Long-chain polyunsaturated fatty acids are highly enriched in the nervous system. Docosahexaenoic acid (DHA $^{2}$; 22:6n-3), in particular, is the most abundant polyunsaturated fatty acid in the brain and is concentrated in aminophospholipids of cell membranes. Numerous studies have indicated that this concentration of DHA in the nervous system is essential for optimal neuronal and retinal functions. Although the underlying mechanisms of its essential function are still not clearly understood, emerging evidence suggests that unique metabolism of DHA in relation to its incorporation into neuronal membrane phospholipids plays an important role. In this review, biochemical mechanisms for enriching and metabolizing DHA in neural cells are discussed in the context of their biological significance in neuronal function.

**Accretion of DHA in Neural Cells**

Accretion of docosahexaenoic acid (4,7,10,13,16,19-22:6) in the central nervous system actively occurs during the developmental period, primarily relying on circulating plasma DHA derived from diet or from biosynthesis in the liver (2). However, local biosynthesis of DHA also occurs in the brain, providing an alternative source of DHA for its accumulation in the brain (3). It is well established that DHA can be biosynthesized from α-linolenic acid (18:3n-3; 9,12,15-18:3), a shorter chain n-3 fatty acid precursor, through chain elongation and desaturation processes (4) (Fig. 1). Linolenic acid is desaturated to 18:4n-3 (6,9,12,15-18:3) by Δ6-desaturase, chain-elongated to 20:4n-3 (8,11,14,17-20:4), and subsequently converted to eicosapentaenoic acid (20:5n-3; 5,8,11,14,17-20:5) by Δ5-desaturase in the endoplasmic reticulum (ER). Mammalian Δ5- and Δ6-desaturases have been identified and cloned (5). However, Δ4-desaturase, responsible for making 22:6n-3 directly from 22:5n-3, an elongation product of 20:5n-3, has been identified only in microalgae (6). In mammals, 22:5n-3 is further elongated to 24:5n-3 (9,12,15,18,21-24:5) followed by desaturation by Δ6-desaturase to 24:6n-3 (6,9,12,15,18,21-24:6). Subsequently, 24:6n-3 is transferred to peroxisomes and converted to 22:6n-3 by removing two carbon chains by β-oxidation. DHA thus formed is transferred back to the ER and quickly incorporated into membrane phospholipids by esterification during de novo synthesis or by a decylation-reacylation reaction. Because biosynthesis of both fatty acids and phospholipids occurs in ER, a particular fatty acid intermediate can be either incorporated into phospholipids or further chain-elongated/desaturated, although the regulation of these processes is still poorly understood. Long-chain n-6 fatty acids are biosynthesized from linoleic acid (18:2n-6) using the analogous pathway and the same enzyme system. In most tissues, the commonly observed long-chain n-6 fatty acid is arachidonic acid (AA) (20:4n-6). Docosapentaenoic acid (22:5n-6, DPA n-6), produced by further elongation and desaturation of AA and subsequent peroxisomal β-oxidation, is rather a minor component, and yet it accumulates in the brain in place of DHA when the DHA supply is inadequate, especially during developmental periods (7). The distinctive fatty acid profile in the brain enriched with DHA or DPA n-6 may reflect the brain-specific uptake and/or regulation of fatty acid synthesis and esterification into membrane phospholipids. The liver is considered to be the primary site for biosynthesis of DHA, which becomes available to brain uptake through subsequent secretion into the circulating blood stream. Among neural cells, consisting of neurons, astrocytes, microglia, and oligodendrocytes, the capacity to synthesize DHA has been demonstrated only in astrocytes (3). Despite the fact that neurons are major targets for DHA accumulation, they cannot produce DHA because of lack of desaturase activity. Cerebromicrovascular endothelia can also elongate and desaturate shorter carbon chain fatty acids. However, they cannot perform the final desaturation step to produce either 22:5n-6 or 22:6n-3 (8). DHA synthesis in astrocytes is negatively influenced by the availability of preformed DHA (9) and thus may represent a quantitatively minor source for the neural DHA accretion when the circulating DHA supply is adequate. Incorporation of circulating DHA across the blood brain barrier appears to be an important route for maintaining adequate levels of DHA in the brain. In agreement with this notion, it has been shown that constant basal turnover of esterified DHA in the brain with unesterified DHA in plasma occurs at an estimated rate of 2–8% per day in adult rats (10). Generally, it is difficult to deplete DHA from the neural membranes of adult mammals even with a diet low in DHA, presumably because of preferential uptake of DHA into the brain to support the basal turnover. In the case of insufficient supply of n-3 fatty acids during development, the loss of DHA does occur but is compensated with DPA n-6 through reciprocal replacement (7), suggesting a requirement of very long-chain, highly unsaturated fatty acids in neural membranes. In pathophysiological conditions caused by aging, Alzheimer disease, and alcohol.
MINIREVIEW: Novel Metabolism of Docosahexaenoic Acid

**FIGURE 1. Biosynthesis of docosahexaenoic and docosapentaenoic acids.**

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**Neuronal Uptake of DHA**

Whether brain DHA is derived from the circulating plasma pool or biosynthesized locally, astrocytes, which are situated in close contact with neurons, appear to play an important role in supplying DHA to neurons. DHA can be released readily from astroglial membranes under basal and stimulated conditions, and supplied to neurons (14–16). Despite its high abundance in neuronal membranes, DHA is not easily released but is tenaciously retained in the neuronal membranes under the conditions in which AA can be released (15). Considering the fact that astroglia support neurons by providing neurotrophic factors (17), DHA supplied by astroglia may also be trophic. Indeed, DHA has been shown to promote neuronal survival (18) and differentiation (19) in both transformed and primary neuronal cells in culture. The phamacological evidence suggests that DHA release from astrocytes is mediated by a Ca\(^{2+}\)-independent phospholipase A\(_2\) (iPLA\(_2\)), whereas cytosolic PLA\(_2\) is involved in AA release, at least under stimulated conditions (20). The release of DHA from astroglia and subsequent neuronal uptake may be important processes for modulating DHA levels in neuronal cells. Nevertheless, the mechanism for neuronal uptake via DHA transfer between these cells is not clearly understood. Rapid diffusion of free fatty acids across the cell membranes has been demonstrated using model membranes as well as adipocytes (21). Likewise, the involvement of proteins such as fatty acid translocase, plasma membrane-bound fatty acid-binding protein, and fatty acid transport proteins has been identified (22). Whether similar mechanisms are operative for DHA uptake in neural cells remains to be elucidated. It has been shown that long-term exposure to ethanol can decrease astroglial release (23) and neuronal incorporation of DHA (24). The inhibited neuronal DHA uptake is consistent with the loss of DHA in neural cells, particularly in brain synaptic plasma membranes after long-term ethanol exposure (13), although ethanol can also induce DHA catabolism by oxidation, as demonstrated from the liver (25).

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**DHA and Neuronal Phosphatidylserine (PS) Accumulation**

Under normal conditions, DHA does not exist in free fatty acid form, but is esterified at the sn-2 position of phospholipids. In neural membranes, DHA is found enriched in aminophospholipids, phosphatidylethanolamine (PE) and PS, particularly as the 1-stearoyl-2-docosahexaenoyl (18:0,22:6) species (26). This lipid profile is rigorously maintained and may be important for normal brain function in view of the fact that the lipid environment can affect functions of membrane protein or membrane-interacting proteins (27). In animal cells, PS is not synthesized de novo but formed by serine base exchange reaction from pre-existing membrane PC or PE using PS synthase1 (PSS1) or PS synthase2 (PSS2), respectively (28) (Fig. 2). Mammalian PSS1 and PSS2 enzymes have been identified and cloned from murine liver and Chinese hamster ovary cells (28). The presence of a serine base exchange enzyme in the brain with apparent molecular mass of 100 KDa, which is higher than that of PSS1 and PSS2, has been also reported (29). The serine base exchange reaction is energy-independent and occurs in a Ca\(^{2+}\)-dependent manner in the ER/microsomes and ER-related mitochondria-associated membranes. Considerable serine base exchange activity has been identified also in the plasma membranes of neuronal cell bodies (30) and synaptosomes (31). Both microsomal and plasma membrane serine base exchange activity has been reported to be higher in neuronal cells than in glial cells (30). Although PS can be recycled to PC or PE by base exchange reactions, PS is transferred to mitochondria and converted to PE by PS-decarboxylase (PSD). In fact, mitochondrial decarboxylation is considered the primary means of metabolizing PS, based on the fact that PS serves as the major precursor for PE in mammalian cells (32). Therefore, the substrate preference in PS synthesis or decarboxylation is an important determinant for the accumulation of specific PS molecular species in neural membranes. Substrate preference for DHA-containing phospholipids has been demonstrated for microsomal PS (18:0,22:6 > 18:0,22:5:6 > 18:0,18:1 = 18:0,20:4n-6 species) (33) and mitochondrial PSD (18:0,22:6 > 18:0,18:1 > 18:0,20:4n-6PS) in the brain (34). The preference for producing 18:0,22:6 species by both PSS and PSD suggests that DHA accumulation in PS is most likely because of favored PS biosynthesis from DHA-containing substrates rather than its unfavorable degradation by decarboxylation. Notably, the PS synthesis from phospholipids containing DPA \(n\)-6, which compensates for the loss of DHA after dietary deficiency of n-3 fatty acids, is also active in the brain, although the conversion is not as effective as for DHA species (33). Favorable PS synthesis from DPA \(n\)-6 species appears to occur specifically in the brain, given that this species is a poor substrate in the liver (33).

Although phospholipid levels are tightly regulated in living exposure, the loss of DHA from the adult brain has also been reported (11–13).
cells, the PS pool can be modulated by the DHA status uniquely in neuronal cells. In neural tissues such as the brain cortex, hippocampus, retina, and olfactory bulb, DHA is highly concentrated and the PS level is also high (26, 35, 36), in agreement with the preferred PS biosynthetic activity toward DHA species (33). In contrast, non-neural tissues such as liver and adrenal, where DHA is a rather minor component, contain relatively low levels of PS. Depletion of DHA by n-3 fatty acid deficiency has been shown to decrease PS selectively from neural tissues despite the compensatory replacement by DPA-n-6 (36), because DPAn-6 cannot fully support the original level of PS biosynthesis (37). Nevertheless, provision of DPAn-6, the second best substrate for PS synthesis in the brain, may play an important role in preventing a further decrease of PS and thus maintaining relatively high levels of PS in neural tissues even under DHA-depleting conditions. Conversely, DHA enrichment in vitro can induce PS accumulation specifically in neuronal cells, primarily because of the accumulation of 18:0,22:6-PS. The PS level in neuronal cells in culture has been shown to increase by supplementing cells with DHA and DPAn-6 but not with oleic acid (18:1n-9) (18, 38). In agreement with brain microsomal PS biosynthetic activity, DHA is more effective for inducing PS accumulation in neuronal cultures in comparison with DPAn-6. Non-neuronal cell lines such as CHO-K1, NIH3T3, and HEK-293 do not appear to increase total PS in response to DHA or DPAn-6, suggesting that the phospholipid profile is differently regulated in neuronal cells (39). Silencing pss1 and pss2 genes, which encode PSS1 and PSS2 enzymes, respectively, affects neither the PSS enzyme level nor the DHA-induced PS accumulation in neuronal cells, although the mRNA levels can be manipulated successfully (18). Similarly, overexpressing these genes in neuronal cells produces no effect on PS accumulation (39), suggesting that modulation of the PS pool in neuronal membranes according to DHA availability may be a unique mechanism that allows the disruption of membrane homeostasis in living cells. Besides n-3 fatty acid deficiency, long-term exposure to ethanol can also decrease PS levels in neural tissues as has been demonstrated from developing rat brain hippocampi after maternal alcohol consumption (40). However, unlike the case with n-3 fatty acid deficiency, where PS reduction is due to depletion of preferred substrates, PS decrease by ethanol is caused by impaired PS biosynthetic activity (41).

Phosphatidylserine represents the major negatively charged phospholipid class in mammalian cell membranes. Although PS is a constitutive component of cell membranes, PS participates in cell signaling by interacting with important signaling proteins for their activation (18). The extent of membrane interaction of protein kinase C and Raf-1 kinase depends on the PS content in the membrane (38, 42). It has been also demonstrated that DHA prevents neuronal apoptosis by facilitating Raf-1 (38) and Akt translocation/activation (18) through its capacity to increase PS in neuronal membranes. The PS-dependent facilitation of Akt translocation/activation may be particularly important for cell survival under suboptimal conditions such as in trophic factor-depleted conditions where PIP3 generation is limited. Under such adverse conditions, cell death due to reduced PIP3 signaling may be rescued by the high content of PS in neuronal membranes. In this regard, the capacity to concentrate membrane PS by DHA is an important determinant for modulating survival signaling. DPAn-6 accumulated in place of DHA in n-3 fatty acid deficiency is not as effective as DHA in accumulating PS and translocating Akt and thus is less effective in supporting neuronal survival (18). Likewise, the PS reduction in neuronal membranes after long-term ethanol exposure accompanies the promotion of neuronal apoptosis (24). Considering that neurons do not regenerate easily, neuronal cells appear to employ a unique mechanism to ensure their survival, by maintaining a high level of membrane PS through DHA enrichment.

Metabolism of DHA via Oxygenation

Polyunsaturated fatty acids released from neural cell membrane phospholipids can be oxygenated through cyclooxygenation, lipoxygenation, and cytochrome P450-dependent monooxygenation, and many of these products subsequently participate in the signal transduction processes (43). Lipoxygenase (LOX)-metabolites generated from AA, in particular, have been implicated in the modulation of synaptic function (44). Because DHA is a substrate for LOX, LOX-metabolites of DHA may also serve as signaling molecules under physiological conditions. It has been shown that 5- and 12-LOX genes are expressed in the brain (45, 46) and that rat brain homogenates

FIGURE 2. PS biosynthesis and degradation in mammalian cells. In the ER, DHA is incorporated into membrane PC or PE by de novo synthesis, or by deacylation/reacylation, and subsequently converted to PS by serine base exchange reaction. The PS thus formed is mostly decarboxylated to PE in mitochondria. PEMT, PE methyltransferase.
are capable of hydroxylating AA and DHA (47). However, the racemic stereochromic nature of the hydroxylated products strongly suggests that these are formed by peroxidative, non-enzymatic processes (47). 12-Lipoxygenase activity is expressed in the brain microvessels (48) and the pineal, which also contains 15-LOX activity (49). It appears that expression of LOX activity in the brain parenchyma may require certain stimuli or pathological conditions. It has been reported that glucocorticoid stimulation causes an increase in 5-LOX mRNA expression and membrane translocation of 5-LOX protein in the brain, which may represent its activation (50). Free radical-mediated peroxidation also occurs in the brain, generating a family of prostaglandin-like compounds, F2-isoprostanes and F4-neuroprostanes, from AA and DHA, respectively (51). These products are formed by the initial oxidation of AA or DHA esterified in the membrane phospholipids and subsequently released as free form. Although F2-isoprostanes and F4-neuroprostanes are detected in the normal human brain and cerebrospinal fluid, their level is elevated in neurological disease states (51). F4-Neuroprostanes, in particular, are elevated in Alzheimer disease in some regions of the brain cortex (52), suggesting that peroxidation products of DHA may serve as potential biomarkers for oxidative stress in neurodegenerative diseases.

Under pathological conditions such as ischemia, seizure, trauma, or infection, release of AA and DHA is rapidly induced in the brain as a result of PLA2 activation (53). Concurrently, microglia are activated and leukocytes are recruited into brain tissues from the circulating blood stream. Subsequent cell-cell interaction may generate diverse oxygenated metabolites from the liberated AA and DHA, such as 5- or 15-LOX products, in the brain. In addition, activated microglia generate the reactive oxygen species, increasing oxidative stress in the neural tissues. It has been demonstrated that DHA can be transformed by a 15-LOX-like enzyme to (10,17S)-docosatriene (54), which is also termed neuroprotectin D1 (NPD1) because of its neuroprotective properties. The production of NPD1 in the brain has been shown to be induced by ischemia-reperfusion, inhibiting leukocyte infiltration and proinflammatory gene expression (55). In an Alzheimer disease model, NPD1 has been shown to suppress Aβ-42-induced neurotoxicity by inducing neuroprotective and anti-apoptotic gene expression (56). Although the biosynthetic activity has been detected in glial cells (54), the production of NPD1 by cell-cell interaction may play an important role in supporting neuronal survival under pathological conditions.

Conclusions

Through specific accumulation and metabolic mechanisms operating in neural cells, DHA influences signaling events that are vital to neuronal survival and differentiation, as depicted in Fig. 3. DHA, supplied from the blood stream or biosynthesized in astroglia, is provided to neurons and rapidly incorporated into membrane phospholipids. Incorporation of DHA results in increased PS levels in neurons because of preferred PS synthesis from DHA-containing phospholipid substrates. The biochemical function of DHA in promoting PS accumulation in neuronal membranes is an important underpinning of the maintenance of neuronal survival. Specifically, Akt and Raf-1 translocation/activation is facilitated by the high concentration of PS in neuronal membranes. PS-dependent acceleration of Akt translocation is particularly important under suboptimal conditions, where the generation of survival signals such as PIP₃ is limited. The Raf-1 translocation facilitated by DHA may contribute to 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 (57). Transformation of DHA to NPD1 is protective, rescuing neuronal cells from cell death under pathological conditions. In conclusion, neuronal accretion of DHA and PS during development is required to prevent inappropriate cell death and to support neuronal differentiation. The loss of DHA and PS, or interference in their accumulation by nutritional deprivation or in pathological states, may diminish protective capacity in the central nervous system, with significant implications for neuronal dysfunction. Because PS-dependent signaling is a target for the neurotrophic action of DHA, it is important to understand the nature of specific regulation in neuronal PS accumulation. The metabolic regulation at both lipid and protein levels and post-translational modifications of PSS as well as the presence of other serine base exchange enzymes,

FIGURE 3. Involvement of DHA metabolism in neuronal survival and differentiation. R, receptor; PI3-K, phosphatidylinositol 3-kinase. GF, growth factor.
which are specific to the brain, need to be explored further. Elucidating the molecular mechanisms underlying the protein interaction with DHA-containing phospholipids, as well as identifying new target signaling proteins and metabolites involved in DHA protection, is another fruitful area for future research.

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