller, 2004; Goldberg and Barres, 2000). cAMP and BDNF are intrinsic factors that are known to contribute to axonal regeneration (Tanaka et al., 2007). Facilitation of cAMP signaling by the addition of dibutyryl-cAMP or a variety of growth factors (brain-derived neurotrophic factor (BDNF) and neurotrophin-3) overcomes inhibition produced by myelin-associated protein and myelin (Cai et al., 1999; Gao et al., 2003). BDNF upregulates levels of cAMP in neuronal cultures. Treatment with BDNF activates their specific receptor tyrosine kinases (Trk A, Trk B, Trk C) that are expressed in various types of neurons and forms a complex with the p75 neurotrophin receptor. The activated receptor tyrosine kinases then transiently activate ERKs, which in turn modulate phosphodiesterases, enzymes that hydrolyze cAMP and neutralize cAMP-mediated signaling (Tanaka et al., 2003). Neurotransmitters, adhesion molecules, and neuromodulators modulate cAMP-mediated signaling. Thus, cAMP contributes to survival of injured neurons, neuronal differentiation and axonal regeneration, and formation of neurite (Tanaka et al., 2007), and protects neurons from acute neural trauma.

Studies on effects *n*–3 fatty acids in TBI indicate that dietary *n*–3 fatty acids supplementation helps brain to cope with the pathophysiological effects of TBI (Wu et al., 2003, 2004a,b). Thus, FPI-mediated increase in oxidative stress and impaired learning ability in the Morris water maze can be prevented by *n*–3 fatty acids supplementation. BDNF is a member of the nerve growth factor family of trophic factors. As stated above, it plays multiple functions including a role in the promotion of neuronal survival and nerve fiber elongation in both the central and the peripheral nervous systems. FPI also reduces levels of BDNF, synapsin I, and CREB. It is well known that BDNF facilitates synaptic transmission and learning ability by modulating synapsin I and CREB. Supplementation of *n*–3 fatty acids in the diet not only normalizes levels of BDNF, synapsin I, and CREB, but reduces oxidative damage, and restores learning and memory disability (Wu et al., 2003, 2004a). The inhibition of TBI-mediated oxidative stress indicates a benevolent effect of *n*–3 fatty acid-containing diet on maintenance neuronal plasticity. In contrast, consumption of high saturated fat diet reduces levels of BDNF, to the extent that compromises neuroplasticity, impairs cognitive function, and aggravates the outcome of TBI (Wu et al., 2004b, 2005). Supplementation of the high-fat diet with vitamin E dramatically reduces oxidative damage, normalizes levels of BDNF, synapsin I, and CREB, produced by high-fat diet consumption. The molecular mechanism associated with *n*–3 fatty acid-mediated modulation of BDNF may involve binding to the cell surface Trk receptors, a family of three receptor tyrosine kinases, each of which can be activated by neurotrophins, such as nerve growth factor (NGF), BDNF, and neurotrophins 3 and 4 (NT3 and NT4) (Huang and Reichardt, 2003; Rao et al., 2007). The cytoplasmic domains of Trk receptors contain several sites of tyrosine phosphorylation that recruit

intermediates in intracellular signaling cascades. Trk receptor signaling activates Ras, Rap-1, and the Cdc-42-Rac-Rho family, as well as pathways regulated by MAP kinase, PtdIns 3-kinase, and phospholipase-C-γ-PKC cascade (Huang and Reichardt, 2003). In general, signals emanating from Trk receptors support survival, growth, and synaptic strengthening. It is likely that *n*–3 fatty acid supports neural cell survival and maintenance of neuroplasticity through modulating MAP kinase, PtdIns 3-kinase, and phospholipase-C-γ-PKC cascade. These studies suggest that consumption of high-fat diet impairs synaptic plasticity and cognitive function through BDNF-mediated mechanism. Thus, consumption of high-fat diet has harmful effects, whereas supplementation of *n*–3 fatty acid-enriched diet produces beneficial effect on TBI through the maintenance of BDNF-mediated neuroplasticity associated with learning and memory (Wu et al., 2003, 2004a,b, 2005; Vaynman et al., 2004).

8.9 Glycerophospholipid and Fatty Acid Alterations in Epilepsy

Epilepsy is a devastating neurological disorder of multiple causes in which the normal pattern of neuronal activity is disturbed due to overactive electrical discharges, producing a temporary communication problem among nerve cells and resulting in recurrent unprovoked seizures. These processes result in strange sensations, emotional and behavior alterations, muscle spasms, and loss of consciousness. At morphological and molecular levels, epilepsy involves long-lasting neuroplastic changes in the expression of neurotransmitters and their receptors, ion channels, sprouting, reorganization of synapses, as well as reactive gliosis (Spencer, 2007). It is well known that epileptic seizures in rat and human brain produce marked increase in levels of exicitatory amino acids in the focal, compared to nonfocal, regions of the cerebral cortex (Nadi et al., 1987; Sherwin et al., 1988; Bazan et al., 2002). Increase in excitatory amino acid levels results in overstimulation of excitatory amino acid receptors producing influx of Ca^{2+} in rat hippocampus during seizure activity (Griffiths et al., 1983; Meldrum, 1993; Liu et al., 1997; Wasterlain et al., 1993; Bazan et al., 2002). The accumulation of Ca^{2+} not only regulates activities of many Ca^{2+}-dependent enzymes (PLA_2 and C, calpains, nitric oxide synthases, nonreceptor protein tyrosine kinases Src and Fyn and a serine-threonine kinase, Ca^{2+}-calmodulin-dependent protein kinase II, and calcineurin), but also modulates the expression of many genes associated with neuroplasticity, gliosis, and cognitive functions (Wasterlain et al., 1993; Bazan et al., 2002; Zagulska-Szymczak et al., 2001). Levels of free fatty acids and diacylglycerols in rat brain rise rapidly with the onset of epileptic seizures, indicating the activation of $cPLA_2$ and PLC (Visioli et al., 1994). Collective evidence suggests that epileptic seizures are accompanied by uncontrolled neuronal firing partially facilitated by the temporary changes in fatty acid composition of neural membranes. Alterations in

neural membrane fatty acid composition along with the generation of non-enzymic ARA oxidation products may be closely associated with the pathogenesis of epilepsy (Bazan et al., 2002). Thus, epileptic seizures induction results in generation of F_2-IsoP and IsoF (Patel et al., 2008). IsoF, but not F_2-IsoP, formation coincides with mitochondrial dysfunction and oxidative stress. Seizure-mediated generation of F_2-IsoP coincides with the peak hypoxia phase, whereas IsoF formation coincides with the "reoxygenation" phase. These results demonstrate seizure-mediated increase in IsoF formation, and its correlation with changes in hippocampal pO_2 and mitochondrial dysfunction may be closely associated with the pathogenesis of epilepsy (Patel et al., 2008).

8.10 Effect of *n*– 3 Fatty Acids on Epilepsy

The *n*–3 fatty acids-enriched diet alleviates and/or reduces the frequency of epileptic seizures in animal models and patients with epilepsy. Animal and human studies indicate that *n*–3 fatty acids decrease seizure thresholds and lower inflammatory mediators in animal models and patients with epilepsy. Thus, a randomized, placebo-controlled parallel group trial (57 patients), which is completed in a 12-week double-blind phase, indicates that daily *n*–3 fatty acid supplementation reduces seizure frequency over the first 6 weeks of treatment in the *n*–3 fatty acid supplement group, but this effect is not sustained. The supplementation of *n*–3 fatty acids results in a significant increase in EPA and DHA levels with a reciprocal reduction in ARA and linoleic acid levels (Yuen and Sander, 2004; Yuen et al., 2005, 2008). Similarly, a 3-month pilot randomized double-blind placebo-controlled study using ^{31}Phosphorus neurospectroscopy demonstrate that in chronic refractory epilepsy, *n*–3 fatty acid supplementation may be associated with reduction in neural membrane glycerophospholipid breakdown, an improvement in brain energy metabolism, and an increased level of glycerophospholipids in membranes and/or vesicle bilayers in neural cells. The unfavorable biochemical changes have been reported to occur in the placebo group (Puri et al., 2007). Recent studies on seizure resistance fat-1 transgenic mice and wild-type controls indicate that *n*–3 fatty acids inhibit seizures induced by pentylenetetrazol (Taha et al., 2008). It is shown that increased brain levels of *n*–3 fatty acids in transgenic fat-1 male mice increases latency to seizure onset by 45%, relative to wild-type controls. Compared with wild-type littermates, transgenic fat-1 mice have significantly higher levels of docosahexaenoic acid and total *n*–3 fatty acids in brain total lipid extracts and glycerophospholipids. It is clearly shown that DHA levels in brain are positively correlated to seizure latency ($p < 0.05$) (Taha et al., 2008). Collective evidence from animal and human studies indicates that *n*–3 fatty acids produce anticonvulsant effects and can be safely used for the treatment of epilepsy (Mostofsky et al., 2004). This suggestion is supported by the beneficial

effects of ketogenic diet (a high-fat, adequate-protein, low-carbohydrate diet) in children with refractory seizures (Freeman et al., 1998; Kossoff, 2004).

Although the molecular mechanism involved with the beneficial effects of *n*–3 fatty acids on epilepsy is not fully understood, *n*–3 fatty acids may act by inducing the expression of neuronal uncoupling proteins (UCPs), a collective upregulation of numerous energy metabolism genes, and mitochondrial biogenesis. In addition, DHA not only attenuates epileptic activity mainly through the frequency-dependent blockade of Na^+ channels in rat hippocampal slices (Young et al., 2000) but also blocks PTZ-mediated unhealthy brain excitation and stimulation that induces convulsions in hippocampal neurons (Sevik et al., 1998). The effects of *n*–3 fatty acids in neural cell may limit ROS generation, increase energy production, and stabilize synaptic functions. Impaired energy production promotes onset and progression of epileptic seizures, and sustained ATP levels are critical to cellular homeostasis and may have both direct and indirect influence on pathogenic mechanisms associated with epilepsy. Although it is unlikely that a single mechanism can explain the clinical benefits of *n*–3 fatty acids, coordinated neurochemical changes in signal transduction processes may stabilize synaptic function and increase the resistance to seizures throughout the brain (Yuen et al., 2005; Puri et al., 2007; Taha et al., 2008).

8.11 Glycerophospholipids and Fatty Acids in Kainic Acid-Induced Neural Cell Injury

Systemic administration of kainic acid (KA) into adult rats induces seizures and increases $cPLA_2$ immunoreactivity in neurons at 1 and 3 days after injection. The $cPLA_2$ immunoreactivity is also increased in astrocytes at 1, 2, 4, and 11 weeks after KA injection (Fig. 8.4) (Sandhya et al., 1998). It is proposed that increased $cPLA_2$ activity in neurons in KA-induced toxicity may be involved in neurodegeneration (Sandhya et al., 1998; Farooqui et al., 1997), whereas elevation in $cPLA_2$ in astrocytes may be associated with gliosis (Farooqui et al., 2001). Like $cPLA_2$, increase in activity of PLC, NOS, caspase-3, and calpains activities also occurs during KA-mediated neurotoxicity (Fig. 8.4) (Farooqui et al., 2001). During KA-induced toxicity, elevated levels of ARA and eicosanoids may produce a variety of detrimental effects on neural membrane structures, activities of membrane enzymes, and neurotransmitter uptake systems (Farooqui et al., 1997). Thus, KA receptor-mediated degradation of neural membrane glycerophospholipids, calcium overload, and oxidative stress can disrupt cellular homeostasis and can threaten cellular integrity and viability (Farooqui et al., 2001, 2003).

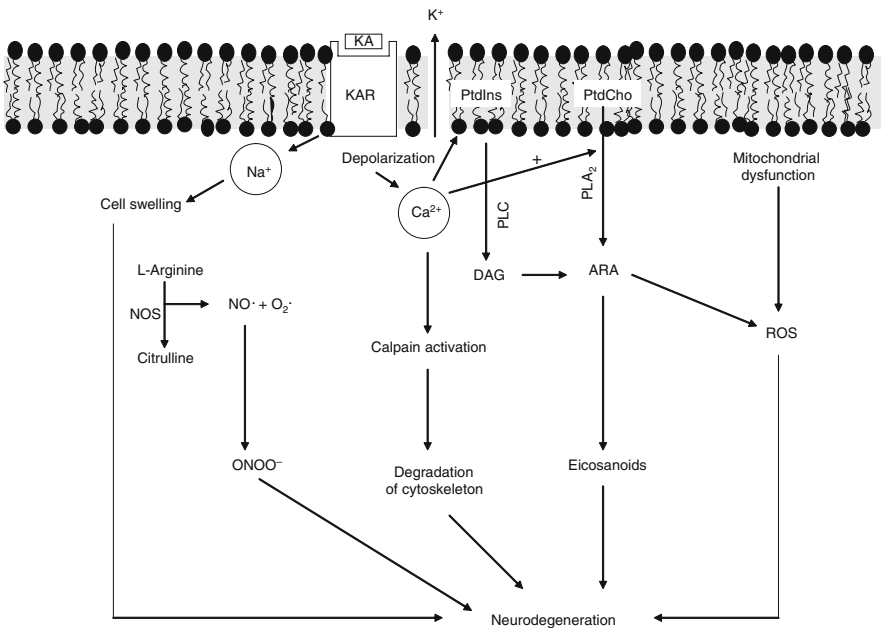

Fig. 8.4 Kainic acid-mediated neurotoxicity in brain. Cytosolic phospholipase A_2 (cPLA$_2$); phospholipase C (PLC); arachidonic acid (ARA); nitric oxide synthase (NOS); peroxynitrite ($ONOO^-$); kainic acid (KA); kainic acid receptor (KAR); diacylglycerol (DAG); inositol-1,4,5-trisphosphate (Ins*P*3); and reactive oxygen species (ROS)

8.12 Effect of *n*–3 Fatty Acids in Kainic Acid-Induced Neural Cell Injury

Supplementation of diet with DHA has been reported to protect from KA-induced retinal damage in 3-week-old female Wistar rats (Mizota et al., 2001). DHA levels in the serum and retina are significantly increased after DHA supplementation. The levels of ARA in serum are decreased with DHA supplementation. Levels of ARA in retina are not affected by DHA supplementation. The b-waves of the electroretinograms recorded after KA injections are significantly attenuated in both groups of rats. However, the attenuation is significantly less in the DHA-supplemented rats than in gum arabic-supplemented control rats. Furthermore, numbers of cells in the inner nuclear layer and ganglion cell layer are significantly higher in DHA-supplemented rats than gum arabic-supplemented control animals. Collective evidence suggests that DHA supplementation can partially block KA neurotoxicity in the rat retina, and DHA plays a role in modulating neuronal excitability by reducing KA-induced responses in the retina (Mizota et al., 2001). Studies on the effect of DHA on hippocampal cultures indicate that DHA protects neurons against glutamate-mediated

cytotoxicity. It increases cell viability, inhibits nitric oxide generation and calcium influx, and upregulates activities of antioxidant enzymes, such as glutathione peroxidase and glutathione reductase. DHA does not increase the levels of glutathione in cell cultures (Wang et al., 2003).

8.13 Interactions Among Excitotoxicity, Oxidative Stress, and Neuroinflammation Following Acute Neural Trauma

Excitotoxicity, oxidative stress, and neuroinflammation are closely linked with the pathogenesis of ischemia, traumatic brain, and spinal cord injuries (Högyes et al., 2003; Farooqui et al., 2007b; Farooqui and Horrocks, 2007). These processes are downregulated by *n*–3 fatty acids and their metabolites (Fig. 8.5). Thus, experimental data on cell culture and animal models are

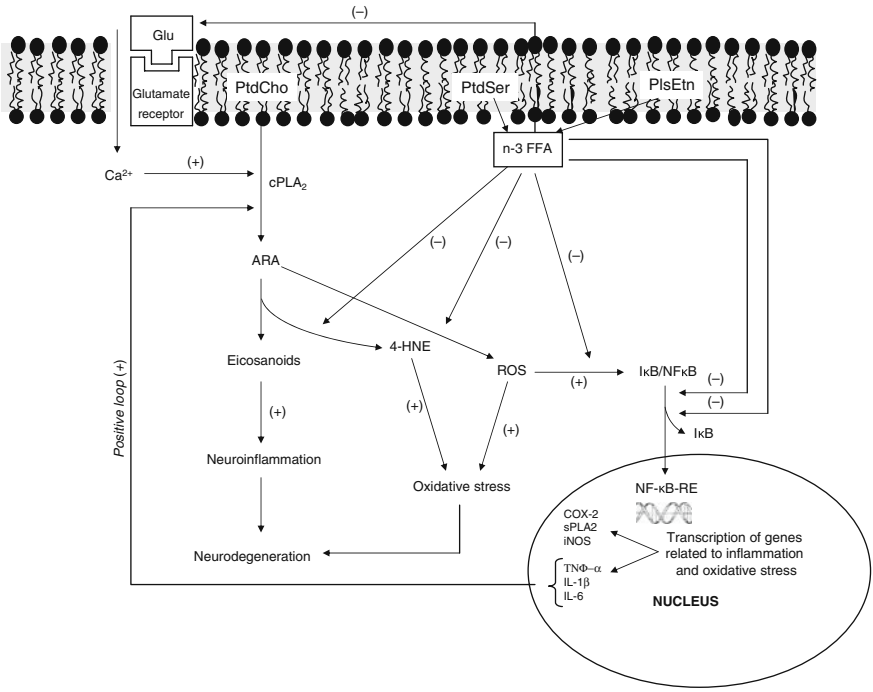

Fig. 8.5 Antiexcitotoxic, antioxidants, and antiinflammatory effects of *n*–3 fatty acids in brain. Cytosolic phospholipase A_2 ($cPLA_2$); secretory phospholipase A_2($sPLA_2$); arachidonic acid (ARA); 4-hydroxynonenal (4-HNE); reactive oxygen species (ROS); inhibitory form of nuclear factor kappa B (IκB/NF-κB); nuclear factor κB-response element (NF-κB-RE); inhibitory subunit of NF-κB (IκB); tumor necrosis factor-α (TNF-α); interleukin-1β (IL-1β); interleukin-6 (IL-6); cyclooxygenase-2 (COX-2); inducible nitric oxide synthase (iNOS); and *n*-3 fatty acids (*n*–3 FFA); positive sign (+) represents stimulation; and negative sign (–) represents inhibition

consistent with neuroprotective effects of *n*–3 fatty acids. Although the clinical evidence that *n*–3 fatty acids may prevent or slow the course of acute neural trauma is controversial, recently there have been several developments (Farooqui et al., 2007b, 2008). Neurons are more susceptible to excitotoxic, oxidative, and inflammatory injuries than glial cells. In brain and spinal cord, glutamate and ROS not only contribute to neuronal injury by intensifying the expression of inflammatory and stress-sensitive genes, including genes for cytokines and chemokines (Hayes et al., 2002; Farooqui and Horrocks, 2006), but also by activating mechanisms that result in a microglia and astrocytes-mediated secondary neuronal damage (Block and Hong, 2005; Farooqui and Horrocks, 2007; Farooqui et al., 2007b). These activated glial cells are histopathological hallmarks of acute neural trauma and are closely associated neurodegenerative processes (Farooqui et al., 2007b). The direct contact of activated glia with neurons per se is not necessary for neurodegeneration. Immune mediators, e.g., nitric oxide, eicosanoids, ROS, and proinflammatory cytokines and chemokines, released by activated microglial cells and astrocytes may act as endogenous neurotoxins that facilitate neurodegeneration. Neuritic beading, focal bead-like swellings in the dendrites and axons are neuropathological features of ischemic injury, traumatic brain and spinal cord injuries, and epilepsy as well as some neurodegenerative diseases. This neuritic beading is a characteristic feature of neuronal cell dysfunction produced by abnormal signaling of *N*-methyl-D-aspartate (NMDA) receptor (Takeuchi et al., 2005). The molecular mechanism associated with neuritic beading that precedes neurodegeneration is not fully understood. However, a rapid drop in intracellular ATP levels is known to occur. Detailed investigations indicate that actual neurite beads contain collapsed cytoskeletal proteins and motor proteins arising from impaired neuronal transport secondary to cellular energy loss. The loss in intracellular ATP levels is caused by the inhibition of mitochondrial respiratory chain complex IV activity downstream of NMDA receptor signaling. Blockage of NMDA receptors nearly completely prevents mitochondrial dysfunction and neurotoxicity, suggesting that NMDA receptor antagonists may be an effective therapeutic approach for acute neural trauma (Takeuchi et al., 2005; Gilgun-Sherki et al., 2006; Wang et al., 2006; Farooqui et al., 2008).

From the therapeutic point of view, it is becoming increasingly evident that antioxidants are almost ineffective in providing neuroprotection in ischemic and neurotraumatic injuries. *n*–3 fatty acids have antiexcitotoxic, antioxidant, anitiinflammatory properties and are the oldest molecules consumed by humans (Simopoulos, 2006; Farooqui et al., 2008). The discovery of resolvins and neuroprotectins, which are lipid mediators of EPA and DHA metabolism, is very important from therapeutic point of view. These metabolites antagonize the effects of eicosanoids, modulate leukocyte trafficking, and downregulate the expression of cytokines in glial cells and modulate interactions among neurons, astrocytes, oligodendrocytes, microglia, and cells of the microvasculature (Serhan, 2005a,b; Bazan, 2005, 2006, 2007). The infusion of neuroprotectin D_1

(NPD_1), following ischemic reperfusion injury or during oxidative stress in cell culture, downregulates oxidative stress and apoptotic DNA damage. NPD_1 also upregulates the antiapoptotic Bcl-2 proteins, Bcl-2 and bclxL, and decreases the expression of the proapoptotic proteins, Bax and Bad. Furthermore, this metabolite inhibits caspase-3 activity, and blocks IL-1-mediated expression of COX-2 (Bazan, 2005). Similarly, recent studies show that exposure of retinal pigment epithelial cells to oxidative stress activates stereospecific docosahexaenoate-oxygenation pathways leading to the formation of NPD_1 and resolvins in the presence of aspirin (Bazan, 2007). Based on above studies, it is proposed that generation of resolvins and NPD_1 and resolvins is an internal neuroprotective mechanism for preventing brain tissue damage (Serhan, 2005b; Bazan, 2005; Lukiw et al., 2005). In addition, *n*–3 fatty acids also downregulate NF-κB activity (Farooqui and Horrocks, 2007), and suppress genes induced by proinflammatory cytokines and other mediators released by glial cells (Gilgun-Sherki et al., 2006; Wang et al., 2006). The effectiveness of *n*–3 fatty acids in protecting against acute neural trauma depends on their ability to cross the blood–brain barrier, their potential in terms of subcellular distribution in mitochondria, plasma membrane, and cytoplasm. A clear appreciation of the potential therapeutic ability of *n*–3 fatty acids would emerge only when in vivo importance of interactions among excitotoxicity, neuroinflammation, and oxidative stress is realized and fully understood at the molecular level (Farooqui and Horrocks, 2007; Farooqui et al., 2007b, 2008).

8.14 Conclusion

Acute neural trauma includes ischemic injury, traumatic brain injury, spinal cord injury, and epilepsy. Ischemia is a metabolic insult caused by severe reduction or blockade in cerebral blood flow. In contrast, mechanical impact and shear forces cause traumatic head and spinal injuries. Ischemic and traumatic injuries to brain and spinal cord are characterized by ATP depletion, release of glutamate and free fatty acids, mitochondrial dysfunction, overproduction of ROS and RNS, accumulation of eicosanoids, upregulation of cytokines, alterations in ion homeostasis, and changes in the cellular redox. The above-mentioned neurochemical changes in acute traumatic injuries may result in neurodegeneration. Other factors that prone neural cells to acute neural injury include inherited genetic abnormalities and problems in the immune system. *n*–3 fatty acids have antiatherogenic, antithrombotic, antioxidant, antiexcitotoxic, and antiinflammatory properties.

DHA-enriched diet significantly increases spatial learning ability through the upregulation of pro-brain-derived neurotrophic factor (BDNF) and mature BDNF. In addition, levels of the activated forms of CREB and synapsin I are also elevated by the DHA-enriched diet. Furthermore, DHA-enriched diet not only downregulates hippocampal oxidized protein levels but also upregulates

hippocampal Akt and CaMKII. Collective evidence suggests that DHA enriched diet enhances BDNF-mediated synaptic plasticity, a process that promotes mental health and reduces risk of neurological disorders.

The dietary intake of *n*–3 fatty acids also causes suppression of proinflammatory cytokines (TNF-α, IL-1, and IL-2) and decrease in adhesion molecule expression. These effects take place at the level of gene expression, and may be responsible for antagonism of the effects of ARA-derived mediators or through more direct actions on the intracellular signaling pathways leading to activation of transcription factors such as NF-κB. *n*–3 fatty acids down-regulate the activity of NF-κB. Based on these effects, it is proposed that the principle mechanism of neuroprotective action(s) of *n*–3 fatty acids may be the suppression of TNF-α and IL synthesis and release, generation of resolvins and neuroprotectins, and modulation of hypothalamic–pituitary–adrenal antiinflammatory responses. In addition, *n*–3 fatty acids also influence the biophysical state of the neural cell membranes. Thus, there appears to be a close interaction between the central nervous system, endocrine organs, cytokines, and dietary *n*–3 fatty acids. This may explain why these fatty acids are beneficial in the management of conditions such as ischemia, traumatic injury to brain and spinal cord, and epilepsy.

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Chapter 9
Status and Potential Therapeutic Importance of *n*–3 Fatty Acids in Neuropsychiatric Disorders

9.1 Introduction

Neuropsychiatric disorders include both neurodevelopmental disorders and behavioral or psychological difficulties associated with some neurological disorders. Examples of neuropsychiatric disorders are schizophrenia, some forms of bipolar affective disorders, autism, mood disorders, some forms of manic depression, attention-deficit disorder, dementia, tardive dyskinesia, atypical spells, irritability, and organic mental disorder (Table 9.1). These disorders are closely associated with the abnormalities in cerebral cortex and limbic system (thalamus, hypothalamus, hippocampus, and amygdale). Although epidemiology studies indicate that genetic factors play an important role in the risk for neuropsychiatric disorders, vulnerability genes have not yet been identified in unequivocal form (Harrison and Owen, 2003). One of the most important characteristic of neuropsychiatric disorders is the impairment of cognitive processing, which refers to signal transduction-mediated processes associated with everyday problem-solving behavior. This includes the ability to learn and store the memory, to retrieve stored memory for further use, and to apply the stored memory to efficiently solve problems (Gallagher, 2004). The impairment of cognitive process may be caused by overexpression or underexpression of certain genes or other unknown factors that result in behavioral symptoms, such as thoughts or actions, delusions, and hallucinations, which are the hallmarks of many neuropsychiatric disorders including schizophrenia and bipolar disorders. In addition, there is substantial evidence indicating that depressive illness is not a mere neurochemical disease, but is linked to gray matter atrophy due to the reduced number/size of neurons and glia in brain. Importantly, neurogenesis (birth/maturation of functional new neurons) continues to occur throughout the lifetime in human adult brains (e.g., hippocampus). The neurogenesis is impaired by multiple not-fully defined factors, such as aging, chronic stress-induced increase of glucocorticoids, and excitotoxicity, accounting for brain atrophy in patients with depressive illness and neurodegenerative diseases (Wada et al., 2005; Farooqui and Horrocks, 2007).

Neuropathological and neuroimaging studies also reveal a number of anatomical and neurochemical alterations in neurocircuits in specific brain area of

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Table 9.1 Status of fatty acid and glycerophospholipid metabolism in neuropsychiatric disorders

Disorder	Glycerophospholipids/free fatty acid metabolism	References
Schizophrenia	Abnormal	Yao and Kammen (2004)
Bipolar disorders	Abnormal	Yildiz et al. (2001)
Dyslexia	Abnormal	Richardson (2006)
Autism	Abnormal	Bell et al. (2004)
ADHD	Abnormal	Young et al. (2004)
Sleep disturbances	Abnormal	Irmisch et al. (2007)
Chronic fatigue syndrome	Abnormal	Maes et al. (2005)
Child problem behavioral syndrome	Abnormal	Krabbendam et al. (2007)

neuropsychiatric disease patients. Cellular aberrations, such as decreased neuronal size, increased cellular packing density suggesting a disruption in neuronal connectivity, particularly in the dorsolateral prefrontal cortex, and distortions in neuronal orientation, abnormalities of cerebral white matter, oligodendrocytes, and myelin along with alterations in signal transduction processes have been observed in immunocytochemical, ultrastructural, and neurochemical studies (Arnold and Trojanowski, 1996; Blitzer et al., 2005). In addition, neuochemical studies indicate that in neuropsychiatric diseases several neurotransmitter systems are simultaneously altered within a single microcircuit, and each transmitter system shows circuitry changes in more than one region. Changes in microcircuits and neurotransmitters (synthesis and transport) may not only vary on a region-by-region basis but also from one disease to another. Both macro- and microcircuitry within the specific brain system (such as limbic system) may serve as "triggers" for the onset of neuropsychiatric condition (Benes, 2000; Harrison, 1999). Brain imaging studies also indicate alterations in cerebral blood flow and glucose utilization in the limbic system and prefrontal cortex of patients with major depression. Furthermore, depressed patients have decreased glucose metabolism in a number of brain regions, and this hypometabolic state correlates negatively with severity of depression (Ito et al., 1996; Kimbrell et al., 2002).

Aging, genetic vulnerability, alterations in blood flow, oxidative stress, neuroinflammation, and environmental changes are major risk factors for the development of both neurological and neuropsychiatric diseases such as schizophrenia, bipolar affective disorders, autism, mood disorders, depression, and attention-deficit disorder (Fig. 9.1). In addition, certain neurodegenerative diseases, such as Parkinson disease (PD) and Alzheimer disease (AD), are accompanied by neuropsychiatric symptoms due to age-related changes in neurotransmission, neuroplasticity, and signal transduction processes (Hornykiewicz, 1987; Becker et al.,1997; Blitzer et al., 2005), and these symptoms have

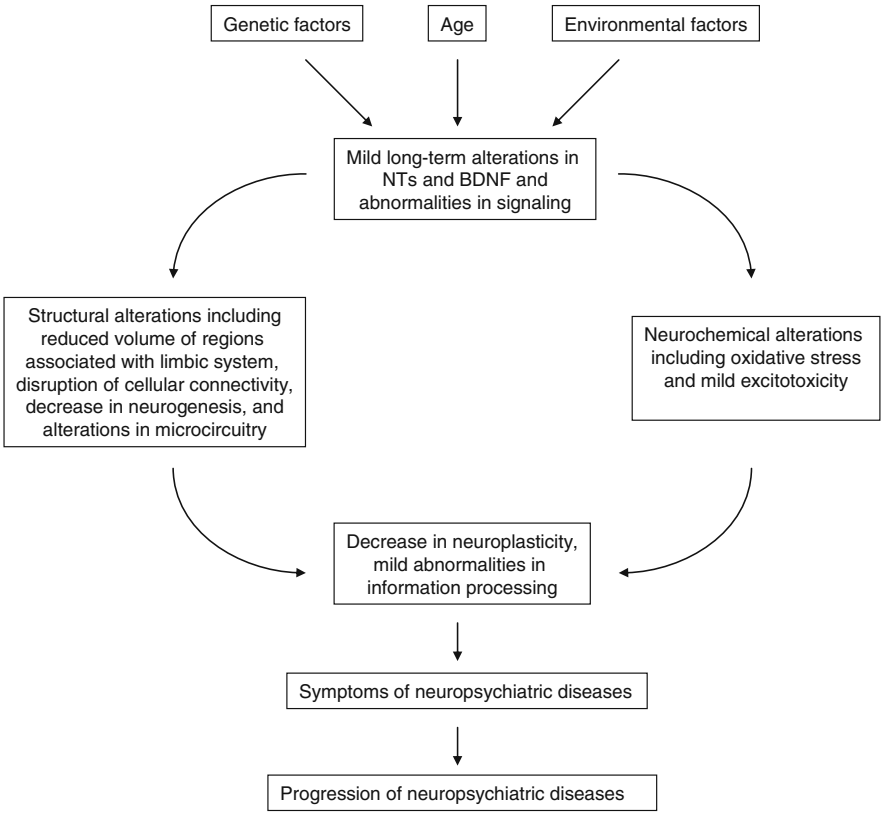

Fig. 9.1 Hypothetical diagram showing contribution of risk factors in the etiology of neuropsychiatric diseases. Neurotransmitters (NTs) and brain-derived neurotrophic factor (BDNF)

important clinical implications for both patients and physicians. In addition, the expression of apolipoprotein D (ApoD), a 24–29 kDa glycoprotein, is also increased in human neurodegenerative (AD and PD) and neuropsychiatric disorders (schizophrenia and bipolar disorders), suggesting that ApoD may act as a marker for the pathology of neurodegenerative and neuropsychiatric diseases (Thomas et al., 2003). ApoD is upregulated 500-fold at the site of the sciatic nerve crush injury in the rats, and its immunoreactivity is markedly increased in kainic acid neurotoxicity (Ong et al., 1997; Boyles et al., 1990; Farooqui et al., 2008a). In vitro studies indicate that ApoD can carry ARA and sterols (Vogt and Skerra, 2001), and may be associated the clearance and/or repair of damaged neural membranes, perhaps quenching harmful material released by neural cells in response to damage or recruiting lipids to expanding membranes. In the etiology of neurodegenerative and neuropsychiatric disorders in which ApoD is elevated, oxidative stress has been reported to play an

important role (Andersen, 2004). It is proposed that expression of ApoD by glial cells may provide local trophic support or sequester ARA, thereby tempering or slowing inflammation. ApoD also exhibits complex regulation by antipsychotic drug treatment, and may represent a distinguishing mechanism of typical versus atypical drugs. The precise role of apoD in the CNS and disease remains to be elucidated, but it is suggested that it plays an important role in the regulation of ARA signaling and metabolism, providing further support for phospholipid membrane pathology in schizophrenia (Thomas et al., 2003). Thus, like neurodegenerative disorders, neuropsychiatric diseases are multifactorial diseases and may involve several factors. They include mild long-term deficit in several neurotransmitters, deficiency of *n*–3 fatty acids, neurotrophic factors (BDNF), upregulation in expression of ApoD, and alterations in certain genes. Most neurodegenerative (see Chapter 8) and neuropsychiatric diseases have a characteristic late-onset pattern and therefore predominantly affect the elderly human population.

9.2 Oxidative Stress and Neuroinflammation in Neuropsychiatric Diseases

Oxidative stress, triggered by numerous unexpected environmental, social, and pathological stimuli occurring during the life of animals and humans, may disturb normal physiological equilibrium (homeostasis) and contribute to neuropsychiatric disorders. Although some oxidative stress is good and essential for neural cell survival, chronic and long-term stress causes alterations in immune and endocrine systems, producing detrimental changes in animal and human brain (Farooqui et al., 2008a). Recent animal model studies demonstrate that exposure to even brief periods of intense stress is sufficient to cause significant structural remodeling of the principle projection neurons within the rodent prefrontal cortex (Holmes and Wellman, 2008). In addition, several lines of evidence indicate that stress-mediated alterations in prefrontal cortex neuronal morphology may contribute to deficits in rodent executive functions, such as working memory, attentional set-shifting and cognitive flexibility, as well as emotional dysregulation in the form of impaired fear extinction (Mizoguchi et al., 2000; Holmes and Wellman, 2008). The molecular mechanism of stress-mediated changes in prefrontal cortex morphology and function are not fully understood. However, exposure to stress may cause oxidative/nitrosative and neuroinflammation-mediated changes in brain tissue in general and prefrontal cortex in particular. These changes are mediated by catecholamines, glucocorticoids, and glutamate, and are shown to be balanced by antiinflammatory pathways in the brain. In particular, acute restraint stress is followed by the upregulation of COX-2 and subsequent generation of proinflammatory prostaglandin, PGE_2, release in the cortex. Concomitantly, the synthesis of antiinflammatory prostaglandin, 15d-PGJ_2,and the activation of its nuclear target, PPARγ, are also produced.

This constitutes a possible endogenous antiinflammatory mechanism of defense against excessive oxidative stress and inflammation. In healthy brain, proinflammatory and antiinflammatory pathways remain in homeostasis and without antiinflammatory responses through the expression of antiinflammatory transcription factors and generation of lipid mediators, such as lipoxins, resolvins, and neuroprotectins; brain tissue would be a sitting duck for various types of neuropsychiatric and neurodegenerative diseases and microbial, viral, and prion infection (Farooqui et al., 2007; Farooqui and Horrocks, 2007). Generation of nitric oxide (NO) and an excessive production of pro-oxidants through the activation of PLA_2 and COX-2 enzymes in various brain areas are major causes of impaired neuronal functional and morphological damage to neural membranes (Farooqui and Horrocks, 2007). The stress-mediated overactivation of above signal transduction pathways depends not only on the activation of NMDA and catecholamine receptors but also on the activation of the transcription factor NF-κB. In the case of inducible NO synthase (iNOS), release of TNF-α also promotes for its expression. Collectively, these studies suggest that chronic stress and inflammation mediated by hyperstimulation of catecholamines, glucocorticoids, and glutamate receptors and cross-talk among these receptors may contribute to the pathogenesis of neuropsychiatric diseases (Farooqui and Horrocks, 2007).

9.3 Dysregulation of Neurotransmission in Neuropsychiatric Diseases

In general, neuropsychiatric diseases are characterized by dysregulation of dopaminergic, glutamatergic, serotonergic, cholinergic, and GABAergic neurotransmission at many different levels including the synthesis, storage, release, reuptake, and inactivation of neurotransmitters (Harrison, 1999). Two types of neurotransmitter receptors, namely iontropic and metabotropic, are found in neural membranes. Iontropic receptors are ligand-gated ion channels that modulate ionic currents and neural membrane potential. In contrast, metabotropic receptors are linked to the regulation of second messengers (cAMP, diacylglycerol, PtdIns, and eicosanoids) through G-protein-coupled or G-protein-independent mechanisms (Fig. 9.2). Iontropic receptors mediate fast synaptic responses throughout the CNS, whereas metabotropic receptors are associated with slow synaptic responses leading to neuromodulation and long-term regulation (Svenningsson et al., 2004; Valjent et al., 2005). Alterations in iontropic and metabotropic neurotransmitter systems have been reported to occur within discrete neurocircuits in the brain of neuropsychiatric disease patients, and psychotic symptoms, such as hallucinations, delusions, paranoid ideation, anxiety, depression, hyperactivity, and autism, are probably caused not only by altering neurotransmitter metabolism, storage, and release

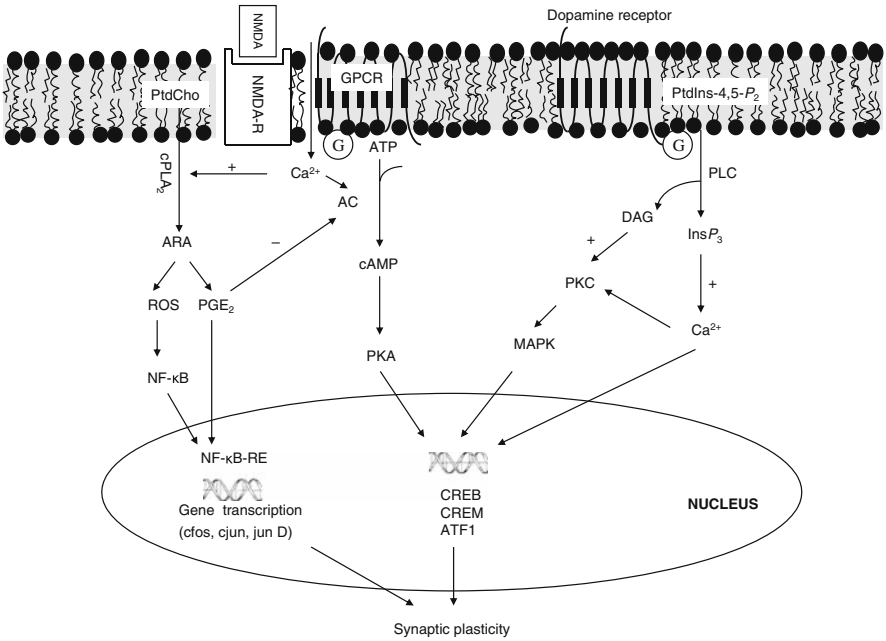

Fig. 9.2 Hypothetical diagram showing receptor-mediated signal transduction process involving iontropic NMDA and metabotropic dopamine receptors. *N*-methyl-D-aspartate receptor (NMDA-R); phosphatidylcholine (PtdCho); cytosolic phospholipase A_2 (cPLA$_2$); arachidonic acid (ARA); reactive oxygen species (ROS); prostaglandins (PG); nuclear factor kappa B (NF-κB); nuclear factor kappa B response element (NF-κBRE); phosphatidylinositol-4,5-bisphosphate (PtdIns-4,5-P_2); phospholipase C (PLC); diacylglycerol (DAG); protein kinase C (PKC); mitogen-activated kinase (MARK); inositol 1,4,5-trisphosphate (Ins*P*3); G-protein-coupled receptor (GPCR) that uses PGE_1 and PGE_2 as agonists; Adenylyl cyclase (AC); cyclic AMP (cAMP); protein kinase A (PKA); protein kinase C (PKC); cAMP response element binding protein (CREB); cAMP responsive element modulator (CREM); activating transcription factor 1 (ATF1); proto-oncogene belonging to the immediate early gene (c-fos, c-jun, and jun D)

but also by cross-talking among neurotransmitter receptors in specific brain regions at distinct neurocircuits.

Levels of neurotransmitters are modulated by diet. This is not surprising considering that neurotransmitters, such as serotonin and dopamine, are derived from nutrient precursors. Serotonin is derived from the amino acid, tryptophan, while dopamine is derived from the amino acid, tyrosine (Fernstrom, 1994). Similarly, levels of fatty acids and fatty acid composition of neural membrane glycerophospholipids are also regulated by diet. Although ARA and DHA are enriched in human brain, despite their abundance, these fatty acids cannot be synthesized de novo by human brain. They, or their precursors, must be ingested from dietary sources and transported to the brain (Horrocks and Farooqui, 2004). These days, our diet has a ratio of *n*–6 to *n*–3 fatty acids

of about 20:1. The Paleolithic diet on which human beings evolved, and lived for most of their existence, had a ratio of 1:1 with high antioxidants (Simopoulos, 2006; Cordain et al., 2005; Weylandt and King, 2005; Farooqui et al., 2007). Changes in eating habits, natural versus processed food, and agriculture development within the past 100 to 200 years have caused changes in the *n*–6 to *n*–3 ratio. *n*–6 fatty acid-enriched diet promotes not only the pathogenesis of cardiovascular disease but also neurodegenerative and neuropsychiatric diseases. In contrast, diet-enriched in *n*–3 fatty acids exerts cardioprotective, immunosuppressive, neuroprotective, and psychoprotective effects (Simopoulos, 2006; Farooqui et al., 2007). Thus, DHA and EPA are the most important dietary supplements that can promote and maintain synaptic plasticity and neurogenesis in the aging brain circuits, and can reduce oxidative stress and neuroinflammation through the generation of lipoxins, neuroprotectins, and resolvins (Serhan, 2005; Marcheselli et al., 2003; Bazan, 2005; Farooqui, 2009; Farooqui and Farooqui, 2009). In addition, DHA and EPA also facilitate optimal membrane fluidity, promote appropriate neurotrophic support, and inhibit production of cytokines and expression of adhesion molecules (Farooqui, 2009; Farooqui and Farooqui, 2009). In contrast, ARA initiates the generation of cytokines, especially IL-1β, the most potent proinflammatory cytokine that induces stress and anxiety-like behavior in rodents (Song, 2002; Song et al., 2008). This cytokine acts on hypothalamus and facilitates the release of corticotrophin-releasing factor, which through its action with adrenocorticotropic hormone mediates the secretion of glucocorticoids from adrenal gland. IL1β also stimulates neurotransmitter systems, thereby increasing the turnover of noradrenaline, serotonin, and dopamine (Song et al., 2003, 2008). DHA enrichment in neural membranes also increases gap junction coupling capacity in cultured astrocytes (Champeil-Potokar et al., 2006). Accumulating evidence suggests that *n*–3 and *n*–6 fatty acids play different role in the neuroendocrine-immune network, and the ratio of *n*–6 to *n*–3 fatty acid is an important dietary factor in reducing oxidative stress and inflammation and promoting neuroprotective and psychoprotective effects on human brain. These observations support the view that diet may also contribute to the etiology of neuropsychiatric and neurodegenerative diseases.

9.4 BDNF-Mediated Signaling in Neuropsychiatric Disorders

In addition to abnormalities in neurotransmission, neuropsychiatric diseases are characterized by inappropriate neurotrophic support, alterations in lipid mediators, cytokines, and transcription factors. Brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin in the brain, promotes growth and maintains intracellular connections. It regulates neuronal neuronal survival, differentiation, and plasticity by modulating signal transduction processes mediated by dopaminergic, cholinergic, glutamatergic, and serotonergic neurotransmission in the CNS. The action of BDNF on neural cells is mediated by

two receptors: TrkB and p75NTR. Interactions of mature BDNF (mBDNF) to TrkB triggers tyrosine phosphorylation in its cytoplasmic domain, resulting in activation of at least three signaling pathways: MEK-MAPK, phosphatidylinositol-3-kinase (PtdIns-3-K), and phospholipase C-γ (PLC-γ) (Kaplan and Miller, 2000; Chao, 2003; Mayr and Montminy, 2001; Huang and Reichardt, 2001) (Fig. 9.3). The majority of BDNF-induced functions are attributed to signaling through TrkB. In contrast, pro-BDNF preferentially interacts with p75NTR and mediates a different set of intracellular signaling cascades, including nuclear factor-κ B (NF-κB), c-jun kinase, and sphingomyelin hydrolysis (Gentry et al., 2004).

It is well known that dopaminergic and serotonergic receptors are coupled to adenylate cyclase and PKA. PKA is a tetrameric enzyme composed of a regulatory subunit dimmer and two catalytic subunits bound to each regulatory subunit. Its activation through G-protein-coupled dopaminergic and serotonergic receptors results in generation of cAMP from ATP. The binding of two

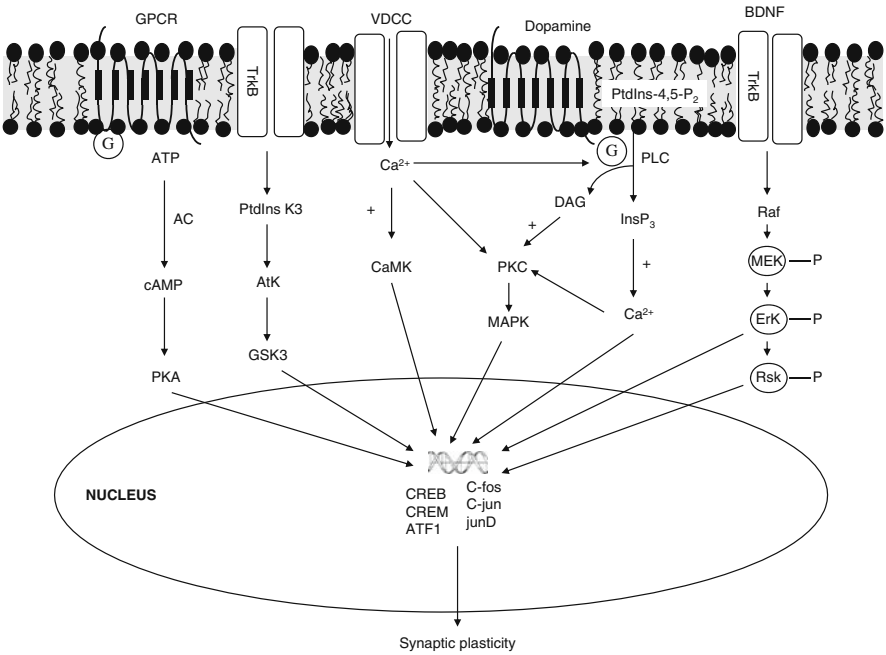

Fig. 9.3 Diagram showing receptor-mediated signal transduction process involving BDNF and seven transmembrane-spanning G-protein-coupled receptor (GPCR). Adenylyl cyclase (AC); cyclic AMP (cAMP); protein kinase A (PKA); cAMP response element binding protein (CREB); cAMP responsive element modulator (CREM); activating transcription factor 1 (ATF1); proto-oncogene belonging to the immediate early gene (c-fos, c-jun, and jun D); mitogen-activated kinase (MARK); brain-derived neurotrophic factor (BDNF); tyrosine kinase receptor (TrkB); extracellular signal regulated kinase (MEK); extracellular signal-regulated kinase (Erk); Raf proto-oncoproteins (Raf); and ribosomal S6 kinase (Rsk)

molecules of cAMP with each regulatory subunit results in dissociation of holoenzyme. The dissociated regulatory subunit dimer remains in the cytoplasm and catalytic subunits are translocated to the nucleus, where they phosphorylate and activate a number of substrate proteins associated with a wide variety of physiological responses, including memory formation, differentiation, neurotransmitter synthesis and release, and synaptic plasticity. Permeability of Ca^{2+} through NMDA receptor is regulated by PKA signaling cascade. PKA inhibitors block the relative fractional Ca^{2+} influx through NMDA receptor in rat hippocampal neurons in culture and hippocampal slices from mice (Skeberdis et al., 2006). Ca^{2+} influx through NMDA receptors is essential for synaptogenesis, experience-dependent synaptic remodeling, and plasticity. In slices, PKA inhibitors markedly block NMDA receptor-mediated Ca^{2+} influx in activated dendritic spines with no significant effect on synaptic current. PKA inhibitors also depress the early phase of NMDA receptor-dependent long-term potentiation (LTP) at hippocampal Schaffer collateral-CA1 synapses. These observations link PKA-dependent synaptic plasticity to Ca^{2+} signaling in spines and thus provide a new mechanism whereby PKA regulates the induction of LTP (Skeberdis et al., 2006). In addition, activation of PKA also causes activation or repression of gene transcription (Borrelli et al., 1992; Sassone-Corsi, 1995). PKA phosphorylates CREB protein, nuclear receptors, and high mobility group containing proteins. This phosphorylation modulates their dimerization or DNA-binding properties (Fimia and Sassone-Corsi, 2001).

The transactivation domain of the CREB protein contains two major domains. The glutamine-rich Q2 domain, which binds to general transcription factor, TAFII130/135, is sufficient for the recruitment of a functional RNA polymerase II complex leading to basal transcriptional activity. The other domain is kinase-inducible domain. It modulates signal-mediated activation of CREB-inducedtranscription (Mayr and Montminy, 2001; Johannessen et al., 2004). The recruitment of the coactivators CREB-binding protein (CBP) and p300 after signal-mediated phosphorylation of this domain at serine-133 strongly enhances CREB-dependent transcription. Transcriptional activity of CREB can also be potentiated by phosphoserine-133-independent mechanisms. Growth-factor-related kinases (e.g., ribosomal S6 kinase, which is downstream of extracellular signal-regulated kinases (ERK) also phosphorylate at Serine-133 and activate it (Fig. 9.2)) (Mayr and Montminy, 2001; Lonze and Ginty, 2002). CREB can also be phosphorylated at Ser-142 by CaMKII, which generally leads to a decrease in activity; however, positive effects of this phosphorylation on transcription have been observed as well (Johannessen et al., 2004). In addition, CREB can be phosphorylated at Ser-129 by glycogen synthase kinase 3β (GSK-3β), a multifunctional enzyme (serine/threonine kinase) involved in the modulation of many aspects of neuronal function, including cell membrane to nucleus signaling, gene transcription, translation, cytoskeletal organization, cell cycle progression, and neural cell survival (Woodgett, 2001). Inhibitory control of GSK-3β has been identified to be crucial for the

phosphoinositide 3′-kinase (PtdIns 3′K)-protein kinase B (Akt)-mediated cell survival. Inhibition of GSK-3β results in mood stabilizer-like behavior in rodent models, and genetic association studies implicate GSK-3β as a possible modulator of particular aspects of bipolar disorder including response to lithium. Recently, direct inhibition of GSK-3β has emerged as a possible option in the pharmacotherapy of several neuropsychiatric disorders (Koros and Dorner-Ciosseik, 2007).

Damage to limbic regions contribute to the etiology of depression, and antidepressants act by reversing such damage and promoting adaptive neuroplasticity. Attempts have been made to understand the molecular mechanism involved in damage associated with depression and antidepressant-mediated plasticity (Nair and Vaidya, 2006). The transcription factor CREB and the BDNF are major targets of diverse classes of antidepressants. Given the role of CREB and BDNF in neuronal plasticity, it is proposed that these transcription factors may play an important role in modulating depression and mood. In neuropsychiatric disorders, a decrease in brain BDNF levels in limbic structures and decrease in BDNF signaling correlate with stress-induced depressive behaviors (Vaidya et al., 1997; Duman, 2004). Chronic administration of antidepressants increases levels of BDNF. This growth factor stimulates neuroplasticity and survival of neural cells in adult brain. Enhancing the cAMP signal-transduction cascade increases the activity and expression of CREB, which in turn upregulates BDNF (Nestler et al., 2002). Several factors modulate the expression of BDNF. Thus, BDNF is increased by enhancing cAMP through the inhibition of phosphodiesterases (PDE), specifically PDE4. The expression of BDNF is inhibited not only by physical and psychological stress (Russo-Neustadt, 2003) but also by the consumption of diet enriched in saturated fat and sucrose (Molteni et al., 2002; Russo-Neustadt, 2003; Wu et al., 2003), and voluntary exercise and learning activities enhance BDNF expression (Nestler et al., 2002). Based on these studies, it is proposed that decreased levels of BDNF may contribute to the atrophy of certain limbic structures (hippocampus and prefrontal cortex). In addition, decrease in BDNF support in brain may be an underlying mechanism responsible for decreased capacity of brain to adaptive changes and increased vulnerability to neurotoxic damage. In rodents, BDNF signaling plays a critical role in the mechanism of antidepressant drug action. BDNF-mediated signaling is both necessary and sufficient for the behavioral effects caused by antidepressant drugs. Thus, abnormal BDNF signaling can influence neuronal differentiation and synaptic function leading to alterations in hippocampal neural cell circuiting, which is reflected in abnormal brain function.

Alterations in the brain serotonin metabolism may contribute to anxiety disorders, bulimia, chronic impulsive/aggressive behavior, and violent suicide (Arango et al., 2002), and these disorders are treated with drugs that augment 5-HT neurotransmission in the brain (Vaswani et al., 2003). BDNF not only has trophic effects but also modulates the structural plasticity and survival of

central serotonergic neurons (Siuciak et al., 1994). This suggests that BDNF-mediated neuronal plasticity is a critical factor in neuropsychiatric disorders, and antidepressant treatments through enhanced BDNF signaling may improve the ability of critical brain circuits to respond optimally to environmental demands, a process that may be critical in the recovery from depression, mood, and bipolar disorders (Castren and Rantamaki, 2008).

9.5 Abnormalities in Essential Fatty Acid Levels and Neuropsychiatric Disorders

Changes in essential fatty acid content in neural membranes modify neural cell function and induce preclinical and postclinical effects, not only by altering microenvironment that modulates neural membrane receptors, ion channels, and enzymes but also by acting as precursors for intra- and intercellular lipid mediators (Fenton et al., 1999; McNamara and Carlson, 2006; Horrocks and Farooqui, 2004). The deficiency of essential fatty acids in brain prevents the renewal of membranes resulting in accelerated cerebral aging. Depletion of DHA in neural membranes is reflected in lower membrane DHA/ARA ratio, increased activity of ARA-metabolizing enzymes, and disturbance of downstream metabolic pathways involved in signaling, growth modulation, brain glucose uptake, immune functions, and neurotransmission. In addition, deficiency of essential fatty acids in brain affects neurodevelopment by altering visual, attention, and cognitive functions. Based on these findings, it is proposed that disturbances in DHA/ARA homeostasis may represent biochemical markers in the preclinical phase of neuropsychiatric illnesses (Hibbeln and Salem, 1995).

The ratio between *n*–3 and *n*–6 PUFA modulates various aspects of cholinergic, dopaminergic, serotoninergic, catecholaminergic, GABAergic, and glutamatergic neurotransmission in mammalian brain tissue (Delion et al., 1994, 1996, 1997; Fenton et al., 1999; Horrocks and Farooqui, 2004; Farooqui and Horrocks, 2007). *n*–3 fatty acid deficiency-mediated alterations in neurotransmission result in impaired learning, changes in attention, motivation, and behavioral performance. Several studies have indicated an elevated ARA/EPA ratio among depressed subjects, indicating that depressed patients may generate high levels of ARA-derived eicosanoids, which may increase their risk of heart disease (Maes et al., 1996; Adams et al., 1996). To this end, several factors increase the risk of heart disease among depressed subjects (Fig. 9.4). Although pathophysiological mechanisms leading to more frequent complications of coronary heart disease in patients with depression are not fully explained, higher sympatho-adrenergic stimulation, elevated plasma norepinephrine, and increased platelet aggregation may contribute to depression. Most symptoms of depression can be exacerbated by *n*–3 fatty acid consumption. As stated earlier, isoforms of PLA_2 hydrolyze ARA and DHA from *sn*-2 position of

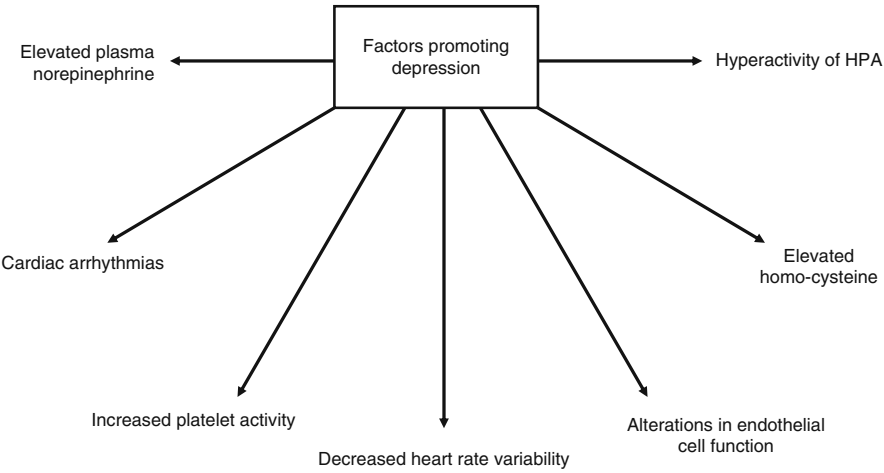

Fig. 9.4 Modulation of depression by cardiovascular factors. Depressive people as well as many heart disease patients have many of above factors. Hypothalamic–pituitary–adrenocortical axis (HPA)

glycerol moiety of neural membrane glycerophospholipids. ARA is metabolized to prostaglandins, which have higher inflammatory potential compared with those generated from the EPA and DHA. PLA_2 activity is coupled with neural membrane fatty acid composition, and plays a central role in the development of neuronal dysfunction associated with neuropsychiatric and neurodegenerative disorders (Farooqui and Horrocks, 2007; Farooqui, 2009). Dietary manipulations with *n*–3 fatty acids modulate densities of dopamine, serotonin, and muscarinic receptors in rat brain. Also, *n*–3 fatty acid supplementation improves mental health rating scores, and there is evidence that the mechanism behind this involves the serotonin receptor complex, supporting the view that a tight relationship exists between essential fatty acid status and normal neurotransmission, and that altered PUFA levels may contribute to the abnormalities in neurotransmission seen in neuropsychiatric disorders (Horrobin, 1998).

9.5.1 Fatty Acids and Glycerophospholipids in Schizophrenia

Schizophrenia is a complex neuropsychiatric disorder with a prevalence of nearly 1% of the world population. Clinically, schizophrenia is characterized by chronic psychotic symptoms and psychosocial impairment. At least two core syndromes of schizophrenia, the positive (hallucinations, delusions, and paranoid ideation) and the negative (apathy and anledonia), are recognized (Liddle, 1987). Not all, but many of these individuals have low levels of

ARA and DHA in membrane glycerophospholipids and also fail the niacin flush test (Glen et al., 1994).

A broad range of neurodevelopmental, structural, neurotransmission, and behavioral abnormalities are associated with schizophrenic patients. Collective evidence suggests that multiple neurotransmitter systems may contribute to the etiology of schizophrenia (Pérez-Neri et al., 2006). Brain tissue is more vulnerable to the toxic effects of free radicals, because they have a high rate of catecholamine oxidative metabolic activity (Farooqui and Horrocks, 2007). Neurotransmitters, like glutamate, can induce the same metabolic processes that increase free radical production and can lead to impaired dopamine-glutamate balance (Farooqui et al., 2008a). Neurodevelopmental abnormalities may result from defective genes and/or non-genetic factors such as prenatal and neonatal infections, birth complications, maternal malnutrition, drug and alcohol abuse, season of birth, sex, birth order, and life style (Horrobin, 1998). These processes involve alterations in neural membrane glycerophospholipids, which are crucial for synaptic remodeling in developing brain and are also involved in learning and memory processes as the developing brain matures into the adult brain (Horrobin, 1998). Genetic and non-genetic factors may also contribute to the cellular metabolic stress that often results in oxidative stress, which triggers the oxidative cell damage contributing to abnormal neural growth and differentiation. Brain tissue is preferentially susceptible to oxidative damage since it is under very high oxygen tension and highly enriched in ROS susceptible proteins, lipids, and poor DNA repair (Mahadik et al., 2006). Levels of apoD are also elevated in the dorsolateral prefrontal cortex and caudate obtained postmortem from subjects with schizophrenia and bipolar disorder compared to controls, suggesting a focal compensatory response of brain tissue to neuropathology associated with psychiatric disorders (Thomas et al., 2003). It is also proposed that ApoD may also act as a sequestering molecule binding excess of ARA in cells. Collective evidence suggests that it is now possible to derive a consistent account of one contributing cause of schizophrenia across multiple levels of analysis, from genes to receptors, functional neuroanatomy, cognition, and symptoms (MacDonald and Chafee, 2006).

Schizophrenic patients have increased levels of lyso-PtdCho in brain, erythrocytes, platelets, and skin fibroblasts, suggesting an increase in activities of brain PLA_2 isoforms (Yao et al., 2000; Ross, 2003). Elevated levels of lyso-PtdCho in schizophrenic patients are significantly reduced after 3 weeks of pharmacotherapy with haloperidol (Schmitt et al., 2001). This effect of haloperidol is caused by a dopamine-mediated decrease in PLA_2activity. Increased activities of PLA_2 isoforms in schizophrenic subjects not only induce changes in membrane glycerophospholipid composition but also alter membrane fluidity. This results in alterations in activity of membrane-dependent proteins such as Na^+-K^+-ATPase, monoamine oxidase, β2- and

α2-adrenergic receptors, and uptake of norepinephrine and serotonin. Disturbances in other membrane-dependent proteins, tyrosine and tryptophan hydroxylase, may also occur and this may explain the dopamine hypothesis (Horrobin, 1998). In addition, a large amount of lipofuscin-like material also accumulates in the oligodendrocytes. The existence of these products within neural cell membranes results in an unstable membrane structure, altered membrane fluidity and permeability, and impaired signal transduction process. Studies on abnormal glycerophospholipid composition in schizophrenia are supported by ^{31}P-Magnetic resonance spectroscopic (^{31}P-MRS) studies of frontal lobe metabolism of living schizophrenic patients. These patients show a reduction in resonances of phosphomonoesters (PME) and/or increase in phosphodiesters (PDE) and ATP in the dorsolateral prefrontal cortex of drug-naïve schizophrenic patients, once again indicating an accelerated glycerophospholipid metabolism (Pettegrew et al., 1991; Stanley et al., 1994; Richardson et al., 2001; Yacubian et al., 2002). It is stated that changes in neural membrane glycerophospholipids may be related to molecular changes that precede the onset of clinical symptoms and brain structural changes in schizophrenia. In contrast, changes in high-energy phosphate metabolism may be state-dependent. The decline in PtdCho and EtnGpl levels in schizophrenic patients is also accompanied by the depletion of ARA and DHA levels in schizophrenia (Horrobin et al., 1991; Yao et al., 2000; Horrobin, 2002; Peet and Horrobin, 1994; Horrobin, 1998).

The cause of the increased PLA_2 isoform activity in schizophrenia is not known. However, several mechanisms are possible. (a) The upregulation of retinoic acid receptor-α (RAR-α) expression (Rioux and Arnold, 2005; Goodman, 1995; LaMantia, 1999; Yao et al., 2000; Ross, 2003; Farooqui et al., 2004). PUFA generated through the activation of PLA_2 isoforms bind to RXR-α and cause excessive stimulation (Lengqvist et al., 2004), resulting in abnormalities in memory formation and consolidation (Das, 2003). (b) RAR-α receptors also upregulate the expression of dopamine D_2 receptors (Samad et al., 1997), suggesting that RAR-α-mediated expression of dopamine D_2 receptors and generation of ARA-derived lipid mediators may contribute to the symptoms of schizophrenia. (c) Finally, increased levels of cytokines in schizophrenia may contribute to the stimulation of PLA_2 isoforms in schizophrenic subjects (Buka et al., 2001; Farooqui and Horrocks, 2006). Collectively, these studies suggest that elevated dopamine and retinoid metabolism along with upregulation of cytokines and the stimulation of PLA_2 isoforms are closely associated with the pathogenesis of schizophrenia (Goodman, 1998; Smesny, 2004; Yao and Van Kammen, 2004; Laruelle et al., 1999; Farooqui et al., 2004), and an accelerated glycerophospholipid metabolism may have significant effects on neuronal development and communication, resulting in behavioral abnormalities in schizophrenic individuals.

9.5.2 *Fatty Acids and Glycerophospholipids in Depression and Bipolar Disorders*

Depression, a potentially life-threatening disease, is a major public health problem. About 13–20% of the world population has some depressive symptoms at any given time, and about 5% of the population is estimated to suffer from major depression. Neuroimaging and postmortem studies have identified abnormalities in brain areas, such as the pre-frontal cortex and limbic system in subjects with major depression (Drevets, 2000, 2003; Botteron et al., 2002). ^{31}P-MRS studies indicate that neural membrane glycerophospholipid abnormalities in depression and bipolar disorders. Depressed bipolar patients have significantly greater PME levels, especially *P*Etn, than control human subjects (Yildiz et al., 2001; Kato et al., 1993), indicating an accelerated glycerophospholipid metabolism in these individuals. Although, PLA_2 activities have not been assayed in brain or peripheral tissue from depressed bipolar patients, an association between major depressive disorders and a polymorphism within $sPLA_2$ has been reported in some patients (Papadimitriou et al., 2003). Similarly, ^{3}H-MRS studies on glycerophospholipid metabolites demonstrate alterations in resonance intensities of choline in both depressed and bipolar patients (Charles et al., 1994; Ende et al., 2000). The choline is primarily derived from phosphocholine, and is used for the synthesis of acetylcholine in brain. Determinations of the rate of oxygen consumption of freshly prepared mitochondria in the presence and absence of various glycerophospholipid degradation products indicate that phosphoethanolamine and ethanolamine inhibit mitochondrial respiration in a dose-dependent manner, whereas choline, PtdEtn, and PtdCho have no effect. This suggests a specific inhibition by ethanolamine and phosphoethanolamine. Although the concentrations of phosphoethanolamine and ethanolamine used in this study are quite high and non-physiological, it is likely that during episodes of depression high concentrations of these metabolites occur in brain tissue (Kato et al., 1993: Modica-Napolitano and Renshaw, 2004), and phosphoethanolamine and ethanolamine-mediated mitochondrial dysfunction can be implicated in the pathophysiology of depression and bipolar disorders (Kato et al., 1993).

Major depression is also accompanied by a significant decrease in *n*–3 fatty acids and/or an increase of the *n*–6/*n*–3 ratio in plasma and/or in the membranes of the red cells. The severity of depression is proportional to low levels of *n*–3 fatty acids or the ratio of *n*–3/*n*–6 fatty acids in plasma and red cell membranes. In addition, other neurochemical perturbations in major depression include alterations in serotonin, norepinephrine, and dopamine, and activation of the inflammatory response system, resulting in an increase of the proinflammatory cytokines (interleukins: IL-1b, IL-6, and interferon g) and eicosanoids (among others, prostaglandin E_2) in the blood and the CSF of depressed patients (Maes et al., 1997; Tuglu et al., 2003). These cytokines bind to their receptors on astrocytes and induce the enhancement

of glycerophospholipid metabolism through the activation of PLA_2. Significantly high calcium levels (Dubovsky et al., 1989) in bipolar disorders may also facilitate PLA_2 stimulation. However, activities of PLA_2 isoforms have not been determined in bipolar disorders.

Secretion of cytokines and generation of eicosanoids have opposite physiological functions according to their *n*–3 or *n*–6 fatty acid precursors. ARA generates proinflammatory prostaglandin E_2 (PGE_2), whereas DHA is metabolized to resolvins and neuroprotectins, which inhibit the formation of PGE_2. It is shown that a dietary increase of *n*–3 fatty acids downregulates the production of IL-1β, IL-2, IL-6, and TNF-α. In contrast, diets enriched in *n*–6 fatty acids significantly upregulates the production of proinflammatory cytokines, like TNF-α. Therefore, it is likely that deficiency of *n*–3 fatty acids may be associated at different levels in the pathophysiology of major depression, on one hand, through their role in the membrane fluidity which influences diverse steps of neurotransmission and, on the other hand, through their function as precursor of pro-inflammatory cytokines and eicosanoids disturbing neurotransmission. This is temping to speculate that *n*–6:*n*–3 imbalance early in life leads to irreversible changes in neural membrane composition of regions involved in limbic system, particularly in hypothalamus. The increase in ALA and reduction in DHA proportions in animals refed ALA in later life are consistent with a dysfunction or downregulation of the conversion of ALA to 18:4*n*–3 by the delta-6 desaturase activity (Li et al., 2006).

Genetic factors also contribute to the pathophysiology of depression. In major depressive disorder (MDD), aberrant genes contribute to depression in several ways: (a) by retarding the synthesis of growth factors that act on brain during development and (b) by regulating release of a neurotransmitter into synapses, and (c) also by modulating neurotransmitter's action or its duration of activity. Serotonergic mechanisms play an important role in the regulation of mood, motor activity, sleep patterns, and depression. Serotonin reuptake is controlled by the serotonin-uptake protein or serotonin transporter (5-HTT) localized within the terminals and dendrites of serotonin-releasing neurons (Wurtman, 2005; Putzhammer et al., 2005). A common functional insertion/deletion polymorphism in the corresponding gene's promoter region is called 5-HTTLPR. This specific genetic locus associated with lower uptake of serotonin and vulnerability to stress-induced depressive symptoms in MDD. According to Wurtman (2005), the gene itself exists as several alleles, the short "S" allele and the long "L" allele. The S variant is associated with less, and the L variant with more, of the uptake protein. The effect of stressful life events on depressive symptoms in young adults seems to be significantly stronger among SS or SL subjects than among LL subjects. Neuroimaging studies show that people with the SS or SL alleles exhibit a greater activation of the amygdala in response to fearful stimuli than those with LL. It is also shown that mutations in the gene that

controls serotonin synthesis in the human brain (tryptophan hydroxylase) also predispose to mood disturbances (Wurtman, 2005).

9.5.3 Fatty Acids and Glycerophospholipids in Dyslexia

Dyslexia, or specific reading disability, is the most common learning neurodevelopmental disorder with a complex, partial genetic basis, but its biochemical defect remains unknown. Dyslexia is the most prevalent childhood cognitive disorders, affecting ~5% of school-age children. Recent studies indicate the involvement of four candidate genes, including dyslexia susceptibility 1 candidate 1 (DYX1C1), roundabout Drosophila homolog 1 (ROBO1), doublecortin domain-containing protein 2 (DCDC2), and KIAA0319. Each gene is implicated in global brain-development processes, such as neural migration and axonal guidance, with the exception of DYX1C1, the function of which is still unknown (McGrath et al., 2006). Recent in situ hybridization studies indicate a very distinct expression pattern of the KIAA0319 gene in the developing cerebral neocortex of mouse and human fetuses. It is clearly shown that interference with rat KIAA0319 expression in utero leads to impaired neuronal migration in the developing cerebral neocortex. These data suggest a direct link between a specific genetic background and a biological mechanism leading to the development of dyslexia (Paracchini et al., 2008; Leonard and Eckert, 2008).

Metabolic alterations in fatty acid and membrane glycerophospholipid metabolism may be associated with neurochemistry of dyslexia (Taylor et al., 2000; MacDonell et al., 2000). ^{31}P-MSR studies on PME and PDE composition in dyslexic patients indicate that the PME peak area is significantly elevated in the dyslexic group, as evidenced by higher ratios of PME/total phosphorus, PME/β NTP, and PME/PDE (Richardson, 2006). No other glycerophospholipid metabolites differ significantly between dyslexic and non-dyslexic adults. The PME peak predominantly contains phosphoethanolamine (*P*Etn) and phosphocholine (*P*Cho), which are precursors of membrane glycerophospholipids. The neurochemical significance of predominance of *P*Etn and *P*Cho is not fully understood. However, it is proposed that intracellular sizes of *P*Etn and *P*Cho pools may be associated with proliferating and non-proliferating stages of neural cells. The PME increase in dyslexia may be due to reduced incorporation of glycerophospholipids into cell membranes, which may induce abnormalities in efficacy of neurotransmitter receptors in dyslexia. These findings are consistent with the hypothesis that membrane glycerophospholipid metabolism is abnormal in dyslexia (Richardson, 2006).

Another hypothesis related to the pathogenesis of dyslexia is the involvement of *n*–3 fatty acids in this disorder. Mothers whose diet during pregnancy has lower levels of *n*–3 fatty acids developed a dark adaptation

defect, and gave birth to children who developed dyslexia. In contrast, mothers whose diet was enriched with *n*–3 fatty acids did not develop the dark adaptation defect and had normal children. Four weeks of a high intake of *n*–3 fatty acids in the first group normalized the dark adaptation defect (Stordy, 1995). This suggests that the pathophysiology of dyslexia involves a defect in the incorporation of *n*–3 fatty acids into membrane glycerophospholipids (Taylor et al., 2000; Richardson, 2006).

9.5.4 Fatty Acids and Glycerophospholipids in Autism

Autism is a complex neurodevelopmental disorder of unknown etiology. It is characterized by deficits in social and language development and communicative abnormalities as well as repetitive and stereotyped behaviors (Widiger and Samuel, 2005). Genetic and environmental factors are closely involved in the etiology of autism (Acosta and Pearl, 2003). Neurexins and neuroligins are transmembrane proteins that modulate excitatory glutamatergic and inhibitory GABAergic transmission in mammalian brain. They not only act as cell adhesion molecules but are also involved in synapse function, and their alterations may be linked to autistic spectrum disorder (Craig and Kang, 2007).

Levels of PtdEtn are decreased while levels of PtdSer are increased in the erythrocyte membranes of children with autism as compared to their non-autistic developmentally normal siblings (Chauhan et al., 2004a; Chauhan and Chauhan, 2006). Highly unsaturated fatty acids in red blood cell glycerophospholipids are decreased in autistic subjects (Bell et al., 2000, 2004), suggesting an abnormality in unsaturated fatty acid-mediated signal transduction may be associated with the pathophysiology of autism. A marked decrease in *n*–3 fatty acids levels has been reported in the plasma of autistic subjects (Vancassel et al., 2001). No changes are observed in levels of *n*–6 fatty acids, and consequently a significant increase in the (*n*–6)/(*n*–3) ratio has been reported in autistic subjects. The decrease in levels of *n*–3 fatty acids is accompanied by higher levels of erythrocyte lipid peroxides and urinary isoprostanes (McGinnis, 2004).

Alterations in glycerophospholipid metabolism and levels are accompanied by increased excretion of 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-iso-$PGF_2\alpha$, and 8-isoprostane-$F_2\alpha$ (8-iso-$PGF_2\alpha$) in urine of autistic children compared to age-matched healthy controls, suggesting that autistic children are vulnerable to oxidative stress (Ming et al., 2005). In addition, alterations in the activities of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, catalase, levels of glutathione levels and mitochondrial and immune dysfunction, are also observed in autism (Chauhan and Chauhan, 2006). Furthermore, increase in levels of lipid peroxidation products in autism indicate that oxidative stress is closely involved with the pathogenesis of autism.

Determination of major antioxidant proteins, transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein), in the serum indicates that these proteins are reduced in autistic children as compared to their developmentally normal non-autistic siblings (Chauhan et al., 2004b).

9.6 Status of *n*–3 and *n*–6 Fatty Acids in Neuropsychiatric Disorders

The oxidation of ARA by the cyclooxygenases generates the "2" series of prostaglandins such as PGE_2, $PGF_{2\alpha}$, and lipoxygenase converts ARA to the "4" series of leukotrienes, such as LTB_4. In contrast, *n*–3 fatty acids, particularly EPA, are not good substrates for the COX and LOX enzyme systems, and their action produces PGE_3 and LTB_5. These eicosanoids are less active interms of their ability to induce inflammation and oxidative stress than ARA-derived eicosanoids (Phillis et al., 2006). In addition, EPA and DHA are also precursors for newly discovered resolvins and neuroprotectins, which are antiinflammatory and inflammation-resolving. DHA- and EPA-derived eicosanoids antagonize the proinflammatory effects of ARA-derived eicosanoids. DHA and ARA are ligands/modulators for the nuclear receptors NF-κB, PPAR, and SREBP-1c, which control various genes of inflammatory signaling and lipid metabolism (Farooqui, 2009). Alterations in *n*–6/*n*–3 fatty acid ratio are known to modulate fatty acid composition of neural cells as well as immune cells resulting in alterations in microglial phagocytosis, T cell signaling, and antigen presentation capability. These effects are mediated at microglial and immune cell membrane level, suggesting important roles of fatty acids in membrane order, lipid raft structure and function, and membrane trafficking (Calder and Grimble, 2002; Cordain et al., 2005; Farooqui and Horrocks, 2007). Collective evidence suggests that the fatty acid composition of human neural and immune cells modulates glutamatergic, dopaminergic, and serotonergic neurotransmissions (Horrocks and Farooqui, 2004; Ross and Turenne, 2002; Evans et al., 2001), and are involved in long-term potentiation, sleep, locomotion, and immune function (Ross and Glen, 2004; Farooqui and Horrocks, 2007). These alterations in these functions may contribute to symptoms of neuropsychiatric diseases.

9.6.1 n–6 *and* n–3 *Fatty Acids in Depression and Bipolar Disorder*

Mass spectrometric studies indicate statistically significant alterations in levels of free fatty acids and phosphatidylcholine in gray and white matter of both depression and bipolar disorder samples compared to controls (Schwarz et al.,

2008). In addition, ceramide levels are also increased in white matter of both neuropsychiatric disorders as compared to control levels. Treatment with antipsychotic drugs indicates that while gray matter PtdCho levels are effected by antipsychotic medication, this treatment has no effect on PtdCho levels in white matter (Schwarz et al., 2008). Determination of lipid profiles in red blood cell samples indicates significant alterations in the concentrations of free fatty acids as well as ceramide. The molecular mechanism associated with abnormal glycerophospholipid and fatty acid metabolism is not known. However, upregulation of cytokines (interleukin-1β, IL-6, IL-2, and interferon-γ) in depression and bipolar disorder may cause an imbalance in the *n*–6 to *n*–3 fatty acid ratio through the activation of PLA_2 activity (Maes et al., 1997). Thus, changes in levels of eicosanoids and cytokines and the *n*–6 to *n*–3 fatty acid ratio in depressed individuals may interfere with serotonin receptor function, a process closely associated with the pathogenesis of depression (Timonen et al., 2004; van West and Maes, 2003).

Levels of *n*–3 but not *n*–6 fatty acids are decreased in peripheral cells of bipolar depressed patients (Edwards et al., 1998; Peet et al., 1998a,b). In contrast, studies on the fatty acid composition of peripheral cells in schizophrenia have been contradictory. Levels of both *n*–3 and *n*–6 fatty acids are reduced in schizophrenia (Yao et al., 1994; Peet et al., 2004; Reddy et al., 2004), whereas others found no reduction in *n*–3 or *n*–6 fatty acids (Vaddadi et al., 1996; Evans et al., 2003). Although the evidence for the deficiency of *n*–3 fatty acids in schizophrenia is week, in bipolar disorders a specific deficiency of *n*–3 fatty acids exists in peripheral cells such as red blood cells (Table 9.2). This view is supported by studies on comparisons of seafood consumption and rates of psychiatric disorders. It is reported that a robust correlation between higher seafood consumption and a lower prevalence of bipolar disorders occurs, but not of schizophrenia (Noaghiul and Hibbeln, 2003). The same correlation, but also including a lower prevalence of suicide, is found in Finland (Tanskanen et al., 2001a,b).

Table 9.2 Status of *n*–3 fatty acids and their supplementation in neuropsychiatric disorders

Disorders	Fatty acid status	Fatty acid supplementation	References
Schizophrenia	*n*–3 fatty acids (↓) *n*–6 fatty acids (↓)	Beneficial	Das (2004)
Bipolar disorders	*n*–3 fatty acids (↓)	Beneficial	Marangell et al. (2003)
Dyslexia	*n*–3 fatty acid (↓)	Unknown	Taylor et al. (2000)
Autism	*n*–3 fatty acids (↓)	Beneficial	Vancassel et al. (2001)
ADHD	*n*–3 fatty acids (↓)	Beneficial	Young et al. (2004)

A downward arrow in parentheses indicates a decrease.

9.6.2 n–6 *and* n–3 *Fatty Acids in Aggressive Disorders and Cocaine Addiction*

Deficiency of *n*–3 fatty acids is also associated with aggressive behavior. Similarly, low levels of plasma DHA may contribute to increased fear and anxiety, components of defensive and violent behaviors (Hibbeln et al., 2004a,b). High dietary intake of DHA is related to a lower likelihood of hostility in young adults (Iribarren et al., 2004). Although the molecular mechanism associated with psychoprotection is not known, it is becoming increasingly evident that interactions between DHA and CB_1receptor may play an important role in antiaggression activities.

There is a relationship between dietary DHA and ARA consumption and elevated cellular levels of corresponding *N*-acylethanolamine, which may act as endogenous cannabinoids. Enrichment of DHA and ARA in two groups of piglet diet results in 10-fold increase in docosahexaenoylethanolamine and 4-fold arachidonylethanolamine, respectively (Berger et al., 2001). This increase in acylethanolamine may be important neurochemically because acylethanolamine with at least 20 carbons and three double bonds are known to bind to CB_1 receptor and produce their effect on brain tissue. It can be speculated that diet enriched in ARA may increase the chances of depression, aggression, and downing of mood because of the generation of arachidonylethanolamine, which binds to CB_1 receptor with higher affinity than the docosahexaenoylethanolamine. DHA-enriched diet, which facilitate the generation of docosahexaenoylethanolamine, may result in mood lifting, thereby decreasing the risk of depression because it binds to CB_1 receptor less tenaciously than arachidonylethanolamine (Berger et al., 2001). A similar process may occur in aggressive behavior of cocaine addicts, who have lower levels of *n*–3 fatty acids, including DHA (Buydens-Branchey et al., 2003a,b). Furthermore, higher consumption of linoleic acid, the *n*–6 precursor of ARA, correlates with higher rates of homicide mortality across countries and time (Hibbeln et al., 2004a).

9.6.3 n–6 *and* n–3 *Fatty Acids in Attention-Deficit/Hyperactivity Disorder*

Attention-deficit/hyperactivity disorder (ADHD) is a neurobehavioral disorder characterized by severe and persistent impulsiveness, inattention, and hyperactivity resulting in long-term educational and social disadvantages (Widiger and Samuel, 2005). It affects about 3–5% of school-aged children with symptoms usually starting before 7 years of age. ADHD patients have significant impairment in family and peer relations, academic functioning, and show high co-morbidity with a wide range of psychiatric disorders, including oppositional defiant disorder (ODD), conduct disorder (CD), anxiety disorder, depression, substance abuse, and pervasive developmental disorder (PDD)

(Anney et al., 2008). ADHD is linked to genetic, environmental, and biological factors that reduce catecholamine transmission to suboptimal levels, and is treated with agents that increase catecholamine transmission. It is suggested that mild alterations in catecholamine metabolism in prefrontal cortex cells have profound effects on the ability of the prefrontal cortex to guide behavior in animals. Optimal levels of norepinephrine acting at postsynaptic α-2A-adrenoceptors and dopamine acting at D_1 receptors are essential to prefrontal cortex function (Arnsten and Li, 2005; Arnsten, 2007). Blockade of norepinephrine α-2-adrenoceptors in prefrontal cortex markedly impairs prefrontal cortex function, and mimics most of the symptoms of ADHD, including impulsivity and locomotor hyperactivity (Arnsten and Li, 2005). Conversely, stimulation of α-2-adrenoceptors in prefrontal cortex strengthens prefrontal cortex regulation of behavior and decreases distractibility. At present, the most effective treatments for ADHD involve the use of therapeutic agents that optimize catecholamine actions on prefrontal cortex (Brennan and Arnsten, 2008).

Pharmacological, neuroimaging, and animal-model studies also indicate that abnormalities in monoaminergic neurotransmission in ADHD (Spencer et al., 2005) are coupled to alterations in activities of enzymes associated with fatty acid synthesis, specifically a Δ^6-desaturase deficit (Ward, 2005). Adult ADHD patients have significantly lower levels of total PUFA, total *n*–3 fatty acids, and significantly higher levels of total saturated fatty acids in erythrocyte membrane glycerophospholipids. The proportion of DHA in serum and erythrocyte membrane glycerophospholipids is not related to symptom severity in these ADHD subjects (Young et al., 2004). The exact cause of the fatty acid alterations is unknown, but a zinc deficiency may be related. However, since the diet is not deficient in *n*–3 fatty acids, genetic and environmental factors together with abnormalities in fatty acid synthesis have been implicated in the pathophysiology of ADHA. Boys with ADHD are inattentive, impulsive, and hyperactive. The disorder appears to be multifactorial. Their plasma contents of ARA, EPA, and DHA are significantly lower than in the control boys (Stevens et al., 1995). They also show some signs of essential fatty acid deficiency, but did have an elevation of *n*–9 eicosatrienoic acid. The deficiency of the essential fatty acids is caused by impaired conversion of LA and ALA into ARA, EPA, and DHA. The boys with lower plasma phospholipid total *n*–3 fatty acids show more behavior, learning, and health problems than boys with higher levels (Stevens et al., 1996). Delta-6 desaturase deficiency may be one cause of ADHD (Arnold et al., 1994). Preliminary data indicate that some children with ADHD have higher rates of *n*–3 fatty acid oxidative breakdown than control boys (Ross, 2003). This observation explains low levels of *n*–3 fatty acids found in these children. Adults with ADHD also have lower DHA levels in plasma and erythrocytes, but without correlation to ADHD symptom severity (Young et al., 2004).

9.7 Effects of *n*–3 Fatty Acid Supplementation in Neuropsychiatric Disorders

As stated above, alterations in glycerophospholipid homeostasis and generation of PDE and PME represent biochemical markers in the preclinical phase of neuropsychiatric disorders and may serve as a trigger in genetically vulnerable individuals (Buretic-Tomljanovic et al., 2008). The assessment of patient's brain phospholipid metabolism by NMR spectroscopy and neuroimaging may help in monitoring the course of neuropsychiatric diseases and treatment response. In addition, niacin skin flush test may provide valuable information. It can be used for the diagnosis of adolescents and young adults with psychotic behavior, or in defining the necessity for long-term therapy.

Modifications of neural membrane glycerophospholipid composition with *n*–3 fatty acid-enriched diets affect densities of dopamine, serotonin, and muscarinic receptors in rats. *n*–3 fatty acid supplementation improves mental health rating scores, and there is evidence that the mechanism behind this involves the serotonin receptor complex (Haag, 2003; du Bois et al., 2005). Collective evidence indicates that a tight relationship exists between *n*–3 fatty acid status and normal neurotransmission, and that altered polyunsaturated levels may contribute to the abnormalities in neurotransmission seen in neuropsychiatric disorders (Haag, 2003; Fenton et al., 2000).

9.7.1 Depression and Bipolar Disorder

Reduction in dietary intake of *n*–3 fatty acids may result in attention-deficit (hyperactivity) disorder, Alzheimer disease, schizophrenia, and depression (Young and Conquer, 2005). Supplementation of diet with individual or combination *n*–3 fatty acids decreases symptoms associated with above neuropsychiatric disorders. Thus far, however, the benefits of supplementation, in terms of decreasing disease risk and/or aiding in symptom management, are not clear. In fact, results are inconsistent and controversial and more research is needed.

Lower *n*–3 fatty acid content in mother's milk and lower seafood consumption by the mother are associated with higher rates of postpartum depression in women (Hibbeln, 2002). Many studies in this area report no statistically different result and claim no effect of dietary differences on depression. A prime example is a study of the correlation of postpartum depression and fish consumption during pregnancy in relatively small groups of women (Browne et al., 2006). Also, in that study, no differentiation was made between oily fish with high DHA and whitefish with low DHA content. None of the women consumed oily fish regularly thus the study was useless. A much better study confirmed that lowered serum *n*–3 fatty acid levels are present in major depression and postpartum depression (De Vriese et al., 2003).

9.7.2 Treatment with Neuropsychiatric Disorders with High-Doses EPA

Supplementation of *n*–3 fatty acids in the diet also improves symptoms of schizophrenia, bipolar disorder, dyslexia, autism, and ADHD (Peet et al., 2004; Das, 2004; Bell et al., 2004; Stevens et al., 2003; Young et al., 2004; Yao and van Kammen, 2004; Richardson and Puri, 2002; Richardson, 2003a,b). High doses of EPA given to schizophrenics markedly enhanced the responsivity to 5-hydroxytryptamine (serotonin). This correlates inversely with severity of the psychosis (Yao and van Kammen, 2004). EPA is well tolerated by humans and has beneficial effects when supplemented in persons with neuropsychiatric disorders. The *n*–3 fatty acids are particularly useful for the management of bipolar disorder and major depression (Sarmiento et al., 2003; Severus et al., 2001). At the same time, the *n*–3 fatty acids reduce the risk of cardiovascular mortality in these patients (Severus et al., 2001). Larger human trials are needed to confirm the above studies.

A group associated with the late David Horrobin used high-dose EPA in several conditions (Horrobin, 2002), including schizophrenia (Horrobin, 2003) and depression (Murck et al., 2004). The exact mechanism by which EPA benefits depression is not understood. Using the olfactory bulbectomized (OB) rat model of depression, it is shown that bulbectomy induces depression-like changes and results in significantly increased locomotor and rearing activities in an "open field," impaired memory in the Morris water maze, increased expression of corticotrophin-releasing factor (CRF), and increased secretion of corticosterone in OB rats compared with sham-operated rats fed a control diet (Song et al., 2009). Neurochemical alterations include decrease in mRNA expression of nerve growth factor (NGF) in the hippocampus, and increase in PLA_2 in the hypothalamus along with increase in interleukin-1β (IL-1β) and prostaglandin E_2 (PGE_2) in the serum and brain of OB rats compared to sham-operated rats fed a control diet (Song et al., 2009). Treatments of OB rats with EPA normalizes the above behavioral alterations, decreases inflammatory changes, and reduces CRF expression and corticosterone secretion. Furthermore, EPA not only decreases serum concentrations of IL-1β and PGE_2 but also increases the levels of mRNA for NGF in OB rats. Anti-NGF treatment blocks EPA-mediated effects on behavior. These results suggest that growth factors like NGF may be associated with the pathogenesis of depression and EPA may improve depression via its anti-inflammation properties and the upregulation of NGF (Song et al., 2009).

9.7.3 Treatment of ADHD with High-Doses EPA

Children with ADHD have been treated with various PUFA including DHA or EPA (Stevens et al., 2003). Positive effects have been observed with *n*–3

long-chain fatty acids. The greatest improvement is observed in oppositional defiant behavior. Another study with children with specific learning difficulties, particularly dyslexia, combined with ADHD reports significant improvement and reduction in ADHD symptoms after 12 weeks of treatment with PUFA (Richardson, 2004). Whilst both *n*–6 and *n*–3 fatty acids are necessary for brain development, *n*–3 fatty acids appear most promising for treatment of childhood developmental and psychiatric disorders (Richardson, 2004, 2006). Alternative treatments for adults with ADHD have also been reported, but most apply to a specific subgroup or have not been tested rigorously (Arnold and DiSilvestro, 2005). Supplementation with zinc ion or with long-chain *n*–3 fatty acids is most promising. Relatively high doses of fish oil will be needed for adults with ADHD (Young and Conquer, 2005). Collectively, above reports along with epidemiological and tissue compositional studies on small clinical trials support the view that *n*–3 fatty acids intake in neuropsychiatric disorders has neuroprotective and psychoprotective effects (Young and Conquer, 2005; Peet and Strokes, 2005; Parker et al., 2008). Meta-analyses of randomized controlled trials also demonstrate a statistically significant benefit in unipolar and bipolar depression. Available data on therapeutic effects of *n*–3 fatty acids are highly heterogeneous, and results remain inconclusive in most neuropsychiatric conditions (Freeman et al., 2006).

9.8 Mechanism of Action of *n*–3 Fatty Acids in Neuropsychiatric Disorders

The molecular mechanism of action of *n*–3 fatty acids in neuropsychiatric disorders remains unknown. However, as stated earlier *n*–3 fatty acids may modulate one or more neurotransmitter systems such as glutamatergic, dopaminergic, and serotonergic neurotransmission (Evans et al., 2001; Ross and Turenne, 2002; Horrocks and Farooqui, 2004), and may also act through their immunosuppression effect (Murck et al., 2004), which is characterized by impairment of antigen presentation and of T helper cell type-1 responses. *n*–3 fatty acids also block the generation of inflammatory cytokines and eicosanoids. Supplementation with *n*–3 fatty acids stabilizes neural membranes and increases membrane fluidity, which promote optimal interactions of neurotransmitters with their receptors (Farooqui and Horrocks, 2007).

Mounting evidence indicates that PtdIns-PKC signaling is altered in hyperactivity in peripheral tissues (platelets), as well as in postmortem brain tissues of patients with schizophrenia, bipolar disorder, and major depressive disorder, and *n*–3 fatty acids act as endogenous antagonists of the PtdIns-PKC signal transduction pathway (McNamara et al., 2006). In addition, *n*–3 fatty acid deficiency is observed in peripheral and central tissues of patients with schizophrenia, bipolar disorder, and major depressive disorder. Based on

detailed investigations on signaling, it is proposed that *n*–3 fatty acid deficiency may contribute to elevated PtdIns-PKC activity in these illnesses.

Serotonergic neurotransmission plays an important role in bipolar depression and is the site of action of several antidepressants (Delgado, 2004). It is interesting to note that *n*–3 fatty acid supplementation increases the concentration of serotonin in pig frontal cortex (De la Presa-Owens and Innis, 1999), whereas *n*–3 fatty acid depletion reduces the ability of fenfluramine, an appetite suppressant, to stimulate serotonin release in rat hippocampus (Kodas et al., 2004). The neurochemical changes in serotonin levels are reversed by supply of the balanced diet provided at birth or during the first 2 weeks of life through the maternal milk. However, alterations in serotonin levels persist if the balanced diet is given from weaning (at 3 weeks of age). This suggests that the provision of essential fatty acids is durably able to affect brain function, and that this is related to the developmental stage during which the deficiency occurs (Kodas et al., 2004). Furthermore, depletion of *n*–3 fatty acids in rats results in the modification of glycerophospholipid composition and neurochemical function of specific cerebral areas such as striatum and frontal cortex. Thus, rats given *n*–3 fatty acid-deficient diet have an increased serotonin 2_A receptor density in the frontal cortex and decreased D_2 receptors (Delion et al., 1997). Similar results have been reported in the frontal cortex of suicide victims (Maggioni et al., 1990).

A diet deficient in *n*–3 fatty acids also produces a decrease in the dopamine concentration in rat frontal cortex (Delion et al., 1996). Inversely, a diet enriched in *n*–3 fatty acids produces a 40% increase in the dopamine concentration in rat frontal cortex (Chalon et al., 1998). Thus, the modulation of brain dopamine levels by dietary *n*–3 fatty acids may have an effect on dopamine-mediated behavior in individuals with neuropsychiatric disorders.

n–3 fatty acid deficiency produces a decrease in NMDA receptor subunits NR2A and NR2B in the cortex of transgenic mice overexpressing the human Alzheimer disease gene APPswe (Calon et al., 2005). Dietary supplementation with *n*–3 fatty acids partly protects these mice from NMDA receptor subunit loss, indicating that dietary *n*–3 fatty acids can influence cognitive processes in Alzheimer disease and other disorders. These fatty acids also increase the resistance of forebrain cholinergic neurons against NMDA-mediated neurotoxicity (Högyes et al., 2003), indicating that *n*–3 fatty acid supplementation increases neural cell resistance against the excitotoxic damage by being incorporated into neural membrane glycerophospholipids. A decrease in *n*–3 fatty acids in the diet affects the aging process by decreasing longevity and learning ability in rats (Yamamoto et al., 1987). *n*–3 fatty acids modulate responses to γ-aminobutyric acid (GABA), and shifts the inactivation curve to a more hyperpolarized potential in both Na^+ and Ca^{2+} currents in primary neuronal cultures (Hamano et al., 1996).

Collective evidence suggests that depletion of *n*–3 fatty acids can increase the risk of developing neuropsychiatric disorders. The neurochemical mechanism underlying this effect is the deficiency of resolvins and neuroprotectins. As stated earlier, these metabolites are antiinflammatory and they antagonize the effects of eicosanoids. They decrease the transcription activation gene for adhesion molecules, chemoattractant, inflammatory cytokines involved in endothelial activation in response to inflammatory stimuli. Furthermore, *n*–3 fatty acids also modulate gene expression (Horrocks and Farooqui, 2004). Thus, microarray studies indicate that a diet enriched in fish oil modulates the overexpression of 55 genes and suppression of 47 genes in brain. These genes include genes controlling synaptic plasticity, cytoskeleton, and membrane association, signal transduction, ion channels, energy metabolism, and regulatory proteins (Farkas et al., 2000; Kitajka et al., 2002; Puskás et al., 2003). DHA stimulates the expression of peroxisomal enzymes needed for the synthesis of plasmalogens (Farooqui and Horrocks, 2001; Brites et al., 2004; André et al., 2005, 2006; Farooqui et al., 2008b).

9.9 Involvement of Genes in Neuropsychiatric Disorders

Recent DNA microarray studies indicate that genes involved in synaptic neurotransmission, signal transduction, and glutamate/GABA regulation are differentially regulated in brains of subjects with schizophrenia and bipolar disorders (Fatemi et al., 2006; Ogden et al., 2004). Thus, 177 putative schizophrenia risk genes have been identified in brain, 28 of which map to linked chromosomal loci (Glatt et al., 2005). These genes include MAG, CNP, SOX10, CLDN11, and PMP22 (Dracheva et al., 2006; Wan et al., 2005). Furthermore, 123 putative biomarkers for schizophrenia have been described in blood, six of which (BTG1, GSK3A, HLA-DRB1, HNRPA3, SELENBP1, and SFRS1) have corresponding differential expression in brain (Glatt et al., 2005). Similarly, genes for DARPP-32, PENK (preproenkephalin), and TAC1 (tachykinin 1, substance P) have been identified in bipolar subjects (Ogden et al., 2004). These studies suggest that molecular mechanisms involved in pleasure and pain may have been recruited by evolution to play a role in higher mental functions, such as mood. The analysis also revealed other high-probability candidates genes (neurogenesis, neurotrophic, neurotransmitter, signal transduction, circadian, synaptic, and myelin related), pathways and mechanisms of likely importance in pathophysiology of bipolar disorders. The continued application of this approach in larger populations should facilitate the discovery of highly reliable and reproducible candidate risk genes and biomarkers that can be used for the identification of neuropsychiatric disorder patients in a human population (Konradi, 2005). This information may be useful for early diagnosis and monitoring the effects of therapeutic drugs for the treatment of neuropsychiatric disorders.

9.10 Conclusion

Neuropsychiatric diseases are characterized by dysregulation of several neurotransmitters at many different levels including the synthesis, storage, release, reuptake, and inactivation, changes in neuroplasticity, abnormalities in processing information along with deficiency in learning and memory. Levels of neurotransmitters are modulated by diet. Thus, serotonin and dopamine are derived from nutrient precursors. Serotonin is derived from the tryptophan while dopamine is derived from the tyrosine (Fernstrom, 1994). Similarly, fatty acid composition of neural membrane glycerophospholipids is regulated by diet. Although supplementation of DHA alters membrane fatty acid composition throughout the body, neural membranes are the most sensitive targets for fatty acid alterations. Thus, in neural membranes incorporation of DHA and EPA promotes and maintains synaptic plasticity and induces neurogenesis in the brain circuits, and reduces oxidative stress and neuroinflammation through the generation of lipoxins, neuroprotectins, and resolvins. In addition, DHA and EPA also facilitate optimal membrane fluidity, promote appropriate neurotrophic support, and inhibit production of cytokines and expression of adhesion molecules. Neuropsychiatric diseases are multifactorial diseases and may involve several factors. They include deficiency of *n*–3 fatty acid and neurotrophic factors (BDNF), and involvement of certain genes. *n*–3 fatty acids are important dietary supplements that not only promote and maintain synaptic plasicity and neurogenesis in the aging brain circuits but reduce oxidative stress and neuroinflammation through the generation of lipoxins, neuroprotectins, and resolvins. A deficiency of *n*–3 fatty acids is closely associated with the pathogenesis of neuropsychiatric diseases.

BDNF has emerged as a regulator of brain development and neuroplasticity. Decrease in BDNF neurotrophic activity may occur through decreased expression of BDNF or through inability of the receptor to transduce signals in the presence of some genetic or environmental factor. The intracellular mechanism involves MAPK cascade and cyclic adenosine 3′,5′-monophosphate cascade.

In addition, etiology of neuropsychiatric disorders also involves genetic and environmental factors. Molecular genetic studies suggest that the genetic architecture of neuropsychiatric disorders is complex, while the handful of genome-wide scans conducted thus far is not conclusive. Microarray analysis has not only identified many candidate gene studies of neuropsychiatric disorders but also provided substantial evidence for implication of several genes in the etiology of these disorders. Genes associated with schizophrenia include BTG1, GSK3A, HLA-DRB1, HNRPA3, SELENBP1, and SFRS1. Similarly genes for DARPP-32, PENK (preproenkephalin), and TAC1 (tachykinin 1, substance P) have been identified in bipolar subjects. Genes for ADHD include dopamine D4 receptor gene (DRD4), the dopamine D5 receptor gene (DRD5), the dopamine transporter gene (DAT), the dopamine beta-hydroxylase gene (DBH), the serotonin transporter gene (5-HTT), the serotonin receptor 1B gene

(HTR1B), and the synaptosomal-associated protein 25 gene (SNAP25). Cloning of these genes may provide important insights into the mechanisms of neuropsychiatric diseases. Collectively, these studies suggest that neuropsychiatric diseases are multifactorial polygenic diseases in which many genes and environmental factors interact and bring about neural cell injury. Supplementation of diet with DHA and EPA improves cognition, learning, and memory by restoring neural membrane integrity. EPA supplementation improves schizophrenic and hyperactive patients, while a combination of EPA and DHA provides benefit in bipolar disorders and autism.

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Chapter 10
Status and Potential Therapeutic Importance of *n*–3 Fatty Acids in Other Neural and Non-neural Diseases

10.1 Introduction

n–3 and *n*–6 fatty acids are the major families of PUFA that are components of the human diet. After ingestion, both *n*–3 and *n*–6 fatty acids are distributed to every cell in the body where they are actively involved in many physiological processes, including modulation of cardiovascular, immune, hormonal, metabolic, neuronal, and visual functions (Farooqui, 2009). At the cellular level, these fatty acids are incorporated in cellular membranes glycerophospholipids. The interaction of agonists with various receptors results in activation of isoforms of phospholipases A_2 and the release of *n*–3 and *n*–6 fatty acids. *n*–3 and *n*–6 fatty acids and their enzymically oxidized products (docosanoids and eicosanoids) act as intracellular signaling and participate in the regulation of gene expression (Farooqui, 2009). Deficiency of *n*–3 fatty acid not only causes many neurological and neuropsychiatric problems (Chapters 7, 8, and 9) but is also associated with many other neural and non-neural diseases, including peroxisomal diseases, rheumatoid arthritis, cystic fibrosis, several types of cancers, chronic obstructive pulmonary disease (COPD), dermatological conditions, immune problems, and gastrointestinal disorders (inflammatory bowel disorders). A variety of biochemical effects of *n*–3 fatty acids have been demonstrated from feeding studies with fish or fish oil supplements in humans and animals. These include effects on triglycerides, high-density lipoprotein cholesterol, platelet function, endothelial and vascular function, blood pressure, cardiac excitability, measures of oxidative stress, pro- and antiinflammatory cytokines, and immune function.

10.2 Effect of Fish Oil on Peroxisomes

Studies on the effect of diet enriched in fish oil in mice indicate that even after prolonged feeding, 0.1% fish oil-enriched diet has no effect on peroxisomes in liver, heart, and kidney. Similarly, feeding 0.8% fish oil-enriched diet effects neither peroxisomal number nor the catalase activity in the liver. Hepatic

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peroxisomal β-oxidation is increased by 50% after 14 days. This is accompanied by reduction in the size of peroxisomes. The 0.8% fish oil-enriched diet also produces a small increase (+25%) in myocardial catalase activity but has no effect on kidneys. Feeding fish oil to mice does not affect fatty acid metabolism in the liver (Van den Branden et al., 1995; De Craemer et al., 1996). These observations are of particular interest to those patients with a peroxisomal fatty acid β-oxidation defect, who display a severe deficiency of DHA. Diets supplemented with low fish oil doses can improve the DHA level without adding a strong load to the disturbed fatty acid metabolism.

10.3 Peroxisomes and Peroxisomal Disorders

Peroxisomes are complex subcellular organelles in nearly all eukaryotic cells. They are involved in numerous metabolic functions, including β-oxidation of very long-chain fatty acids, α- and β-oxidation of branched long-chain fatty acids, synthesis of plasmalogens, cholesterol, leukotrienes, and bile acids (Van Veldhoven and Mannaerts, 1999). Peroxisomes do not contain DNA; the matrix and membrane proteins are encoded by the nuclear genome. Peroxisomal disorders are a group of genetically heterogeneous metabolic diseases that share dysfunction of peroxisomes, such as β-oxidation of very-long-chain fatty acids (VLCFA), synthesis of plasmalogen, and bile acid. Peroxisomal diseases are classified into three categories – peroxisome biogenesis disorders (PBDs), single peroxisomal enzyme deficiencies, and contiguous gene syndrome (Fig. 10.1). PBDs comprise 14 complementation groups, and their responsible genes have been identified. The first group of disorders is characterized by a failure to form intact and normal peroxisomes, resulting in multiple metabolic abnormalities, which are referred to as peroxisome biogenesis disorders (PBD) or as generalized peroxisomal disorders. The second category of disorders is characterized by the deficiency of a single peroxisomal enzyme (Shimozawa, 2007). The contiguous gene syndromes are characterized by multiple unrelated clinical characteristics produced by deletion of the multiple adjacent genes. Each of the individual genes within a contiguous region, when mutated, encodes for a distinct biochemical feature. There has been significant development on the genetic mechanism of diseases not only caused by single peroxisomal enzyme abnormalities but also caused by defects in peroxisome biogenesis.

Peroxisome assembly disorders (Zellweger syndrome and rhizomelic chondrodysplasia punctata) are caused by genetic defects in PEX genes and alterations in proteins encoded by PEX genes, peroxins, which are necessary for the importation of targeted proteins into the peroxisomes (Raymond, 1999). Fourteen PEX genes are required for normal organelle assembly for peroxisome biogenesis. Mutations in each of the 14 PEX genes cause a peroxisomal biogenesis defect (PBD). Affected patients can be classified into two broad

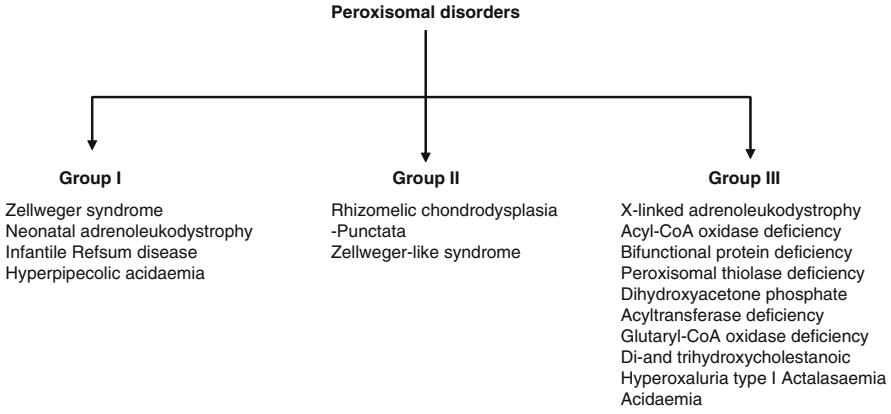

Fig. 10.1 Classification of peroxisomal disorders

clinical spectra: the Zellweger spectrum, which accounts for about 80% of PBD patients and rest of rhizomelia chondrodysplasia punctata (RCDP) spectrum (Krause et al., 2006). The clinical feature of Zellweger spectrum patients extends from Zellweger syndrome as the prototype, and the most severe entity of this group to neonatal adrenoleukodystrophy (NALD) as an intermediate form, and infantile Refsum (IRD) disease as the mildest variant. Characteristic features of ZS patients are dysmorphic features, severe neurological impairment, liver dysfunction, and eye and skeletal abnormalities. Similar but less severe clinical signs are seen in patients with NALD and IRD (Krause et al., 2006).

10.3.1 Zellweger Syndrome

Zellweger syndrome is a rare autosomal recessive disease characterized by defect in neuronal migration, abnormal peroxisomal biogenesis, deficiency plasmalogen, and lower levels of DHA in brain (Table 10.1) and other body tissues compared to normal healthy subjects (Martinez and Mougan, 1999). Zellweger syndrome patients also have a defect in neuronal migration, which is the major cause of the severe mental retardation and seizure associated with this disorder. Studies on glycerophospholipid composition indicate that levels of glycerophospholipids are only slightly altered in peroxisomal disorders compared to age-matched controls (Martinez and Mougan, 1999). Marked differences are observed in the distribution and levels of glycerophospholipids. Thus, PlsEtn is very much decreased, but PtdEtn is increased whereas PtdSer and PtdCho are decreased in the brain tissue from peroxisomal disorders compared to age-matched control. Marked changes are observed in PUFA composition in glycerophospholipids (Martinez and Mougan, 1999). DHA is drastically decreased in all glycerophospholipid classes while ARA is increased in PtdEtn

Table 10.1 Deficiency of DHA in peroxisomal disorders and beneficial effects of DHA on peroxisomal disorders

Disorder	DHA	DHA treatment	References
Zellweger syndrome	Decreased	Beneficial	Martínez and Mougan (1999); Martínez et al. (2000)
Zellweger mouse model	Decreased	Beneficial	Janssen et al. (2000)
Rhizomelic form of chondrodysplasia	Decreased	Not known	Oorthuys et al. (1987)
Infantile Refsum disease	Decreased	Beneficial	Martínez (2000)
Neonatal adrenoleukodystrophy	Decreased	Beneficial	Martínez (2000)
X-linked adrenoleukodystrophy	Decreased	Not known	Martínez (2000)
Adrenomyeloneuropathy	Decreased	Not known	Martínez (2000)
Retinitis pigmentosa	Decreased		Anderson et al. (1999)

and PtdSer. In PlsEtn marked changes are observed in DHA and ARA composition. Both fatty acids are very significantly decreased. The myelin PUFA, adrenic acid, is decreased in PlsEtn, but PtdSer is slightly increased. The changes in fatty acid composition confirm the deficiency of DHA in the brain in peroxisomal disorders (Martinez and Mougan, 1999). Similarly, DHA levels are decreased in the brain of Pex7: ABCD 1 double KO mice (Table 10.1), but DHA levels were normal in visceral organs at birth. All tissues examined in postnatal mutant mice show reduced DHA levels (Faust et al., 2001). Detailed investigation in Pex7:ABCD 1 double KO mouse indicate that plasmalogens not only act as structural glycerophospholipids but also protect neural cells from damage caused by VLCFA accumulation (Brites et al., 2008). VLCFAs disrupt to the structural integrity and stability of neural cells, especially oligodendrocytes that are associated with the generation of myelin sheath. In brain tissue, accumulation of VLCFA also results in gliosis, inflammatory demyelination, and axonopathy, which are modulated by plasmalogens. It is proposed that deficiency of plasmalogens make brain tissue more prone to neural cell damage. In testis, plasmalogens protect spermatocytes from VLCFA-mediated degeneration and apoptosis (Brites et al., 2008). Plasmalogens and DHA are essential for myelination (Horrocks and Sharma, 1982; Farooqui, 2009), so their deficiency in Zellweger syndrome may be responsible for some neurologic as well as retinal problems in these patients.

10.3.2 Adrenoleukodystrophy

Adrenoleukodystrophy (ALD) is an inherited disorder characterized by progressive demyelination in brain and adrenal dysfunction. Biochemically, ALD

is characterized by the accumulation of pathgnomonic amounts of saturated VLCFA, particularly hexacosanoic (C26:0) and tetracosanoic acids (C24:0) in all tissues, including the brain white matter, adrenal glands, and skin fibroblasts, of the patients (Morita, 2007). The accumulation of VLCFA is linked to a mutation in the ABCD1 gene that encodes ABCD1/ALDP, a peroxisomal ABC protein. ABCD1/ALDP is thought to be involved in the active ATP-driven transport of VLCFA-CoA from the cytoplasm into the peroxisomes (Morita, 2007). However, the precise function of ABCD1/ALDP is still unclear. The accumulation of VLCFA is caused by reducing peroxisomal VLCFA β-oxidation and/or increasing fatty acid elongation. To understand the toxic effects of VLCFA in the brain, neural cells have been treated with VLCFA (Hein et al., 2008). It is reported that exposure of oligodendrocytes and astrocytes to docosanoic- (C22:0), tetracosanoic- (C24:0) and hexacosanoic acids (C24:0) causes neural cell death within 24 h. VLCFA induce depolarization of mitochondria in situ and increase intracellular Ca^{2+} level in all three brain cell types (Hein et al., 2008). Interestingly, oligodendrocytes are markedly affected by VLCFAs toxicity. In isolated mitochondria, VLCFAs exert a detrimental effect by affecting the inner mitochondrial membrane and promoting the permeability transition. Collectively, these studies indicate that VLCFAs mediate their toxicity by inducing mitochondrial dysfunction and Ca^{2+} deregulation (Hein et al., 2008). Since the reduction in accumulation of VLCFA in the brain is thought to be crucial for preventing the progression of neurologic symptoms in ALD, compounds that can cross the blood–brain barrier without harm and decrease the VLCFA levels in the brain can be highly attractive agents for effective treatment of ALD patients.

X-linked adrenoleukodystrophy (X-ALD) is a clinically heterogeneous disorder ranging from the severe childhood cerebral form to asymptomatic persons. The disease is caused by mutations in the ABCD1 gene, which encodes a peroxisomal transporter that plays a role in the import of VLCFA or VLCFA-CoA into peroxisomes. X-ALD is characterized by accumulation of VLCFA (>C22:0), particularly in ganglioside and cholesterol ester fractions of brain white matter and adrenal cortex. In addition, exhaustive depletion of cholesterol and sphingomyelin in comparison to controls has also been reported (Paintlia et al., 2003). Failure of activation of VLCFA by peroxisomal very long chain fatty acyl-CoA synthetase prevents their degradation by peroxisomal β-oxidation. X-ALD maps to Xq28 and the gene encodes a peroxisomal membrane protein and not the gene for VLCS (McGuinness and Smith, 1999). The accumulation of VLCFA in white matter of ALD patients is also accompanied by a decrease in levels of PlsEtn, increase in levels of ROS, lipid aldehydes (4-hydroxynonenal and acrolein), and deleterious oxidized proteins (protein carbonyl) in all areas of the cALD brain (Steinberg et al., 2008; Khan et al., 2008). This observation is supported by studies on ALDP knock-out (KO) mice. Low levels of PlsEtn are detected in brain white matter of ALDP KO mice. Treatment of ALDP KO mice with lovastatin upregulates PlsEtn levels in the brain.

Further, in an in vitro study, lovastatin treatment of rat C6 glial cells upregulates PlsEtn biosynthesis and downregulates the cytokine-induced ROS accumulation. Collective evidence suggests that alterations in PlsEtn metabolism and ROS in ALD may be corrected with lovastatin treatment (Khan et al., 2008).

The expression of mRNA for cytokines (IL-1α, IL-2, IL-3, IL-6, TNF-α, and GM-CSF), chemokines (CCL2, -4, -7, -11, -16, -21, -22, CXCL1, CX3CL1, and SDF-2), and iNOS is significantly upregulated compared to normal looking area of the ALD and controls. It is proposed that the accumulation of VLCFA contents in membrane domains may trigger the inflammatory process through activation of resident glial cells (microglia and astrocytes), resulting in loss of myelin and oligodendrocytes (Khan et al., 1998; Paintlia et al., 2003).

10.4 Treatment of Peroxisomal Disorders with DHA

Treatment of Zellweger syndrome patients with purified DHA partially improves visual function, increases levels of plasmalogens, and reduces levels of saturated very long chain free fatty acids (Martínez et al., 1999, 2000; Martínez, 2001). The most constant improvement has been in the normalization of the DHA levels and in the liver function. Some DHA-treated patients also show vision improvement and increase in muscle tone, which may be caused by DHA-mediated reduction in fibrinolytic/matrix-metalloproteinase (MMP) activity (Delbosc et al., 2008). It is reported that DHA decreases vascular smooth muscle cells migration/proliferation by interacting with Notch pathway that modulates expression of interleukin-1β as well as fibrinolytic/MMP activity (Delbosc et al., 2008).

Magnetic resonance imaging (MRI) examination shows improvement in myelination in nine patients. Significantly, the clinical improvement has been most marked in those patients who started the treatment before 6 month of age. Similarly, patients with generalized peroxisomal disorders also show improvements in brain and liver functions (Martínez, et al., 1999). In contrast, recent studies in a mouse model of Zellweger syndrome, peroxisome-deficient mice, indicate that normalization of DHA levels in Zellweger pups does not improve symptoms of Zellweger syndrome. The neuronal migration defect was unaltered, indicating that a DHA deficit is not a major pathogenic factor in these newborn Zellweger mice (Janssen et al., 2000; Infante and Huszagh, 2001). The reason for this discrepancy between human and mouse is not known. Apparently, the peroxisome-deficient mouse is not an exact model for the human condition. The discrepancy may be due to differences in signal transduction between the human disease and its mouse model.

10.4.1 DHA and Adrenomyeloneuropathy

Adrenomyeloneuropathy is a rare inherited metabolic disorder. Adrenomyeloneuropathy patients generally have spinal cord dysfunction, which leads to the initial symptoms that include difficulties in walking or a change in the walking pattern. Like X-ALD, this disease is characterized by the accumulation of VLCFAs, which form cytoplasmic inclusions (cholesterol esterified with VLCFAs) and deficiency of DHA leading to progressive dysfunction in the nervous system, adrenal glands and testes; all of which are sites of VLCFA metabolism (Martínez et al., 2000; Spurek et al., 2004). The gene for adrenomyeloneuropathy is located on the long arm of the X chromosome (Xq28) and encodes a peroxisomal membrane protein belonging to the adenosine triphosphate-binding cassette superfamily of transporter proteins. Administration of monounsaturated fatty acids in adrenomyeloneuropathy patients leads to competitive inhibition between saturated and unsaturated fatty acid precursors in the microsomal elongation pathway, thereby leading to a reduction in endogenous VLCFA synthesis (Spurek et al., 2004). Treatment of adrenomyeloneuropathy patients with DHA also results in beneficial effects (Martínez et al., 2000).

10.4.2 DHA, Retinitis Pigmentosa, and Retinopathy

Retinitis pigmentosa (RP) is a group of genetic (hereditary) diseases that cause degeneration of rods and cones (light-sensing cells) in the retina. Over time, RP patients lose night vision in adolescence, side vision in young adulthood, and central vision in later life because of progressive loss of rod and cone photoreceptor cells. At the molecular level, retinal degenerations involve multiple and complex cell-signaling pathways that are modulated by genes and ultimately result in photoreceptor cell death through apoptosis. RP is very heterogeneous, both phenotypically and genetically.

More than 45 genes for retinitis pigmentosa have been identified (Hartong et al., 2006). These genes modulate components of the phototransduction cascade, proteins involved in retinol metabolism and cell–cell interaction, photoreceptor structural proteins and transcription factors, intracellular transport proteins and splicing factors (Hims et al., 2003). Integral to this demise is the close relationship between photoreceptors and retinal pigment epithelial (RPE) cells. It is well known that photoreceptor cells are enriched in DHA, which is esterified at *sn*-2 position of glycerophospholipids of lipid bilayer, where it provides an optimal environment for protein interaction and signal transduction. Humans and animals with inherited RP have lower blood and tissue levels of DHA than controls (Anderson et al., 1999, 2002; Hodge et al., 2006). The primary defect in RP may not be related to DHA metabolism, but rather some transport mechanism(s), which may lead to the reduction in blood and tissue levels of DHA. The possibility is that mutations in RP may cause

metabolic stress that provokes structural and biochemical changes, such as enrichment of glycerophospholipid metabolism in photoreceptor cells and their rod outer segments (Anderson et al., 1999). Studies on treatment of RP with nutritional interventions (vitamin A palmitate and *n*–3-rich fish) slow progression of RP in many patients (Berson et al., 2004; Hartong et al., 2006; Hodge et al., 2006). Because of the list of newly identified genes, growing knowledge of affected biochemical pathways, and development of animal models, larger trials for RP are needed, especially for genetically defined subsets of patients (Hartong et al., 2006).

Age-related macular degeneration (AMD) along with dementia is another leading cause of irreversible blindness in the elderly (Johnson and Schaefer, 2006; SanGiovanni et al., 2007). DHA is highly concentrated in retinal tissue. It has been reported to prevent or delay the progression of dementia and AMD. Higher intake of specific types of fat – including vegetable, monounsaturated, and polyunsaturated fats and linoleic acid – rather than total fat intake may be involved in a greater risk for developing advanced AMD. It is suggested that DHA-enriched diet is inversely associated with risk for AMD. Although at this point it is not possible to recommend DHA for the treatment of AMD and dementia, data from the Framingham Heart Study suggest that = 180 mg/d of dietary DHA (approximately 2.7 fish servings/wk) is associated with an approximately 50% reduction in dementia risk (Johnson and Schaefer, 2006; SanGiovanni et al., 2007).

In other eye diseases, such as retinopathy of prematurity and diabetic retinopathy, which may cause blindness in childhood and middle age, increasing *n*–3 fatty acid levels by dietary or genetic means reduces the avascular area of the retina by increasing vessel regrowth after injury, thereby reducing the hypoxic stimulus for neovascularization (Connor et al., 2007). Although, the molecular mechanism involved in neovascularization is not known, *n*–3 fatty acid-derived lipid mediators, neuroprotectin D_1, resolvin D_1, and resolvin E_1, may contribute to protection against neovascularization (Connor et al., 2007). It is also proposed that the protective effect of *n*–3 fatty acids and their lipid metabolites may be mediated, in part, through suppression of TNF-α. This inflammatory cytokine is highly expressed in a subset of microglia that is closely associated with retinal vessels. These findings indicate that increasing the sources of *n*–3 fatty acids or their lipid mediators reduce pathological angiogenesis (Connor et al., 2007). Collective evidence suggests that supplementing of diet with *n*–3 fatty acids may protect from retinopathy.

10.5 DHA and Prion Diseases

Prion diseases are characterized by neuronal loss, spongiform degeneration, and glial cell proliferation. They include scrapie, found in goats and sheep, bovine spongiform encephalopathy (mad cow disease) in cattle, and fetal

familial insomnia, Creutzfeldt-Jakob disease (CJD), kuru, and Gerstmann-Sträussler-Scheinker syndrome in humans (Prusiner, 2001; Grossman et al., 2003). Human prion protein (PrP^c) contains 209 amino acids, a disulfide bridge between residues 179 and 214, a glycosylphosphatidylinositol (GPI) anchor, and two sites of non-obligatory N-linked glycosylation at amino acids 181 and 197 (DeArmond and Prusiner, 2003). The conversion of the normal PrP^c (α-helix and random coil structures) to an alternatively folded β-pleated sheet containing isoform (PrP^{sc}) is the main cause of prion diseases (Prusiner, 2001; Grossman et al., 2003). The accumulation of PrPSc within the brain leads to neurodegeneration through an unknown mechanism. Prion infection increases the free cholesterol content of prion-infected ScGT1 and ScN2a cells as well as in primary cortical neurons, a process that is not replicated by the stimulation of cholesterol synthesis (Bate et al., 2008a). The presence of PrP^{sc} not only increases solubilization of free cholesterol in cell membranes but also activates the PLA_2 pathway, which has been implicated in PrP^{sc}formation and in PrP^{sc}-mediated neurotoxicity (Bate et al., 2008a). Thus, pathogenesis of prion diseases may involve PrP^{sc}-mediated alterations in cholesterol homeostasis, which may be involved in the loss of cognitive function in prion diseases (Jeffrey et al., 1992).

Treatment of prion-infected neuronal cells with DHA, EPA, or the cholesterol synthesis inhibitor, simvastatin, decreases the amounts of free cholesterol in membrane extracts. Simvastatin reduces cholesterol synthesis while DHA and EPA stimulate the conversion of free cholesterol to cholesterol esters. Crucially, while simvastatin reduces PrP^{sc} formation, both DHA and EPA significantly increase the amounts of PrPSc in these cells. Unlike simvastatin, the effects of DHA and EPA on PrP^{sc} content are not reversed by stimulation of cholesterol synthesis with mevalonate. Treatment of ScGT1 cells with DHA and EPA also increases the activation of PLA_2 and generation of PGE_2. Finally, treatment of neuronal cells with DHA and EPA increases the amounts of PrP^c expressed at the cell surface and significantly increases the half-life of biotinylated PrP^c, indicating that although treatment with DHA or EPA significantly reduces the free cholesterol content of prion-infected cells, they significantly increase PrP^{sc} generation in three neuronal cell lines (Bate et al., 2008b). DHA or EPA treatment of infected cells stimulates PLA_2, an important enzyme associated with the generation of $PrP^{sc}$and alterations in PrPc trafficking. These results are consistent with the view that DHA- or EPA-mediated alterations in cell membranes may not only increase the amounts of abnormal prion protein but also stimulate $cPLA_2$ activity, upregulate PGE_2 synthesis, increase caspase-3 activity, and reduce neuronal survival, raising the possibility that some DHA or EPA supplements may accelerate neuronal loss in the terminal stages of prion diseases (Bate et al., 2008c).

Usher syndrome, an autosomal recessive gene disorder is characterized by hearing loss and a progressive loss of vision from RP. The hearing loss is thought to be congenital, and ranges from moderate to profound. RP can occur without hearing loss (Jacobson et al., 2008). Many patients with Usher

syndrome have speech difficulties and severe balance problems. Although Usher syndrome have low levels of DHA in the retina (Anderson et al., 1999), DHA trials for this disease have not been performed.

10.6 DHA and Multiple Sclerosis

Multiple sclerosis (MS) is a progressive autoimmune disease in which the immune system attacks the the brain and spinal cord causing demyelination (striping of myelin from the axons). Early symptoms of multiple sclerosis include weakness, tingling, numbness, and blurred vision. Pathologically, MS is chareacterized by the presence of multifactorial inflammatory infiltrates (T cells, B cells, and macrophages) within the brain associated with degradation of myelin, oligodendrocytes, and axons, reactive astrogliosis, and activation of microglia (Reindl et al., 2006; Berger and Reindl, 2007).

The molecular mechanisms associated with lesion formation in multiple sclerosis are unknown. The prevailing view is that macrophages are primary mediators of myelin destruction in the relapsing and remitting forms of this disease. However, recent investigations indicate widespread oligodendrocyte apoptosis in the absence of a clear cellular immune response (Matute and Perez-Cerda, 2005). Development of experimental autoimmune encephalomyelitis (EAE) in rats has been used as an experimental model for MS. It is reported that association of neuroinflammation in brain of rats with EAE compromises the peroxisomal functions (Singh et al., 2004). Degradation of very long chain fatty acids is not only reduced by 47% but also causes the accumulation (C26:0, 40%). A 66% decrease in activity of dihydroxyacetonephosphate acyltransferase (DHAP-AT), first enzyme in plasmalogens biosynthesis, results in reduction in plasmalogen levels (16–30%). Catalase activity is also decreased by 37%. Gene microarray analysis of EAE spinal cord show a significant decrease in transcripts encoding peroxisomal proteins including catalase (folds 3.2; $p<0.001$) and DHAP-AT (folds 2.6; $p<0.001$). Thus, decrease of peroxisomal functions in the brain tissue may have negative consequences for myelin integrity and repair because plasmalogens are major constituents of myelin (Singh et al., 2004). Administration of lovastatin (a cholesterol lowering and antiinflammatory drug) provides protection against loss/downregulation of peroxisomal functions during EAE induction. This suggests that inflammatory mediators may have a markedly negative effect on peroxisomal functions and thus on myelin assembly and that these effects can be prevented by treatment with statins (Singh et al., 2004).

Few studies have been performed on fatty acid composition in neural and non-neural tissues from MS patients. Determination of fatty acid composition in RBC indicates that MS patients show a significant decrease in γ-linolenic acid in PtdCho and stearic acid in PtdEtn fraction. No reduction in LA is observed in either the PtdCho or PtdEtn fractions of the MS subjects (Nightingale et al.,

1990). Studies on fatty acid composition in adipose tissue indicate no detectable EPA in MS and healthy controls. Although DHA is not detected in MS patients, 40% of the controls show measurable levels of DHA. No changes are observed in LA levels in MS patients. Treatment of MS patients with fish oil indicates that *n*–3 fatty acids are incorporated into red blood cells over 5 weeks. The effects of oral supplementation on adipose tissue are followed for several years. Levels of LA are increased at 1 year and levels of DHA and EPA continued to rise through 2-year period (Nightingale et al., 1990). Treatment of nine MS patients with fish oil for 2 years results in a significant reduction in the mean annual exacerbation rate and the mean Expanded Disability Status Scale (EDSS) as compared to pre-study values. The plasma total glycerophospholipid *n*–3 fatty acids are increased and *n*–6 fatty acids decreased significantly, suggesting that dietary intake of fish oil can improve clinical outcome in patients with newly diagnosed MS (Nordvik et al., 2000). In contrast, recent studies indicate that PUFA have no major effect on the main clinical outcome in MS (disease progression), and do not substantially affect the risk of clinical relapses over 2 years (Farinotti et al., 2007).

10.7 DHA and Non-neural Diseases

Components of Western diet contribute not only to the increased risk of atherosclerosis and coronary disease but also contribute to chronic obstructive pulmonary disease (COPD), Crohn's disease (CD), systemic lupus erythematosus (SLE), rheumatoid arthritis (AR), ulcerative colitis (UC), and cystic fibrosis (CF). Many above mentioned diseases are autoimmune diseases are characterized by a high level of cytokines (IL-1 and TNF-α) and the proinflammatory eicosanoids (leukotriene LTB_4) derived from *n*–6 fatty acids. Reduced calorie intake and supplementation of diet enriched in DHA delay the onset of above diseases, primarily, due to increased antioxidant enzyme activities, decreased NF-kB activation, and decreased IL-1, IL-6, and TNF-α mRNA expression (Table 10.2). Furthermore, inclusion of *n*–3 fatty acids and

Table 10.2 Deficiency of DHA in chronic visceral diseases and beneficial effects of DHA on peroxisomal chronic visceral diseases

Disorder	DHA	DHA treatment	References
Chronic obstructive pulmonary disease	Decreased	Beneficial	Shahar et al. (1999)
Crohn's disease	Decreased	Beneficial	Ramano et al. (2005)
Systemic lupus erythematosus	Decreased	Beneficial	Duffy et al. (2004)
Cystic fibrosis	Decreased	Beneficial	Al-Turkmani et al. (2008)
Arthritis	–	Beneficial	Cleland et al. (2003)
Osteoporosis	–	Beneficial	Calder (2008a)

soy protein to rodent diet protects them against bone loss induced by ovariectomy in mice due to NF-kB expression and decreased activation of osteoclasts. The available evidence indicates that increased daily intake of dietary *n*–3 fatty acids reduces the severity of autoimmune disorders, lessens the chance of developing cardiovascular disease, and protects against bone loss during postmenopause (Fernandes et al., 2008).

Consumption of fish oil has beneficial effects on chronic inflammatory disorders, but several weeks are required for beneficial effects to appear. This may be due to the downregulation of proinflammatory cytokine and stimulation of antioxidant enzymes (Ergas et al., 2002; Farooqui et al., 2007). Fish oil supplementation seems advantageous especially in acute and chronic disorders where inappropriate activation of the immune system occurs (Table 10.2). Fish oil has not only a mild effect on active inflammation in rheumatoid arthritis, SLE and Crohn's disease, but it may also prevent relapse (Ergas et al., 2002). In diseases where the inflammation is mild, fish oil may slow or even prevent disease progression. If the disease is already an existing condition and intensity of inflammation is high, increased consumption of fish oil may not be beneficial. Therefore, the use of *n*–3 fatty acids can be recommended to the general public, not only to prevent cardiovascular and cerebrovascular diseases but also to reduce the risk of autoimmunity (Ergas et al., 2002). There have been a number of clinical trials assessing the benefits of dietary supplementation with fish oils in COPD, AR, CD, UC, and SLE in humans. Many of the placebo-controlled trials of fish oil in chronic inflammatory diseases reveal significant benefit, including decrease in disease activity and a reduction in use of antiinflammatory drugs (Simopoulus, 2002).

10.7.1 DHA and Chronic Obstructive Pulmonary Disease

COPD (emphysema or chronic bronchitis) is a serious progressive lung disease characterized by progressive obstruction of the airflow into and out of the lungs and increase in shortness of breath. Other symptoms of COPD can cause coughing that produces large amounts of mucus, wheezing, and chest tightness. COPD involves a chronic inflammatory response, predominantly in small airways and lung parenchyma, which is characterized by increased numbers of macrophages, neutrophils, and T lymphocytes. The inflammatory mediators associated with COPD include inflammatory peptides, ROS and RNS, chemokines, cytokines, and growth factors (Barnes, 2004; Chung, 2005). These mediators are involved in orchestrating the complex inflammatory process that results in small-airway fibrosis and alveolar destruction (Barnes, 2004). Generation of ROS and RNS and depletion of glutathione in COPD is not only accompanied by intense airway wall remodeling, but also by the

expression of IL-1, TNF-α, and IL-8 from macrophages, alveolar, and bronchial epithelial cells (Chung, 2005). TNF-α and IL-1β mediated expression and release of matrix metalloproteinase-9 (MMP-9) from macrophages and bronchial epithelial cells, leads to production of extracellular matrix glycoproteins such as tenascin (Chung, 2005).

Environmental factors, such as cigarette smoking, outdoor and indoor pollution, and childhood respiratory infections, are known to play a major role as risk factors for developing COPD. A fully established genetic risk factor for COPD is α-1-antitrypsin deficiency (Miki and Satoh, 1999). In addition, polymorphisms for the genes including α-1-antichymotrypsin, vitamin D-binding protein, microsomal epoxide hydrolase, cytochrome P450, glutathione *S*-transferase M1 and T1 (GSTM1 and GSTT1), hemeoxygenase, MMP-9, TNF-α, and immunoglobulin A have also been reported in different world populations. It is stated that COPD is a complex polygenic disease in which gene–environment interactions play a very important role (Nishimura, 2003). Collective evidence suggests that pathogenesis of COPD may be a self-perpetuating process leading to a chronic inflammatory state with associated airway remodelling and progressive lung function decline. Noxious substances (toxic oxidants/free radicals molecules, and particulate matters) or gases (NO_2, SO_2), most commonly from smoking, may also be involved in the pathogenesis of COPD.

Bronchial mucosal dendritic cells, the professional antigen-presenting cells, are another important constituent of the chronic inflammatory cell influx found in airways of patients with COPD. Role of bronchial mucosal dendritic cells in the pathogenesis of smoking-induced in COPD is not clear. It is recently proposed that the stimulation of bronchial mucosal dendritic cells mediate B-cell growth and T-cell responses in cigarette smoke-mediated oxidative stress. Nicotine induces the generation of proinflammatory responses that promote chronic inflammation in smokers probably through the proliferation and regulation of the balance of Th1/Th2 T cells (Vassallo et al., 2008; Tsoumakidou et al., 2008).

In addition to cigarette smoking (a major risk factor for COPD), nutritional depletion is another common problem in COPD patients. Nutritional depletion is caused, to a large extent, by an imbalance between low-energy intake and high-energy requirements (Thomas, 2002; Steinkamp, 2003; Schols, 2003; Planas et al., 2005). Nutritional imbalance adversely affects morbidity and mortality of COPD patients. Both metabolic and mechanical inefficiency may contribute to elevated energy expenditure during physical activity, while systemic inflammation involves hypermetabolism at rest. In addition, COPD-specific symptoms and systemic inflammation may impair appetite and dietary intake.

Western societies consume a proinflammatory diet. Typical Western diet consists of 20- to 25-fold more ARA than DHA. ARA consumption promotes the release of eicosanoids, which are major inducers of inflammation in various

tissues including bronchial system and lungs. It is hypothesized that a smoker's risk of developing COPD is inversely related to physiologic levels of EPA and DHA (Shahar et al., 1999). Determination of EPA and DHA in plasma of nonsmokers, current, or former smokers indicates that chances of developing COPD are inversely related to the DHA (but not to the EPA) content of plasma lipid components, indicating that DHA may be a potential agent that may have positive effects on COPD and other chronic inflammatory conditions of the lung (Schwartz, 2000).

The enrichment of DHA in diet in patients with COPD may partially replace ARA from membranes of lungs and inflammatory cells (neutrophils and lymphocytes). This change leads to decrease in the production of eicosanoids. This response alone can be a potentially beneficial antiinflammatory effect of DHA. However, DHA may produce a number of other effects, which might occur downstream to altered eicosanoid generation (Calder, 2002; Schwartz, 2000; Kompauer et al., 2008; Wong, 2005). For example, supplementation of diet with DHA suppresses the expression of proinflammatory cytokines and decreases adhesion molecule expression. These effects not only related to biophysical properties of lungs and inflammatory cell membranes but also at the level of gene expression causing either antagonism of eicosanoids synthesis or other effects on the intracellular signaling pathways, which may lead to the activation of transcription factors such as NF-κB. It is recently shown that DHA downregulates the activity of NF-κB (Calder, 2002; Wong, 2005). DHA and EPA can provide beneficial effects through another mechanism. Thus, oxidized *n*–3 fatty acids (J3-isoprostanes) react directly with the negative regulator of Nrf2, Keap1, initiating Keap1 dissociation with Cullin3, thereby inducing Nrf2-directed gene expression (Gao et al., 2007). Nrf2-based cellular defense pathway protects against oxidative stress. Collectively, these studies suggest that high dietary intake of DHA may protect against COPD (Shahar et al., 1999, 2008; Wong, 2005; Matsuyama et al., 2005; Kompauer et al., 2008).

10.7.2 DHA and Crohn's Disease

Crohn's disease (CD) is a chronic inflammatory disease characterized by swelling, ulceration, and transmural inflammation of the gastrointestinal (GI) tract. Although CD can affect any area of the GI tract, it most commonly affects the lower part of the small intestine. CD is a relapsing disease that ultimately leads to the destruction of the intestinal tissue. The swelling in CD extends deep into the lining of small intestine. It causes pain and makes the intestines empty frequently, resulting in diarrhea. In addition, serious rectal bleeding, weight loss, skin problems, and fever may also occur. The symptoms of CD are similar to irritable bowel syndrome and ulcerative colitis, which is caused by inflammation and ulcers in the top layer of the lining of the large intestine. In CD, all layers of the intestine may be involved. The etiology of CD is unknown.

However, it is proposed that multiple factors including specific genetic (genes encoding immune responses) and environmental factors (*Mycobacteria*, *Yersinia*, *Campylobacter*, *Clostridium*, *Clamidias*, or their products) along with immune dysfunction may contribute to pathogenesis of CD (Louis, 2001; Watt and Satsangi, 2002). Chronic inflammation during the course of CD is mediated by dysregulation of the adaptive immune system leading to immunological imbalance, which is characterized by excessive production and release of cytokines. Another important factor in CD is inflammatory processes cause mucosal damage, and therefore a further disturbance of the epithelial barrier function results in an increased influx of bacteria into the intestinal wall. This may even further accelerate the inflammatory processes (Schmidt and Stallmach, 2005). The onset of disease occurs when the immune system attacks the gastrointestinal tract, and because of this reason, CD is considered as an autoimmune disease. This autoimmune activity causes inflammation in the gastrointestinal tract, and therefore CD is classified as an inflammatory bowel disease.

In CD patients, consumption of fish oil plus antioxidants modifies cellular membrane composition and lowers generation of PGE_2 and interferon-γ by circulating monocytes or macrophages, leading to a decrease in disease activity in CD patients (Trebble et al., 2004, 2005; Romano et al., 2005; Turner et al., 2007). In addition, DHA and EPA may also act by changing the lipid environment in membrane microdomains of tight junction in vitro (Li et al., 2008). Thus, *n*–3 fatty acids effectively block the redistribution of occludin and ZO-1 and distortion of tight junction morphology, reduce transepithelial electrical resistance induced by IFN-γ and TNF-α. A dramatic reorganization of tight junction proteins in epithelial lateral membrane is observed following treatment with above cytokines (Li et al., 2008). These results indicate that *n*–3 fatty acids play an important role in proinflammatory cytokines-induced permeability defects and epithelial barrier dysfunction by modifying lipid environment in membrane microdomains of tight junction (Li et al., 2008). Collectively, these studies suggest that *n*–3 fatty acids are safe, and are effective for maintenance of remission in CD when used in enteric-coated capsules (Trebble et al., 2004; Romano et al., 2005; Trebble et al., 2005, 2007). A number of annoying side effects of fish oil such as belching, halitosis, and diarrhea have also been reported to occur in CD patients during treatment with fish oil.

10.7.3 DHA and Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune chronic inflammatory disease characterized by arthritis, cutaneous rash, vasculitis, and involvement of central nervous system, renal, and cardiopulmonary manifestations (Tsao, 2004; Wong and Tsao, 2006). Patients with SLE generate abnormal antibodies in their blood that target tissues within their own body rather than foreign

infectious agents. Molecular mechanism and causes associated with abnormal autoimmunity in lupus is not known. However, abnormalities in the cytokine network may be closely associated with pathobiology of this disease. Risk factors include genes, viruses, ultraviolet light, and drugs. It is suggested that the immune system in SLE is more easily stimulated by external factors, such as viruses or ultraviolet light. Common symptoms of SLE include joint pain, skin inflammation, and skin lesions, which are aggravated by ultraviolet radiation from sunlight, fatigue, sleep disorders, such as restless legs syndrome and sleep apnea and hair loss.

Anti-DNA antibodies are the serologic hallmark of SLE and important markers for diagnosis and prognosis (Pisetsky, 1992). Recent studies in human and murine lupus indicate that disease susceptibility results from genetic polymorphisms regulating immune responses as well as impairing the clearance of apoptotic cells (Ardoin and Pisetsky, 2008; Pisetsky, 2008). Because the products of dead cells, including nucleic acids, have immunologic activity, this situation can promote antigen-driven antinuclear antibodies responses. Furthermore, immune complexes of antinuclear antibodies can drive the expression and release of proinflammatory cytokines, inducing the "interferon signature" and intensifying disease (Ardoin and Pisetsky, 2008).

n–3 fatty acids reduce T-cell proliferation, and suppress the production of interleukin-1, interleukin-2, and TNF-α by these cells both in vitro and in vivo. Dietary supplementation of EPA and DHA produces prolonged remission of in SLE patients without any side effects. These results suggest that *n*–3 fatty acids, EPA and DHA, are useful in the management of SLE (Das, 1994). Detailed investigations indicate that low doses of dietary fish oil in SLE not only have a therapeutic effect on disease activity but also improve endothelial function, decrease in the number of autoreactive (hyperreactive) T cells, and reduce oxidative stress, and may therefore confer cardiovascular benefits (Wright et al., 2008). At the molecular level, DHA and EPA may act both directly by replacing ARA as an eicosanoid substrate and inhibiting ARA metabolism and indirectly by altering the expression of inflammatory genes through their effects on transcription factor activation. DHA and EPA may also give rise to a family of antiinflammatory mediators termed resolvins and protectins. Thus, *n*–3 PUFA are potentially potent antiinflammatory agents. Reduced calorie intake and supplementation of diet with *n*–3 fatty acids have synergistic effect and delay the onset of SLE, primarily, due to upregulation in antioxidant enzyme activities, downregulation NF-κB activation, and decrease in IL-1, IL-6, and TNF-α mRNA expression in various body tissues (Duffy et al., 2004; Wright et al., 2008).

10.7.4 DHA and Cystic Fibrosis

Cystic fibrosis (CF) is the most common lethal inherited disorder that affects lungs and gastrointestinal system, and caused by mutation in the gene encoding

the CF transmembrane conductance regulator, an ATP-gated chloride channel that is regulated by cAMP-dependent protein kinase (Zeng et al., 1997; Freedman et al., 1999). Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene also disrupt another vital physiological function, Cl^--dependent HCO_3^- transport. ATP and trypsin evoke intracellular calcium signaling in pancreatic duct-derived cells through the activation of purinergic and protease-activated receptors and facilitate Cl^--dependent HCO_3^- transport (Reddy and Quinton, 2001; Namkung et al., 2003). Alterations in CFTR function result in pancreatic insufficiency and ileal hypertrophy that prevents digestion and absorption of food. The defective CFTR gene and its protein product induce body to generate unusually thick, sticky mucus that clogs lungs which results in life-threatening lung infection (Reddy and Quinton, 2001; Namkung et al., 2003).

The pathological hallmark of CF chronic inflammatory response is the massive neutrophil (polymorphonuclear cells, PMN) influx into the airways (Pizurki et al., 2000; Conese et al., 2003). This abnormal neutrophil emigration is probably triggered by the abnormal secretion of chemoattractants by respiratory epithelial cells and polarized lymphocyte T-helper response (Conese et al., 2003). Neutrophils from CF patients respond differently to inflammatory mediators than neutrophils from normal subjects, indicating that they are primed in vivo before entering the CF airways. CF neutrophils secrete more myeloperoxidase and elastase, mobilize less opsonin receptors, and release less L-selectin than non-CF neutrophils (Conese et al., 2003). Blocking surface adhesion molecules ICAM-1, VCAM-1, and E-selectin on CFTR-deficient bronchial epithelial cells with specific monoclonal antibodies retards the adherence of CF apoptotic airway neutrophils. In addition, CF neutrophils show upregulation in cytokine synthesis (IL-6 and IL-8) and an abnormal chemotaxis response (Tabary et al., 2006). Thus, in CF patients adherence of a large number of neutrophils on airway epithelium may be associated with elevated levels of IL-6 and IL-8, and these processes may contribute to sustained and exaggerated inflammatory response in CF airways. It is also suggested that CFTR may be associated with regulation of some neutrophil functions and indicate that alterations in properties of CF neutrophils may depend on genetic factors (Pizurki et al., 2000; Conese et al., 2003).

Biochemical alterations in CF also include alterations in circulating and pancreas, lungs, and ileum levels of ARA and DHA, particularly a decrease in DHA. The imbalance in ARA and DHA levels in CF knock-out mice ($CFTR^{-/-}$ mice) may be related to CFTR function. In $CFTR^{-/-}$ mice, the imbalance in ARA and DHA levels can be reversed by the oral administration of DHA (Freedman et al., 1999, 2000). It is shown that ARA-derived lipid mediator, leukotriene B_4 (LTB_4), is associated with the enhanced influx of neutrophils into the lungs. DHA therapy reverses the increased neutrophil infiltration in the lungs of CF knock-out mice. Like $cftr^{-/-}$ mice, levels of LA and DHA are significantly lower in patients with severe CF (Strandvik et al., 2001). Mechanisms associated with fatty acid alterations in CF are not understood, but recent

investigations on cellular uptake of LA and DHA in a human bronchial epithelial cell model of CF indicate that CF (antisense) cells have reduced levels of LA and DHA compared to wild-type (WT, sense) cells expressing normal CFTR (Al-Turkmani et al., 2008). The uptake of LA and DHA is higher in CF cells compared with WT cells. Subsequent incorporation of LA and DHA into most lipid classes and individual glycerophospholipids is also increased in CF cells. The metabolic conversion of LA to ARA is increased in CF cells, indicating increased flux LA through the *n*–6 pathway (Al-Turkmani et al., 2008). Supplementing CF cells with DHA prevents the production of LA metabolites and corrects the *n*–6 fatty acid defect. Collectively, these studies suggest that low LA level in cultured CF cells is due to its increased metabolism, and this increased LA metabolism can be corrected by DHA supplementation (Al-Turkmani et al., 2008).

Although, oral supplementation of DHA in few DeltaF508 homozygous cystic fibrosis patients shifts the serum glycerophospholipid fatty acids to a less proinflammatory profile, no conclusive clinical improvement is observed in CF patients, and more studies are required on a larger group of patients (Al-Turkmani et al., 2007; Van Biervliet et al., 2008).

Besides above changes in fatty acid levels, CF is characterized by increased production of proinflammatory mediators and oxidant stress, and systemic redox imbalance with reduced glutathione (GSH) (Devlin et al., 2007; Innis et al., 2007; Innis and Davidson, 2008). Glycerophospholipid and fatty acid metabolism is closely related to GSH through the methionine–homocysteine cycle, in which choline via betaine provides methyl groups to regenerate *S*-adenosylmethionine, important in generating PtdCho and PlsCho and amino acid precursors for GSH (Fig. 10.2). Homocysteine metabolism is connected to two pathways: remethylation to methionine, which requires folate and vitamin B12 (or betaine in an alternative reaction); and transsulfuration to cystathionine which requires vitamin B6. The two pathways are coordinated by *S*-adenosylmethionine which acts as an allosteric inhibitor of the methylenetetrahydrofolate reductase and as an activator of cystathionine β-synthase. Supplementation of diet with choline or betaine in children with CF results not only in increased plasma levels of methionine but also in higher *S*-adenosylmethionine (SAM):*S*-adenosylhomocysteine (SAH):oxidized glutathione (GSSG) ratio (Innis et al., 2007; Innis and Davidson, 2008). Chronic malabsorption of PtdCho in CF causes depletion of choline, whose levels may be further compromised by downregulation of de novo choline synthesizing enzymes resulting from a low SAM:SAH in children with CF (Chen et al., 2005; Innis and Hasman, 2006). Enrichment of choline in diet may be an effective intervention through conservation of methionine and increased SAM, which may induce beneficial effects related to choline availability and oxidative stress in patients with CF. Alternatively, supplementation with betaine may provide an effective intervention to reduce homocysteine and oxidative stress and to increase the recycling of homocysteine to methionine (Innis et al., 2007; Innis and Davidson, 2008). Homocysteine levels are also linked with DHA levels in neural and non-neural tissues (Li et al., 2006, 2007).

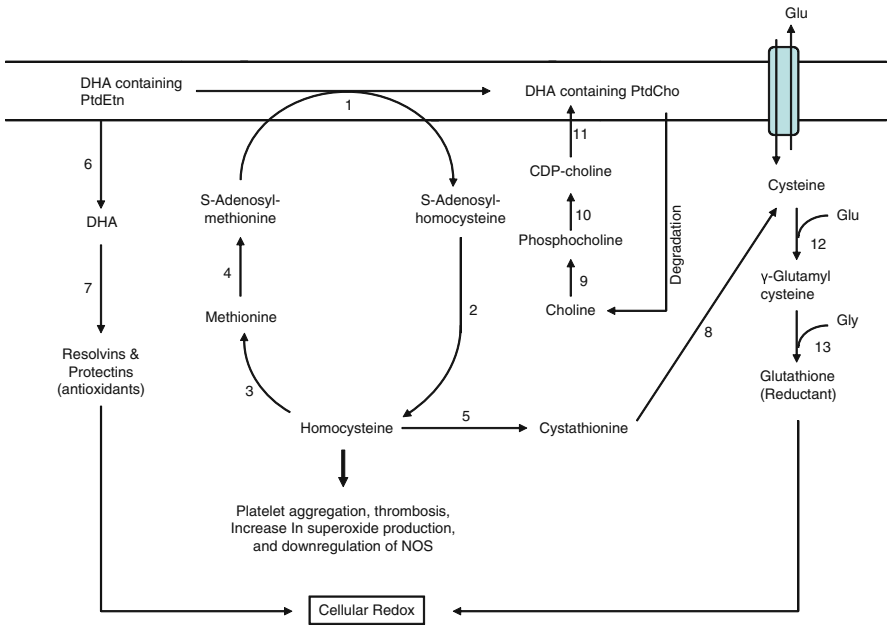

Fig. 10.2 Interrelationship between glycerophospholipid and cysteine metabolism. Phosphatidylethanolamine *N*-methyltransferase (1); *S*-adenosylhomocysteine hydrolase (2); 5-methytetrahydrofolate homocysteine methyltransferase (3); methionine adenosyltransferase (4); cystathionine β-synthase (5); phospholipase A_2 (6); cyclooxygenase (7); cystathionase (8); choline kinase (9); CTP-phosphocholine cytidyltransferase (10); CDP-choline transferase (11); γ-glutamylcysteine synthetase (12); glutathione synthatase (13); glutamate (Glu); glycine (Gly); and nitric oxide synthase (NOS)

Thus, increase in plasma level of DHA through the consumption of DHA-enriched diet and vitamins like folic acid is associated with decrease in plasma homocysteine. This may have cardioprotective and neuroprotective effects in CF patients not only through decrease in homocysteine levels but also through maintenance of optimal redox status of various tissues (Das, 2008; Selley, 2007; Schaefer et al., 2006). Collective evidence suggests that alterations among DHA metabolism, cysteine/homocysteine pathway, and glutathione level are controlled by certain genes that modulate ARA/DHA levels along with oxidant defenses and redox balance in various tissues from CF patients, and these processes may be closely associated with the pathogenesis of CF.

10.7.5 DHA and Arthritis

Arthritis is a group of diseases characterized by inflammation in joints. There are different forms of arthritis; each has a different cause. They include

osteoarthritis, rheumatoid arthritis (RA), and psoriatic arthritis, and artthritis associated with autoimmune diseases. The most common form of arthritis, osteoarthritis, is caused by trauma to the joint, infection of the joint, or age. RA is a chronic autoimmuneinflammatory disease affecting joints of hands, wrists, feet, knees, cubitus, ankles, shoulder, and is manifested by swelling and pain producing functional impairment. Psoriatic arthritis is a chronic autoimmune disease caused by malfunctioning immune system and characterized by inflammation of the skin psoriasis (a serious skin condition) and joints. Arthritis associated with autoimmune diseases is caused by the production of antinuclear antibodies (ANA), which instead of attacking the legitimate infectious, viral invaders, and mutated cells of the body, turn and attack aspects of the healthy body cells and tissues itself. The immune system of patient becomes confused, poorly defending against outside invading agents while attacking healthy tissues and organs.

RA is a multifactorial condition characterized by chronic inflammation of the joints. Diet (*n*–6 fatty acid-enriched diet), environmental factors (infectious agents, smoking, sex hormones), and genes (upregulation of proto-oncogenes and transcription factors) modulate and contribute to the pathogenesis of RA (Kobayashi et al., 2008).

Tissue proteinases synthesized and released by synovia, chondrocytes, and pannus promote cartilage destruction, and cytokine-activated osteoclasts have been implicated in bone erosions. RA synovial tissues synthesize a variety of cytokines and growth factors that induce monocyte differentiation to osteoclasts and their proliferation, activation, and longer survival in tissues. The involvement of immune cells is a general hallmark of RA. In this regard, macrophages, T cells, and their respective cytokines (TNF-α, interleukin-1, interleukin-6, interleukin-17 interferon-γ), chemokines (monocyte chemoattractant protein-1, monocyte chemoattractant protein-4 CCL18), cell adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and matrix metalloproteinases) play a major role in pathophysiology of RA. Expression of these molecules is controlled at the transcription level and activation of a limited number of transcription factors participate in this process (Okamoto et al., 2008; Kobayashi et al., 2008). Chronic inflammation is accompanied by increased osteoclast differentiation and resorptive activity, driven by disregulation of receptor activator of NF-κB ligand (RANKL) and the synergistic activity of inflammatory cytokines. In RA osteoclast survival is increased, but osteoblast differentiation and survival are reduced, with a consequent decrease in bone formation and a net loss of bone (Findlay and Haynes, 2005).

Inflammation in joints is an orthoprotective mechanism that isolates the damaged bone cells from undamaged area. In arthritis joint, lesions are characterized by infiltration of T lymphocytes, macrophages, B lymphocytes, and polymorphonuclear leukocytes (PMN) in the lesion area. The migration of these cells is a co-ordinated multistep process involving chemotaxis, adhesion in the lesion area (Diamond et al., 1999). PMN and macrophages eliminate

antigens by phagocytosis and release free radicals and lytic enzymes into phagolysosomes. This is followed by a process called resolution, which is a turning off mechanism by neural cells to limit tissue injury. In relation to inflammatory processes, main fatty acid that contributes to joint inflammation is ARA. It is metabolized by COX and LOX to proinflammatory lipid mediators (PGE_2 and LTB_4) called eicosanoids. Generation of these proinflammatory lipid mediators is closely associated with the pathophysiology of all forms of arthritis. During inflammatory reaction, lipid mediators not only initiate inflammatory responses but also mediate resolution. There are two phases in inflammatory responses: the first phase of inflammation involves release of ARA and generation of PGE_2 and LTB_4. The second phase of inflammation is associated with the generation of lipoxins (LXA_4 and LXB_4), and the proresolving prostaglandin, PGD_2 (Gilroy et al., 2004; Serhan and Chiang, 2008; Parkinson, 2006). Lipoxins act through ATL receptors (ALX-R), and not only a play role as intiinflammatory mediator but also participate in "immunomodulation" via regulation of macrophage, dendritic cell, and T-lymphocyte effector functions in the setting of polarized T-helper cell responses (Th1 and Th2) (Parkinson, 2006). Collectively, these studies indicate that during inflammatory reaction prostaglandins, leukotrienes, thromboxanes, and lipoxins not only serve as autocrine molecule that contribute to edema but are also associated with resolution of inflammation.

In general, high intake of *n*–3 fatty acids decreases generation of eicosanoids, inhibits expression of proinflammatory cytokines, retards the formation of ROS, and blocks the expression of adhesion molecules (Calder, 2002). As stated earlier, *n*–3 fatty acids act both directly (e.g., by replacing ARA in cellular membranes and decreasing precursors for eicosanoids and competing for COX and LOX) and indirectly (e.g., by downregulating the expression of inflammatory genes through effects on transcription factor activation). *n*–3 fatty acids also give rise to a family of antiinflammatory mediators termed resolvins and protectins (Serhan et al., 2004). Thus, *n*–3 fatty acids are potentially potent antiinflammatory drugs that can be used for the treatment of a variety of acute and chronic inflammatory diseases, including rheumatoid arthritis, inflammatory bowel diseases, and asthma (Calder, 2006a,b, 2008a,b). The molecular mechanism associated with beneficial effects of *n*–3 fatty acids within the synovial joint is not understood. However, it is proposed that *n*–3 fatty acid supplementation of human osteoarthritic cartilage downregulates the expression and activities of enzymes involved in cartilage degradation (aggrecanases and collagenases) (Curtis et al., 2003) as well as COX-2 and IL-1α and TNF-α. Downregulation of these processes may contribute to slowing the progression of degenerative joint diseases (Curtis et al., 2003).

The number of patients with various forms of arthritis has increased rapidly in recent years (Horrocks and Yeo, 1999). This is because of changes in diet. Excessive amounts of *n*–6 fatty acids and a very high *n*–6/*n*–3 fatty acid ratio, as is found in today's Western diets, promote the pathogenesis of inflammatory and autoimmune diseases, whereas increased levels of *n*–3 PUFA (a lower *n*–6/

n–3 ratio) exert suppressive effects (Simopoulos, 2008). A *n*–6/*n*–3 ratio of 2-3/1 suppresses inflammation in patients with RA. Collective evidence suggests that the optimal *n*–6/*n*–3 ratio for beneficial effects may vary from one disease to another (Table 10.3). This view has been recently challenged. It is reported that the ratio of *n*–6/*n*–3 fatty acids has several inherent problems (Harris, 2007,

Table 10.3 *n*–3/*n*–6 ratio and its therapeutic value in chronic degenerative diseases

Disease	*n*–3/*n*–6 Ratio	Risk factor
Heart disease	4:1	Decreased
Colorectal cancer	2.5:1	Decreased
Breast cancer	4:1	Decreased
Rheumatoid arthritis	2–3:1	Decreased
Asthma	5:1	Decreased

Summarized from Mills et al. (2005) and Simopoulos (2008).

2008; Griffin, 2008). Firstly, the *n*–6/*n*–3 fatty acid ratio makes no distinction between ALA and the metabolically more active EPA/DHA. Secondly, the ratio suffers from the fact that the *n*–6 and *n*–3 fatty acids are metabolized with different rates generating different lipid mediators. This can make *n*–6/*n*–3 fatty acid ratio a higher or lower. Finally, ratio may be influenced by the biochemical and physiological activities of *n*–6 and *n*–3-derived lipid mediators. ARA-derived eicosanoids are metabolically more potent in promoting inflammation, platelet aggregation, and immune and vascular reactivity than DHA- and EPA-derived eicosanoids, resolvins, and protectins (Farooqui and Horrocks, 2007; Harris, 2008; Griffin, 2008). These drawbacks can produce considerable variation in the physiological response to a decrease in the ratio and can complicate the interpretation of intervention studies. Furthermore, chronic diseases are multigenic and multifactorial conditions; therefore, it is quite possible that the therapeutic ratio may also depend on the degree of severity of disease, resulting from the genetic predisposition (Simopoulos, 2008). Public use of fish oil (containing 1–2 g DHA and EPA) daily from childhood to old age may have protective effects against many chronic diseases found in Western societies, as well as in the developing countries.

10.7.6 DHA and Osteoporosis

Osteoporosis is a bone disorder characterized by the reduction in bone mineral density, disruption in bone microarchitecture, and alterations in non-collagenous proteins leading to an increased risk of fracture. Fish oil is known to protect inflammation-mediated bone loss in chronic inflammatory diseases, such as RA, periodontitis (inflammation-mediated progressive loss of the

alveolar bone around the teeth), and osteoporosis (Sun et al., 2003; Rahman et al., 2008). The molecular mechanism associated with bone loss is not fully understood. However, it is known that bone mineral density loss correlates with upregulation in RANKL (a receptor activator of NF-κB ligand) expression in activated CD4+ T-cells from corn oil-fed ovariectomized mice, but no changes are observed in fish oil-fed mice (Sun et al., 2003). In vitro addition of *n*–3 fatty acids results in a significant decrease in TRACP activity and $TRACP^+$ multinuclear cell formation from bone marrow cells compared with ARA and LA. DHA and EPA also prevent bone marrow macrophage NF-κB activation mediated by RANKL. Levels of (TNF-α, IL-2, and IFN)-γ are decreased in splenocytes of both sham and ovariectomized fish oil-fed mice, and interleukin-6 is decreased in sham-operated fish oil-fed mice, suggesting that inhibition of osteoclast generation and activation may be one of the mechanisms by which dietary *n*–3 fatty acids reduce bone loss in ovariectomized mice (Sun et al., 2003). Similarly, studies on the effect of *n*–3 fatty acids on RANKL-stimulated osteoclastogenesis using a murine monocytic cell line RAW 264.7 indicate that EPA and DHA have differential effects on RANKL signaling (Rahman et al., 2008). DHA downregulates osteoclast differentiation, while EPA markedly upregulates this process. The differential potential closely correlates with the downregulation of osteoclast-specific genes, including tartrate-resistant acid phosphatase, cathepsin K, calcitonin receptor, matrix metalloproteinase-9, and osteoclast-specific transcription factor, c-Fos, as well as TNF-α to a greater extent with DHA than EPA (Rahman et al., 2008). Further, pretreatment of RAW 264.7 cells with DHA also cause significant downregulation of NF-κB and p38MAPK than EPA. It is suggested that DHA may be much more effective than EPA in alleviating RANKL-mediated proinflammatory cytokine generation, intracellular signaling activation, thereby decreasing osteoclast activation and bone resorption (Rahman et al., 2008).

10.7.7 DHA and Psoriasis

Psoriasis is a non-contagious skin disease, characterized by the appreance of red scaly patches due to inflammation. Marked increase in ARA levels along with upregulation in generation of its proinflammatory metabolites ($LTB_4$and 12-HETE) has been observed in psoriatic lesions (Mayser et al., 2002a). Intravenous injections of Omegavenous, an emulsion of DHA and EPA in psoriasis patients, reduce symptoms of this disease (Mayser et al., 1998). The beneficial effect of DHA and EPA is related to reduction in generation of inflammatory eicosanoids. The rapidity of the response to intravenous injections of Omegavenous makes it as a better procedure of delivering DHA and EPA than oral supplementation (Mayser et al., 2002a). Similarly, alterations in the eicosanoids

metabolism (increase in lipoxygenase products) along with increase in ARA levels are observed in patients with atopic dermatitis (Mayser et al., 2002b). Administration of *n*–3 fatty acids effectively improves the severity of this skin condition. Collective evidence suggests that *n*–3 fatty acids are important therapeutic agents for the treatment of psoriatic lesions and atopic dermatitis.

10.7.8 DHA and Its Clinical Trials in Chronic Diseases

Using fish oil as a source of *n*–3 fatty acids, many clinical trials have been performed for heart diseases (Tziomalos et al., 2007), and these results have been very encouraging. Enrichment of *n*–3 fatty acids in diet not only prevents cardiovascular diseases but also protects from arrhythmia and hypertriglyceridemia (Leaf, 2007). Fish oil supplementation also modestly decreases blood pressure and lowers the levels of homocysteine and reduces levels of fibrinogen.

Although biochemical studies have linked DHA with COPD, data on usefulness of DHA in preventing COPD symptoms are limited (Matsuyama et al., 2005). The relation between the dietary intake of *n*–3 fatty acids and COPD in 8,960 current or former smokers participating in a population-based study of atherosclerosis indicates that high dietary intake of *n*–3 fatty acids has positive effects and may protect cigarette smokers against COPD (Shahar et al., 1994). More double-blind clinical trials are needed in big populations of COPD to judge the usefulness of DHA for COPD treatment. It is also reported that a high intake of fish helps retard the age-related decline in lung capacity in smokers and non-smokers. Long-term trial of fish oil supplementation in adult asthma patients show significant improvements, but shorter trials (less than 1 year) have not confirmed these findings (Schwartz, 2000).

Few trials have been performed of CF patients using fish oil (Katz et al., 1996; Christophe et al., 1992; De Vizia et al., 2003). The CF patients who supplemented fish oil in their diet show a marked increase in their phospholipid levels of EPA and DHA and levels of EPA and DHA return to baseline 2 weeks after discontinuing supplementation. It is suggested that oral supplementation with fish oil and lecithin is effective in increasing the levels of *n*–3 fatty acids in CF patients. Based on several trials, it is concluded that long-term fish oil supplementation (8 months) has positive effects, such as decreasing inflammation, in cystic fibrosis (Katz et al., 1996; Christophe et al., 1992; De Vizia et al., 2003). More trials are needed to judge the beneficial effects of *n*–3 fatty acids in CF patients.

In a clinical trial with 78 patients with CD who had been classified as having a high risk of relapse, half the patients are randomized to given nine enteric-coated 500-mg fish oil capsules (40% and 20% EPA and DHA) daily, the other half are treated with nine placebo capsules daily. Fish oil treatment has very positive results on CD. In control group, 69% of the patients show a relapse in

CD during 1-year study period, only 28% patients in fish oil-treated group show relapse in the disease. At the end of the 1-year period, CD was in still remission in 59% of the patients as compared to only 26% in the control group. It is concluded that fish oil therapy is effective in preventing relapses in patients with CD in remission (Belluzzi et al., 1996).

Seventeen published clinical trials and two meta-analyses have shown that oral supplementation with fish oil or intravenous administration of *n*–3 fatty acid preparation called Omgegaven^{R} reduces symptoms of RA in patients despite ongoing treatments with RA drugs (Stamp et al., 2005; Fritsche, 2006). In addition, fish oil has beneficial effects in occlusive cardiovascular disease, for which patients with RA are at increased risk (Leeb et al., 2006; Kremer, 2000). After 12 weeks of therapy, patients reported not only significant improvements in morning stiffness and joint tenderness but also in a reduction in the amount of medication and in levels of C-reactive protein. In addition, patients show increase in grip strength and enhancement in walking pace. There are no serious side effects. Dietary supplementation with *n*–3 fatty acids in patients with active RA has a modest effect on five out of eight disease variables, without effect on other traditional parameters for monitoring disease activity. A large double-blind clinical trial may help in defining a possible therapeutic role for *n*–3 fatty acids in patients with RA.

EPA is known to only facilitate increase in levels of calcium in the body, but also deposition of calcium in the bones and thus improving bone strength. In addition, EPA- and GLA-deficient subjects are more likely to suffer from bone loss than those with normal levels of these fatty acids. Supplementation of EPA and GLA in women with osteoporosis experienced significantly less bone loss over 3 years than those who were given a placebo (Sun et al., 2003).

10.8 Conclusion

Two distinct families of PUFA, namely *n*–6 fatty acids and *n*–3 fatty acids, are known to occur in human tissues. Humans are evolved on a diet with roughly equal amount of *n*–6 and *n*–3 fatty acids. Introduction of vegetable oils in early 1950s has resulted in an enormous increase in the consumption of *n*–6 fatty acids. The present day Western diet has this ratio of fatty acids has reached anywhere from 15 to 20 times as much *n*–6 acids, compared to *n*–3 acids. A high consumption of *n*–6 fatty acids shifts the physiological state to one that is prothrombotic and proaggregatory as characterized by increase in blood viscosity, vasospasm, and vasoconstriction and reduction in bleeding time. Consumption of *n*–6 fatty acids in vegetable oils elevates eicosanoids and upregulates the expression of proinflammatory cytokines, such as IL-1, IL-6, and TNF-α. In contrast, *n*–3 fatty acids have antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, and vasodilatory properties. Daily use of *n*–3

fatty acids may slow down the intensity of many chronic visceral diseases such as coronary heart disease, hypertension, rheumatoid arthritis, Crohn disease, cystic fibrosis, and chronic obstructive pulmonary disease. There have been few small clinical trials of chronic diseases using fish oil, and results have been very encouraging. Large double-blind clinical trials are necessary to test the efficacy of *n*–3 fatty acid for the treatment of chronic visceral diseases.

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Chapter 11
Perspective and Directions for Future Development on the Effects of Fish Oil Constituents on Brain

11.1 Introduction

Interest in *n*–3 and *n*–6 fatty acids and their beneficial and harmful effects on brain has increased immensely over the past 25 years. These fatty acids are required in large amounts for normal brain development and growth. Because humans do not have desaturase enzymes that facilitate the insertion of *n*–3 or *n*–6 double bonds, both families of fatty acids are obtained from the diet and are regarded as essential fatty acids. DHA status of the newborn and breastfed infant depends on the maternal intake of DHA and varies widely because infants obtain *n*–3 fatty acids (DHA) directly from mother's milk and in adult life from dietary fish and fish oil or from its precursor α-linolenic acid (ALA) in liver. Mammalian liver also uses ALA to make another long-chain *n*–3 fatty acid called EPA, which while beneficial in many ways is not as essential to body functions as is DHA. The rate of conversion of ALA into DHA and EPA is very low. Humans can convert only 5–10% of dietary ALA into DHA and EPA. Similarly, during infancy humans obtain *n*–6 fatty acid (ARA) from mother's milk and in adulthood either from plant sources or synthesized from linoleic acid (LA) in liver. Fatty acid compositional studies indicate that in brain ARA is evenly distributed in gray and white matter PtdCho, PtdEtn, and PtdSer, while DHA is highly concentrated in neuronal membranes, most so in synaptic plasma membrane PlsEtn and PtdSer. Among subcellular structures, the highest levels of DHA are found in synaptosomes, mitochondria, and microsomes (Farooqui, 2009). ARA, DHA, and EPA incorporate and not only modulate biophysical and biochemical properties of neural membrane but also markedly affect the signal transduction processes associated with optimal brain function and gene expression. In general, DHA is immunosuppressive and slows growth, while ARA stimulates growth. This suppression of growth by DHA is mediated through a decrease in ARA-derived PGE_2.

Both DHA and ARA act as precursors for DHA- and ARA-derived lipid mediators, such as docosanoids and eicosanoids, respectively. The incorporation and proportions of DHA and ARA markedly influence neural membrane properties such as fluidity, flexibility, elasticity, permeability, and gene expression (De Caterina and Massaro, 2005; Deckelbaum et al., 2006). The metabolic

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utilization of DHA differs from that of ARA metabolism. Oxygenation of ARA generates proinflammatory mediators such as prostaglandins, leukotrienes, and thromboxanes, whereas DHA generates antiinflammatory lipid mediators such as resolvins, protectins, and neuroprotectins, which directly or indirectly suppress the activity of NF-κB (Farooqui and Horrocks, 2006; Phillis et al., 2006; Farooqui, 2009). The non-enzymic DHA-derived lipid mediators include 4-HHE, neuroprostanes, neuroketal, and neurofurans, whereas ARA-derived lipid non-enzymic lipid mediators are 4-HNE, isoprostanes, isoketal, and isofurans. ARA-derived enzymic and non-enzymic lipid mediators are prothrombotic, proaggregatory, and proinflammatory. In contrast, DHA-derived lipid mediators, docosanoids, not only downregulates proinflammatory cytokines but also have antiinflammatory, antithrombotic, antiarrhythmic, hypolipidemic, vasodilatory, and antiexitotoxic properties (Simopoulos, 2002a; Farooqui, 2009). Increased consumption of DHA results in a decrease in the level of ARA in neural membrane glycerophospholipids, thereby decreasing the level of substrate for the production of proinflammatory eicosanoids and cytokines. The antiinflammatory effects of DHA may also be due to downregulation of iNOS in microglial cell and peritoneal macrophages in a dose-dependent manner. This inhibition is accompanied not only by inhibition of the oxidative stress-sensitive NF-κB activation but also by upregulation of GSH (Farooqui, 2009). Collectively, these studies suggest that because of differences in their mode of action, excess of DHA-enriched diet can interfere with ARA metabolism through their short-lived lipid mediators, which act as local hormones or vice-versa.

11.2 Chronic Diseases and Dietary *n*–6/*n*–3 Ratio

n–6 fatty acids are not inherently unhealthful. The problem is that the diets in the Western countries contain extremely high levels of omega-6 fatty acids. Thus, Western diet has very high amounts of saturated fat and with a ratio of ARA to DHA of about 20:1. The Paleolithic diet on which human beings have evolved, and lived for most of their existence, had a ratio of 2-1:1, and was high in fiber, rich in fruits, vegetables, lean meat, and fish. (Simopoulos, 2002a, 2006, 2008; Cordain et al., 2005). The intake of *n*–6 fatty acids has increased from 10 g/day in the late 1970s to 15 g/day by the 1990s. Changes in eating habits, natural versus processed food, which is enriched in corn-based livestock, and increase in the consumption of vegetable oil in the past 20 years along with decrease in consumption of sea food have altered ARA to DHA ratio in favor of ARA (Simopoulos, 2000, 2006; Kris-Etherton et al., 2000; Weylandt and Kang, 2005; Kang and Weylandt, 2008). As stated above, the high intake of ARA-enriched food not only elevates levels of prostaglandins, leukotrienes, and thromboxanes, but also upregulates the expression of proinflammatory cytokines, such as TNF-α and IL-1β. ARA-enriched diet also increases levels of

isoprostanes, which have vasoconstrictive effects in pulmonary artery, coronary arteries, cerebral arterioles, retinal vessels, and portal vein (Montuschi et al., 2007). In contrast, consumption of DHA-enriched diet has antiinflammatory effects that are partly mediated by repression of genes that code for proinflammatory cytokines (Fig. 11.1). DHA-enriched diet also elevates the levels of docosanoids that have antioxidant and antiapoptotic effects in neural and non-neural tissues (Bazan, 2005a,b; Serhan, 2005). Furthermore, as stated in earlier chapters, both DHA and ARA interact with the genome through several mechanisms. They regulate the activity of several transcription factors, including PPAR, LXR, HNF-4, NF-κB, and SREBP (Jump, 2004). DHA and ARA also modulate gene expression indirectly through their interactions with COX, LOX, PKC, or sphingomyelinase signal transduction pathways, as well as pathways that involve changes in membrane lipid/lipid raft composition, affecting G-protein receptor or tyrosine kinase-linked receptor signaling (Jump, 2004; Horrocks and Farooqui, 2004; Farooqui, 2009). Thus, levels of DHA- and ARA-derived lipid mediators in neural and non-neural tissues are partly regulated by diet (Horrocks and Farooqui, 2004; Farooqui, 2009). This

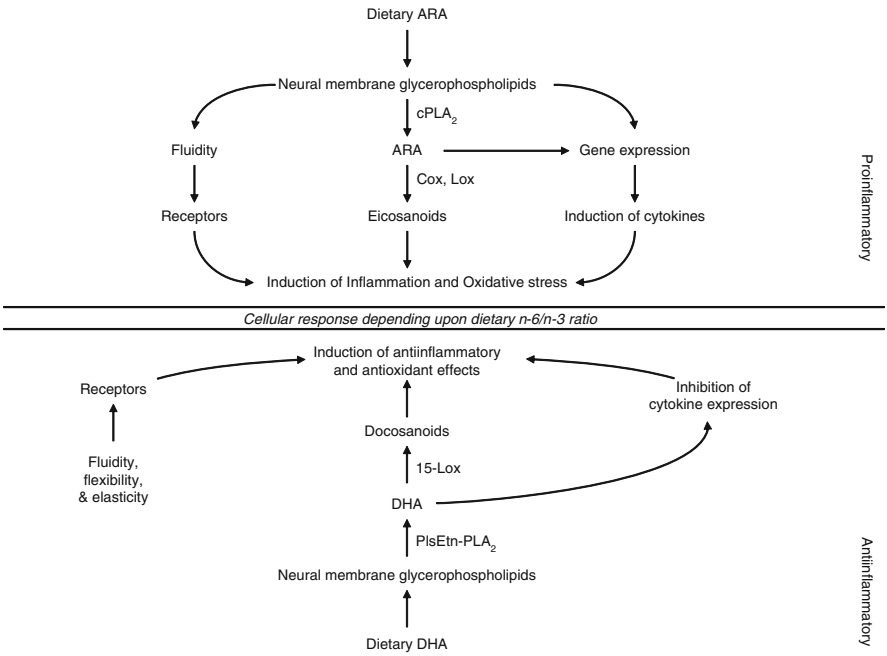

Fig. 11.1 Diagram showing interaction between dietary arachidonic acid and docosahexaenoic acid metabolism. Cytosolic phospholipase A_2 ($cPLA_2$); cyclooxygenase (COX); lipoxygenase (LOX); plasmalogen-selective phospholipase A_2 ($PlsEtn\text{-}PLA_2$); and 1-lipoxygenase (15-LOX). Diet with high *n*–6/*n*–3 fatty acid ratio promotes inflammatory and neurodegenerative effects, whereas diet enriched in *n*–3 fatty acids contribute to antiinflammatory and neuroprotective effects

suggests that the ratio between *n*–6 and *n*–3 fatty acids must be properly maintained in human diet (Fig. 11.1). The primary goal of future research on essential fatty acids should be how to increase the levels of *n*–3 fatty acids in human tissue including brain through diet, and avoid not only cardiovascular, inflammatory, and autoimmune diseases but also neuropsychiatric and neurodegenerative diseases. Epidemiological, biochemical, and clinical studies indicate that in colorectal cancer a *n*–6/*n*–3 ratio of less than 2.5:1 decreases rectal cell proliferations; in cardiovascular disease a ratio of less than 4:1 decreases the rate of mortality; a ratio 2-3:1 reduces inflammation in patients with rheumatoid arthritis; and in asthma a ratio of 5:1 reduces many symptoms of this disease (Chajès and Bougnoux, 2003; Simopoulos, 2002a; Merendino et al., 2003; Mills et al., 2005). In general, a balanced ratio of *n*–6/*n*–3 fatty acid in various body tissues is essential for optimal growth and development. Collective evidence suggests that ARA-enriched diet promotes chronic visceral (cardiovascular, inflammatory, and autoimmune diseases) as well as neurodegenerative diseases, whereas a diet enriched in DHA exerts cardioprotective, anticarcinogenic, immunosuppressive, and neuroprotective effects (Simopoulos, 2004, 2006, 2008; Farooqui, 2009). In addition, humans consuming smaller amounts of *n*–3 fatty acids have a higher prevalence of major depressive disorders. Their plasma levels of *n*–3 fatty acids are lower than normal subjects. DHA deficiency in depressive patients is associated with dysfunctions of neuronal membrane stability and transmission of serotonin, norepinephrine, and dopamine, which may be linked to the pathogenesis of mood and cognitive dysfunction in depression. On the other hand, EPA is important in balancing the immune function and physical health by reducing ARA level on cell membrane and prostaglandin E_2 (PGE_2) synthesis.

Most of chronic visceral diseases and neurodegenerative diseases are accompanied by oxidative stress and inflammation. In these diseases, years of slowly progressing and clinically undetectable biochemical changes set the stage for devastating clinical consequences that cannot be treated with present day available drugs. However, regular use of *n*–3 fatty acids throughout life may protect humans from chronic visceral and neurological disorders. DHA and EPA in fish oil reduce oxidative stress and inflammation in several ways. Firstly, fish oil decreases the formation of ARA by blocking the activity of Δ^5-desaturase. Secondly, it inhibits the synthesis of eicosanoids (Calder, 2004, 2005, 2008) and induces the synthesis of resolvins and neuroprotectins. Lastly, DHA in fish oil serves as ligand for several transcription factors. It not only decreases the production of cytokines, ROS, but also downregulates the expression of adhesion molecules (Serhan, 2005, 2006; Bazan, 2005a,b; Calder, 2005). Thus, DHA supplementation may restore signal transduction processes by protecting neurons from harmful effects of ARA metabolite-mediated oxidative stress and neuroinflammation, and DHA plays an important role in normal neurological and cognitive functions (Horrocks and Farooqui, 2004). Levels of DHA are markedly decreased in neural membranes obtained from brains of aged healthy elderly

people and also from patients with neurological disorders (Bechoua et al., 2003; Horrocks and Farooqui, 2004). Depletion of DHA also induces transgene-dependent caspase activation and deficits in the neuroprotective insulin signaling pathway (Akbar and Kim, 2002). Numerous epidemiological studies indicate that increased fatty fish consumption and high DHA intake are associated with a reduced risk of AD (Kalmijn et al., 2004).

11.3 Expression of Genes in Animals and Plants to Improve *n*–6 to *n*–3 Fatty Acids Ratio

To produce a desirable increase in *n*–3 fatty acids levels in vivo, one has to consume large amount of fish or high doses of *n*–3-containing fish oil for several months. The appearance of *n*–3 fatty acids in various body tissues, especially in brain, from the consumed *n*–3 fatty acid-enriched food involves digestion, absorption, transport, and glycerophospholipid metabolism. These processes slow down the efficacy of dietary intervention. In addition, anti-inflammatory activity of deodorized fish oil varies significantly among commercially available fish oil preparations due to variations in EPA/DHA content (Bhattacharya et al., 2007). The efficacy of fish oil may also vary with health status of subjects. For example, patients with gastrointestinal problems may not be able to ingest and absorb *n*–3 fatty acid-enriched food or supplements optimally (Kang et al., 2001). In addition, encapsulated fish oil supplements may not be appropriate for lifetime daily use, because of possible caloric excess. With increase in world population, the sources of edible fish in the oceans are decreasing due to overfishing and environmental pollution (mercury and polychlorinated biphenyl, PCB); thus, it is important to develop alternative sources of *n*–3 fatty acids.

Recently, a *fat-1* gene encoding an *n*–3 fatty acid desaturase has been cloned from *Caenorhabditis elegans*. Expression of this enzyme in plant, *Arabidopsis*, results in a protein that converts *n*–6 fatty acids into *n*–3 fatty acids (Spychalla et al., 1997). The expressed protein encoded by a fat-1 cDNA shows 32–35% identity with both desaturases (FAD2 and FAD3) of *Arabidopsis* and produces an abundance of EPA and DPA specifically in the brain and reduces ARA in *fat-1* gene-carrying mice. Gene expression profile, RT-PCR, and protein microarray studies in the hippocampus and whole brain of wild-type and fat-1 transgenic mice show that genes and proteins associated with neuroinflammation, apoptosis, neurotransmission, and neuronal growth and synapse formation are specifically modulated in fat-1 mice (Zhu et al., 2008; Ménesi et al., 2009). Compared to wild-type mice, fat-1 mice are protected from dextran sulfate-mediated colitis and display diminished growth of implanted B16 melanoma cells (Xia et al., 2006; Hudert et al., 2006). Fat-1 mice have higher levels of *n*–3 fatty acids and PGE_3 compared to wild-type animals in the tumor and

surrounding tissues (Xia et al., 2006). Furthermore, the phosphatase and tensin homolog (PTEN) gene that encodes a tumor suppressor protein is significantly upregulated in the fat-1 mice. In vitro addition of EPA or PGE_3 retards the growth of B16 cell line and upregulates the expression of PTEN, which may be partially attenuated by inhibition of PGE_3 formation. This suggests that PGE_3 may act as an antitumor mediator. Collectively, these studies demonstrate an anticancer (antimelanoma) effect of EPA through, at least in part, activation of PTEN pathway mediated by PGE_3 (Xia et al., 2006). These results explain not only the therapeutic importance of *n*–3 fatty acids in neurodegenerative, neurotraumatic, and neuropsychiatric diseases, but also provide rationale for treating chronic visceral diseases, such as cancer (Xia et al., 2006; Hudert et al., 2006; Farooqui, 2009; Ménesi et al., 2009).

Attempts to introduce *fat-1*gene in mice and pork have met with success, and genetically engineered fat-1 mice and pork have been produced. These *fat-1* gene transgenic mice and pork are capable of producing *n*–3 fatty acids from the *n*–6 type, leading to abundant *n*–3 fatty acids with reduced levels of *n*–6 fatty acids in their organs and tissues, without the need of a dietary *n*–3 supply. The transgenic animals have a balanced ratio of *n*–6 to *n*–3 fatty acids in their tissues and organs independent of dietary levels of DHA and ARA (Kang et al., 2004; Ma et al., 2006; Kang, 2007; Prather, 2006). Thus, transgenic animals provide an opportunity to investigate the metabolism and roles of DHA- and ARA-derived enzymic and non-enzymic lipid mediators, along with mechanisms associated with cardioprotection and neuroprotection.

In transgenic mice that endogenously synthesize *n*–3 fatty acids from *n*–6 fatty acids, an increase in *n*–3 fatty acids leads to production of antiinflammatory resolvins, resulting in an effective reduction in inflammation and tissue injury in colitis (Hudert et al., 2006). The endogenous increase in *n*–3 fatty acids and *n*–3 fatty acid-derived lipid mediators do not decrease levels of leukotriene B_4 and prostaglandin E_2. It is proposed that observed inflammation protection may be mediated not only by the decrease in NF-κB activity and expression of TNF-α but also through downregulation of iNOS and IL-1β. These results clearly show that the fat-1 transgenic mouse is a new experimental model for the study of *n*–3 fatty acid-derived lipid mediators. They add insight into the molecular mechanisms associated with inflammation protection provided by *n*–3 fatty acid-derived resolvins and protectins (Hudert et al., 2006).

Both *n*–3 fatty acids and calorie-restriction (CR) exert antiinflammatory effects in animal models of autoimmunity and inflammation (Bhattacharya et al., 2006; Farooqui and Farooqui, 2009). Studies on the synergistic effects of *n*–3 fatty acids and CR on LPS-mediated inflammatory responses using fat-1 transgenic mice indicate that there is no difference in body weights between wild-type and fat-1 mice on ad libitum (AL) or CR (40% less than AL) diets. Splenocytes prepared from these animals on AL and CR diet have lower *n*–6/*n*–3 fatty acid ratio. Splenocytes from fat-1/CR mice also show significant

reduction in NF-κB (p65/p50) and AP-1 (c-Fos/c-Jun) DNA-binding activities following LPS treatment. LPS treatment in splenocytes from fat-1/CR mice also produces a significant reduction in IL-6 and TNF-α secretion (Bhattacharya et al., 2006). The inhibition of LPS-mediated effects is more pronounced in fat-1/CR mice when compared to fat-1/AL or WT/CR mice. Collectively, these studies indicate that *n*–3 lipids with moderate CR may confer protection in autoimmune and inflammatory diseases (Bhattacharya et al., 2006; Farooqui and Farooqui, 2009).

The discovery of transgenic animals with *C. elegans* gene *fat-1*also indicates that this technology may be adapted to enrich *n*–3 fatty acids in cattle, sheep, and chicken and in products such as meat, milk, and eggs (Kang et al., 2004; Kang, 2007; Prather, 2006). More studies are needed on the development of *fat-1* gene-containing cattle, sheep, pork, and chicken meat, and long-term studies are required on effects of transgenic meat in large human population.

In recent years, investigators are developing new techniques to modify the genetic makeup of plants to improve their properties. This has led to the production of a new generation of crops, grains, and their by-products for feed. Attempts are made to produce *n*–3 fatty acids in transgenic plants that can produce a sustainable and clean supply of *n*–3 fatty acids (Napier, 2006; Robert, 2006; Moghadasian, 2008). Agricultural production of oils is highly efficient process. It has the potential to be sustainable. The transfer of genes from marine microalgae and other microorganisms into oilseed crops has shown that the production of terrestrial *n*–3 fatty acid oils is indeed possible (Robert, 2006). However, nothing is known about the health-promoting effects of *n*–3 fatty acids from transgenic plants. The safety assessment of genetically modified plants-derived *n*–3 fatty acids should be compared with *n*–3 fatty acids of animal origin in order to identify intended and unintended differences, which subsequently should be assessed with respect to their potential impact not only on the environment, safety for humans and animals, but also on nutritional quality.

11.4 Unsolved Problems of DHA and ARA Metabolism

Considerable information is available on enzymes of ARA metabolism and ARA-derived lipid mediators. Many enzymes (cytosolic phospholipases A_2, COX, and LOX) that metabolize ARA have been isolated and characterized from neural and non-neural tissues (Phillis et al., 2006; Farooqui and Horrocks, 2007), but very little information is available on enzymes that release and metabolize DHA. DHA-releasing phospholipase A_2 has been partially characterized from brain (Farooqui et al., 1995). No information is available on brain 15-LOX, the enzyme that facilitates the synthesis of docosatrienes and resolvins (Wittwer and Hersberger, 2007). These lipid mediators possess potent

antiinflammatory, proresolving, and antifibrotic actions in vivo (Bazan, 2005a,b; Serhan, 2005, 2006). In addition, they prevent platelet aggregation, lower blood pressure, reduce LDL-C, ameliorate the adverse actions of homocysteine, activate telomerase, and have antiarrhythmic action and cytoprotective properties (Das, 2008).

Nitric oxide interacts with *n*–6 and *n*–3 PUFA and generates their respective nitroalkene derivatives that can be detected in plasma (Das, 2006). Nitroalkene derivatives of *n*–3 and *n*–6 fatty acids produce many physiological effects, including induction of vascular relaxation, inhibition of neutrophil degranulation, and formation of superoxide. In addition, fatty acid nitroalkene derivatives inhibit platelet activation, and act as endogenous ligands for PPARγ and decay in the blood to release NO (Das, 2006). This suggests that PUFA not only form precursors to various eicosanoids, resolvins, LXs, and NPD_1, but also react with various other molecules to form novel compounds with significant neurochemical and neurophysiological activity (Das, 2006). More studies are needed on physiological effects of nitroalkene derivatives of *n*–6 and *n*–3 fatty acids to understand their neurochemical and neurophysiological significance.

11.4.1 Characterization of Enzymes Associated with DHA and ARA Metabolism

As stated above, considerable progress has been made on incorporation, biosynthesis, catabolism, roles, and involvement of DHA, EPA, and ARA in chronic visceral and neurological disorders, but little information is available on metabolism and role of DHA-, EPA-, and ARA-derived lipid mediators. Only handful of phospholipases A_2, COX, LOX, and EPOX have been purified and characterized from brain tissue (Phillis et al., 2006; Farooqui et al., 2006). Molecular biological approaches, such as cloning of the cDNA for isoforms of phospholipases A_2, COX, LOX, and EPOX and functionally expressing them in different types of neural and non-neural cells would advance the understanding of role and metabolism of neuroprotectins, protectins, resolvins, lipoxins, and other eicosanoids at cellular and subcellular levels in neural and non-neural tissues and promote the understanding of neurochemical effects of docosahexaenoic acid.

11.4.2 Development of Antisense Oligonucleotides and RNAi

Regulation of phospholipases A_2, COX, LOX, and EPOX is a very important area for future research work. Specific inhibitors, antisense oligonucleotides, and RNAi for isoforms of phospholipases A_2, COX, LOX, and EPOX are needed for further characterization of these enzymes and should be synthesized (Bennett, 1998; Pirollo et al., 2003; Heng and Cao, 2004). Better drug delivery

systems that target brain need to be developed to protect isoforms of phospholipases A_2, COX, LOX, and EPOX inhibitors, antisense oligonucleotides, and RNAi from in vivo degradation or detoxification. The delivery of inhibitors, antisense, and RNAi should be performed through drug delivery systems that not only protect them from detoxification but must reach the site where neurodegenerative processes are taking place (Yoshikawa et al., 1999). This would enhance the efficacy of phospholipases A_2, COX, LOX, and EPOX inhibitors, antisense oligonucleotides, and RNAi. The effects of these inhibitors on genes expression and DHA-derived lipid mediators can be monitored by microarray procedures and lipidomics (Bosetti et al., 2005). These studies can lead to better therapeutic agents for the treatment of chronic visceral and neurological disorders involving DHA and ARA metabolism alterations.

11.4.3 Characterization of Receptors for Neuroprotectins and Resolvins

Few reports are published on identification and characterization of receptors for neuroprotectin, resolvins, protectins, and lipoxins in non-neural cells, and no information is available on these receptors and factors that regulate them in neural cell membranes (Bazan, 2005a,b; Serhan, 2005, 2006). New information is available on the E-series resolvins (RvE_1, RvE_2), D series resolvins (RvD_1, RvD_2), and the protectins including neuroprotectin D_1/protectin D_1 (NPD_1/PD_1) as well as their aspirin-triggered epimeric forms. Members of each new family show potent stereospecific actions in modulating resolution and endogenous anti-inflammation mechanisms (Serhan and Chiang, 2008). Very little information is available on non-enzymic lipid mediators of arachidonic acid metabolism (isoprostanes and isofurans) and docosahexaenoic acid metabolism (neuroprostanes and neurofurans) in brain tissue (Fam and Morrow, 2003; Roberts II et al., 1998). Thus, more information is needed on isolation, characterization, and quantification of enzymic and non-enzymic lipid mediators of DHA and ARA in neural and non-neural tissues of normal subjects and in various tissues of patients with chronic visceral diseases and neurological disorders. The development of specific inhibitors of phospholipases A_2, COX, LOX, and EPOX that can cross the blood–brain barrier without harm would be important for developing new therapies for chronic visceral and neurological disorders.

Minute amounts of neuroprotectin, resolvins, protectins, and lipoxins in neural and non-neural tissues at the cellular and subcellular levels and in biological fluids can be detected by lipidomics, proteomics, and genomics procedures (German et al., 2007; Watson, 2006; Soule et al., 2006; Bowers-Gentry et al., 2006). Lipidomics analysis has been used for detection and characterization of F_2-isoprostanes, prostaglandins, leukotrienes, lipoxins,

hydroxyeicosatetraenoic acids, neuroprotectins, protectins, and resolvins, and 4-HNE in neural and non-neural cells (Serhan, 2005, 2006; Adibhatla et al., 2006; Milne et al., 2006; Morrow, 2006; Lu et al., 2006; Perluigi et al., 2005). Using proteomics more studies are required on determination of levels of DHA-, EPA-, and ARA-derived lipid mediators in various tissues of normal subjects and patients with chronic visceral diseases and neurological disorders. Establishment of automatic systems including databases and accurate analyses of DHA-, EPA-, and ARA-derived lipid mediators will facilitate the identification and characterization of key biomarkers associated with chronic visceral and neurodegenerative diseases (Lu et al., 2006).

11.5 Nutrigenomics/Nutrigenetics/Transcriptomics Approaches to *n*–3 Fatty Acids

Nutritional science defining the interactions of nutrients with genes is called as nutrigenomics. Nutrigenomics not only deals with the study of how different foods may interact with specific genes to increase the risk of common chronic diseases but also provides molecular basis of how common chemicals in the diet regulates health by altering the expression of genes and the structure of an individual's genome (Caramia, 2007, 2008; Gollies, 2007; Mariman, 2006). Transcriptomics is defined as the study of the complete set of mRNA transcripts (transcriptome) produced by the genome at any one time. Unlike the genome, which is roughly fixed for a given cell line (excluding mutations), the transcriptome can vary with external environmental conditions (Kato, 2008). Nutrigenetics involves the genetic basis of the different individual responses to the same nutritional stimulus. The identification of diet–gene interactions through nutrigenomics /nutrigenetics not only provides an opportunity to develop dietary interventions that may lead to evidence-based dietary strategies for restoring health and fitness, but obviate the effects of genetic factors for preventing diet-related diseases and provide important clues about gene expression and gene modulation by environmental factors (Caramia, 2007, 2008; Gollies, 2007; Mariman, 2006).

In recent years, we have been empowered with technological advances in lipidomics, proteomics, and genomics. The use of "omics" technology in nutritional science can provide information about the effects of genetic variation and gene–nutrient interactions in the management of not only chronic visceral diseases, such as coronary heart disease, hypertension, cancer, diabetes and obesity, and the role of nutrients in gene expression, but also on the effects of nutrients on neurotraumatic, neurodegenerative, and neuropsychiatric diseases. At the same time, clinical methods for profiling almost all metabolic products in a single sample of blood or urine are being designed and developed (metabolomics). Relations between diet and

nutrigenomic along with metabolomic profiles have become important components of research that can revolutionize clinical practice in nutrition (Zeisel, 2007).

Consumers in the Western world are questioning the quality and safety of their diets. Much of this interest is due to the accumulating information on bioactive food components, which can influence a number of key molecular events that are associated with health and disease resistance. Variation in the ability of food components to upregulate or downregulate gene expression may play a major role in vulnerability or resistance to disease process. Since many food components are known to influence phosphorylation/dephosphorylation and other posttranslational events, it is likely that these protein modifications may account for vulnerability or resistance to disease process. These food components can not only influence one's ability to achieve one's genetic potential, but can also have a significant influence on the quality of life as measured by both physical and cognitive performance, and modify the risk and/or severity of a variety of disease conditions (Milner, 2004; Milner, 2007). Accumulating evidence from past 50 years indicates that dietary habits and life style may act as determinant of premature death not only in chronic visceral diseases, such as heart disease and cancer, but also in neurological diseases such as stroke and Alzheimer disease (Chapter 7).

Information obtained from Nutrigenomics/nutrigenetics approaches has enormous implication for nutrition research both in the prevention and in the management of chronic visceral and neurodegenerative diseases. Attempts are underway to define the contribution of genes and their interaction with nutrients in the development of the individual. Knowledge of genetic susceptibility to chronic visceral and neurodegenerative diseases will help in identifying those at higher risk for disease, as well as their response to dietary food (Simopoulos, 2002b; Afman and Müller, 2006). According to Simopoulos, the prospect of targeting specific dietary treatment to those predicted to gain the most therapeutic benefit clearly has important clinical and economic consequences, particularly in diseases of high prevalence such as coronary artery disease, hypertension, osteoporosis, diabetes, Alzheimer disease, and cancer (Simopoulos, 2002b; Afman and Müller, 2006; Miggiano and Gagliardi, 2006). With recent advances in genomic, proteonomics, and lipidomics, continued efforts, and investments in research offer a golden opportunity not only for identification of biomarkers but also in understanding chronic visceral and neurodegenerative disease processes at the molecular level, preventing intrinsic and environmental risks to health, and developing new approaches to improve the quality of life worldwide. Furthermore, knowledge of genetic susceptibility to chronic visceral and neurodegenerative diseases will help identify individuals at higher risk for diseases, as well as their response to diet (Simopoulos, 2002b; Afman and Müller, 2006). The molecular profiling via genomics, proteomics, and metabonomics will not only open new windows to study disease states and

biological systems but also provide exciting opportunities for novel applications in clinical research as well as in drug discovery and development.

Another successful recent technology is transcriptome analysis (Kato, 2008). This technology provides substantial information on the novel function of food factors. At present, the nutrigenomics database has several hundred publications on "omics" studies (Kato, 2008). Furthermore, the transcriptomics approach is being applied to food safety issues. This new technology will not only facilitate the safety evaluation of newly developed foods but also will help in clarifying the molecular mechanism of toxic effects resulting from the excessive intake of a nutrient (Kato, 2008).

Thus, nutrigenomics/nutrigenetics/transcriptomics approach holds the promise of not only revolutionizing the clinical and public health nutrition practices but also promoting the establishment of: (a) genome-informed nutrient and food-based dietary guidelines for disease prevention and healthful aging, (b) individualized medical nutrition therapy for the management of chronic diseases, and (c) better targeted public health nutrition interventions that not only maximize benefit and minimize adverse outcomes within genetically diverse human populations but will be tasty and flavorful (Stover and Caudill, 2008). Rapid advancements in the field of nutritional nutrigenomics/nutrigenomics will not only include filling fundamental gaps in knowledge of nutrient–genome interactions in health and diseases but also provide information on the potential benefits of customizing nutrition prescriptions based on genetics. In the near future, nutritionists and dietitians will have the opportunity of making genetically driven dietary recommendations aimed to improving human health and retard chronic human diseases (Stover and Caudill, 2008).

Although the public health applications of nutrigenomics /nutrigenetics/ transcriptomics approaches in Western countries are probably at least 10–15 years away, but clinical applications of nutrigenomics/nutrigenetics/transcriptomics are likely to be more immediate, probably resulting in "designer diets" for individuals with particular polymorphisms. It is hoped that in the coming 50 years, studies on the effect of diets, particularly having lower dietary *n*–6/*n*–3 fatty acid ratios, on gene-expression patterns, organization of the chromatin, protein expression, and lipid mediator profiles, using genomics, proteomics, lipidomics, and metabolomics of tissue and biological fluid samples from patients with chronic visceral and neurodegenerative diseases, will show major progress for the treatment of these disorders. New classes of therapeutic agents based on diet, antioxidants, and lower *n*–6/*n*–3 fatty acid ratio should be developed. Thus future progress on nutrigenomics (Ordovas and Corella, 2004; Afman and Müller, 2006; Miggiano and De Sanctis, 2006) will increase our understanding of the influence of nutrition on genetic expression, metabolic pathways, and homeostatic control in normal human subjects and how this regulation is disturbed in the early phases of chronic visceral and neurodegenerative diseases (Valenzuela and Nieto, 2001). Dysregulation

of metabolism in early human development may result in enhanced susceptibility to chronic visceral and neurodegenerative diseases caused by a sustained deficiency of certain dietary factors, such as antioxidants and *n*–3 fatty acids in the daily Western lifestyle, with a high calorie diet from childhood to old age in human subjects.

11.6 Conclusion

Long-chain polyunsaturated essential fatty acids are classified in two families. *n*–6 fatty acids are found in plant seeds, whereas *n*–3 fatty acids found in marine vertebrates. ARA belongs to *n*–6 fatty acid family, whereas DHA and EPA are included in *n*–3 family. In brain tissue, ARA is evenly distributed in gray and white matter glycerophospholipids (PtdCho, PtdEtn, and PtdSer), while DHA is highly concentrated in neuronal membranes, most so in synaptic plasma membrane glycerophospholipids (PlsEtn and PtdSer). ARA, DHA, and EPA incorporate, and not only modulate biophysical and biochemical properties of neural membrane (permeability, fluidity, thickness, and elasticity) but also markedly affects the signal transduction processes associated with optimal brain function. Thus, dietary intake of ARA, DHA, and EPA is important for normal neural function. In the brain, ARA, DHA, and EPA and their metabolites modulate neurotransmitter release, gene expression, immunity and inflammation, learning and memory, apoptosis, and activities of membrane-bound enzymes, ion channels, and receptors. ARA, DHA, and EPA compete for same COX and LOX, and therefore levels of their lipid mediators are regulated by their amount present in diet. The Western diet is enriched in ARA (ARA/DHA ratio of 20:1), which is metabolized to proinflammatory and vasoregulatory prostaglandins, leukotrienes, and thromboxanes. The autocrine and paracrine mediators are powerful regulators of aggregation, vasoconstriction and vasodilatation, and inflammation. In contrast, DHA is metabolized to resolvins, protectins, and neuroprotectins, which have antiinflammatory, antioxidant, and antiapoptotic effects. Intake of food enriched in ARA also stimulates the expression of proinflammatory cytokines. In contrast, consumption of DHA-enriched diet represses genes that code for proinflammatory cytokines.

Inadequate amounts of DHA in diet are linked to a wide variety of abnormalities ranging from visual acuity to learning and memory. DHA deficiency is associated with many chronic visceral diseases as well as neurodegenerative and neuropsychiatric disorders. Supplementation of DHA improves cognition not only through its effects on physicochemical properties of cerebral membranes but also through modulation of genes and generation of docosanoids. The involvement of DHA in so many neurological disorders suggests that optimal consumption of this fatty acid is necessary for normal brain function. Long-term DHA supplementation may not only restore signal transduction processes associated with

behavioral deficits and learning activity but also produce several neuroendocrinological and immunological effects on brain tissue.

Nutrigenomics/nutrigenetics/transcriptomics approaches to public health in Western world will allow the development of "designer diets" having balanced and disease resistant nutrition for individuals. Future wholesome approach to health will personalize the preventive medicine and medical therapy. Widespread applications of nutrigenomics/nutrigenetics/transcriptomics for personalized medicine will require associations of gene expression pattern with diagnoses, treatment, and clinical data. This will help in the discovery and development of drugs for chronic visceral and neurodegenerative diseases. Collective evidence suggests that there is a great need for more information and better understanding of nutrigenomics/nutrigenetics/transcriptomics before complete public acceptance for wholesome approach to health.

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