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

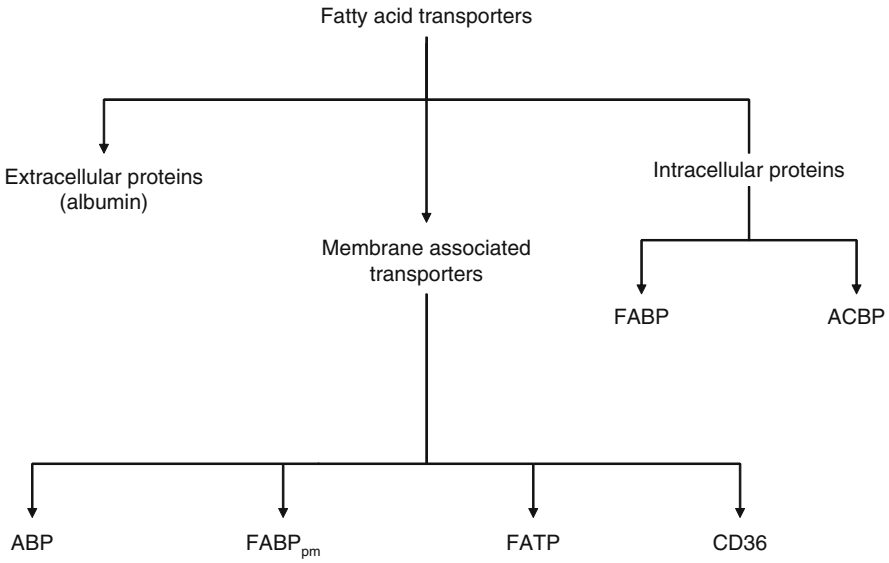

Fig. 2.1 Proteins associated with fatty acid transport. Albumin-binding protein (ABP); plasma membrane fatty acid-binding protein; fatty acid-transport protein (FATP); fatty acid translocase (CD36); cytoplasmic fatty acid-binding protein (FABP); and acyl-CoA-binding protein (ACBP)

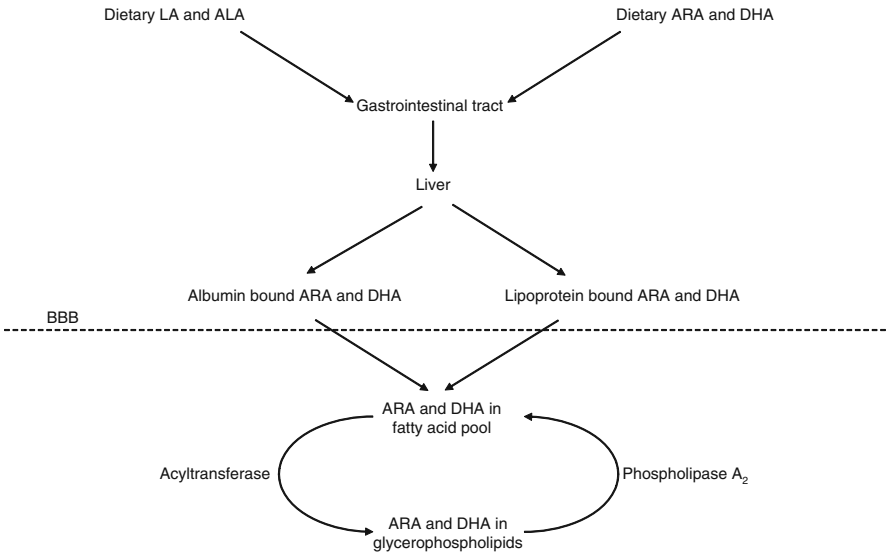

Fig. 2.2 Transport of ARA and DHA to brain

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

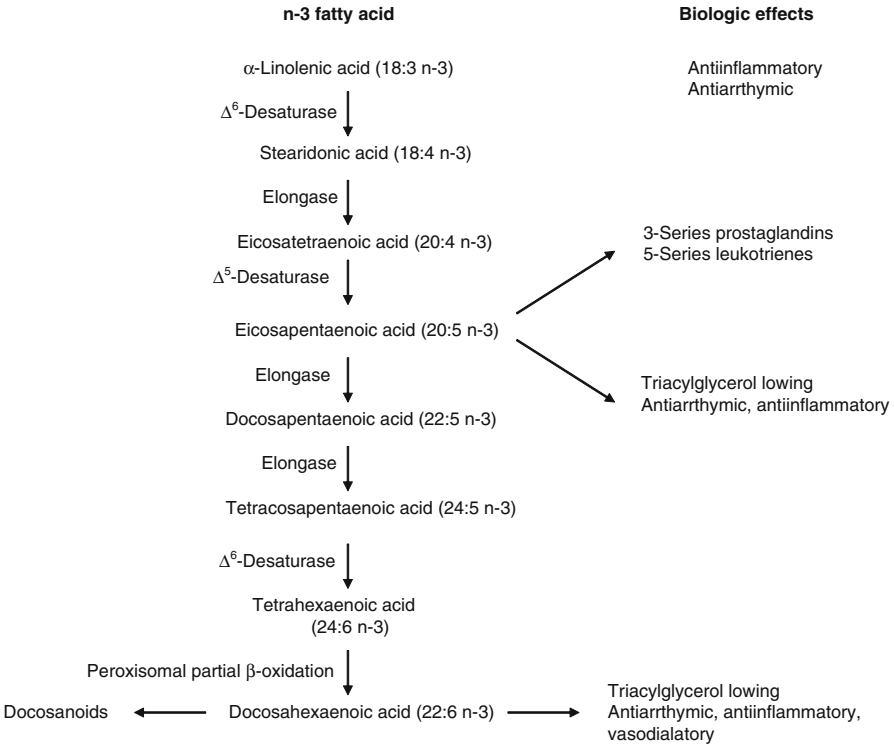

Fig. 2.3 Synthesis of DHA from ALA in liver and biologic effects of DHA

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

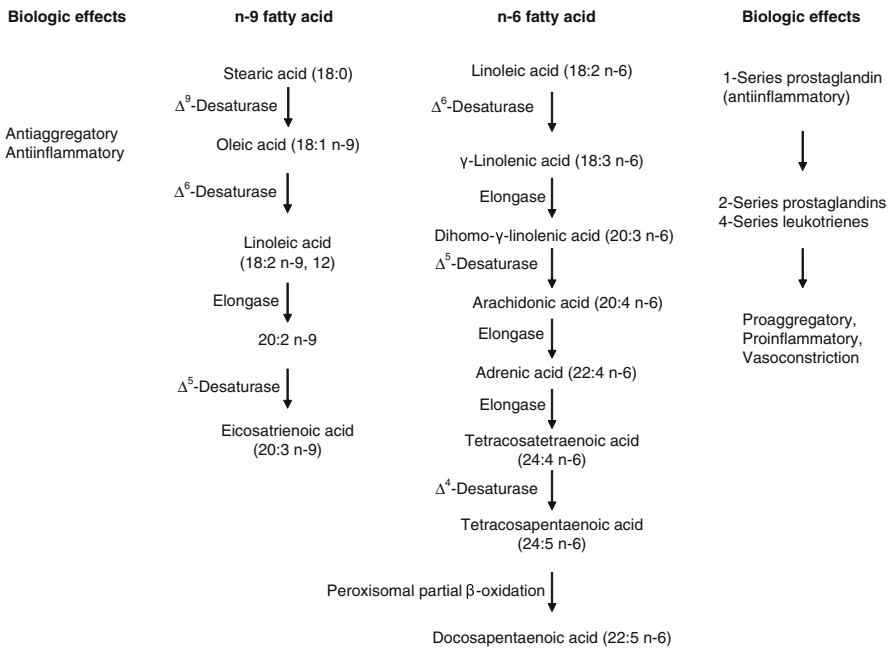

Fig. 2.4 Synthesis of ARA from LA and conversion of stearic acid into eicosatrienoic acid in liver along with their biologic effects

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).

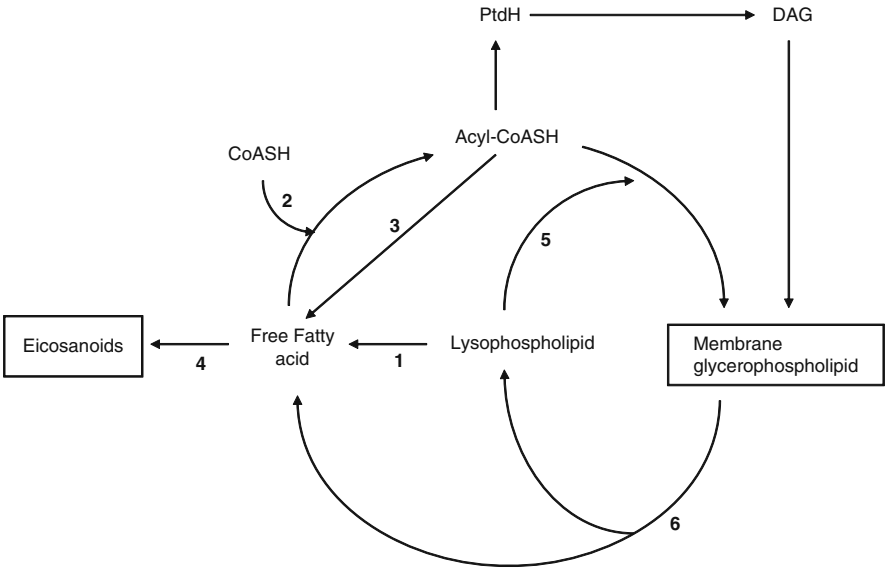

Fig. 2.5 Deacylation–reacylation of glycerophospholipids in brain. Lysophospholipase (1); acyl-CoA synthetase (2); acyl-CoA hydrolase (3); cyclooxygenase and lipoxygenase (4); acyl-CoA:lysophospholipid acyltransferase (5); and phospholipase A_2 (6)

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

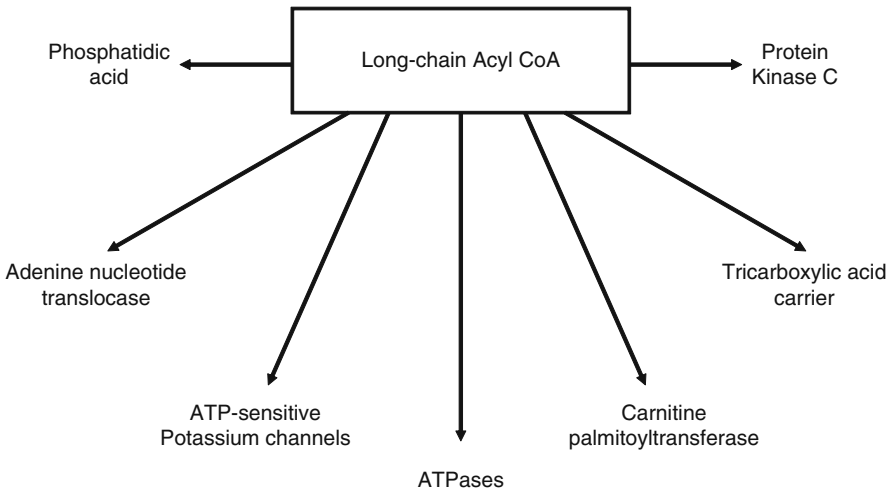

Fig. 2.6 Role of acyl-CoA in the modulation of various processes in brain

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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