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Synapse formation and cognitive brain development: effect of docosahexaenoic acid and other dietary constituents

Richard J. Wurtman*

Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Abstract

The brain is unusual among organs in that the rates of many of its characteristic enzymatic reactions are controlled by the local concentrations of their substrates, which also happen to be nutrients that cross the blood-brain barrier. Thus, for example, brain levels of tryptophan, tyrosine, or choline can control the rates at which neurons synthesize serotonin, dopamine, or acetylcholine, respectively. The rates at which brain cells produce membrane phospholipids such as phosphatidylcholine (PC) are also under such control, both in adult animals and, especially, during early development. If pregnant rats are fed the 3 dietary constituents needed for PC synthesis—docosahexaenoic acid, uridine, and choline—starting 10 days before parturition and continuing for 20 days during nursing, brain levels of PC, and of the other membrane phosphatides (per cell or per mg protein), are increased by 50% or more. In adult animals, this treatment is also known to increase synaptic proteins (eg, synapsin-l, syntaxin-3, GluR-l, PSD-95) but not ubiquitous proteins like *β*-tubulin and to increase (by 30% or more) the number of dendritic spines on hippocampal neurons. Docosahexaenoic acid currently is widely used, in human infants, to diminish the negative effects of prematurity on cognitive development. Moreover, docosahexaenoic acid, uridine (as uridine monophosphate), and choline are all found in mother's milk, and included in most infant formulas. It is proposed that these substances are part of a regulatory mechanism through which plasma composition influences brain development.

1. Introduction

1.1. *ω*-3 fatty acids and the brain

The phospholipids in brain membranes contain many different fatty acids (cf, reference [1]). However, one such compound, the *ω*-3 polyunsaturated fatty acid docosahexaenoic acid (DHA), is both uniquely abundant among them [2] and particularly important in the development and maintenance of brain mechanisms underlying cognitive functions [3]. Thus, cognitive development among breast-fed full-term infants, or in full-term or preterm infants given supplemental DHA, is described as being superior to that in infants consuming formula diets that lack DHA [4]; and the consumption, by term infants of a formula supplemented with DHA (plus the *ω*-6 fatty acid arachidonic acid [AA]) during the first 17 weeks of life increased test scores on the Mental Development Index, assessed a year or more later [5]. At the other end of life, DHA levels in plasma phosphatidylcholine (PC) are inversely correlated with the risk of developing dementia, as shown in aged participants (average, 76 years) enrolled in the Framingham Heart Study and followed for 9 years [6]; and high intakes of fish or of DHA have been described by most investigators as protective against age-related cognitive decline and the risk of developing Alzheimer disease [7-9]. Docosahexaenoic acid administration has also been found to produce dose-related improvements in cognitive functions in various experimental animals (cf, reference [10]).

A number of hypotheses have been proposed to explain the beneficial effects of DHA consumption on brain functions and, particularly, cognition. These include, among others, changing the "fluidity" of neuronal membranes [11], and thereby alternating the activities of receptors, ion channels, G proteins, and other proteins embedded in the membranes; being transformed to active metabolites [12] such as "neuroprotectin D1" (10,17S-docosatriene), which reportedly suppresses A-*β*-42 neurotoxicity [13] or to the prostaglandin-like F4-neuroprostanes

STATEMENT OF CONFLICT OF INTEREST: The author is a member of the Scientific Committee of the Collège International de Recherche Servier (CIRS).

* Tel.: +1 617 253 6731; fax: +1 617 253 6882.
E-mail address: dick@mit.edu.

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[14]; promoting neurogenesis by causing the differentiation of neuronal stem cells [15]; activating syntaxin-3, a synaptic membrane protein that promotes neurite outgrowth [16]; decreasing the AA content of brain phospholipids [17]; or forming DHA-rich diacylglycerols that are preferentially used for synthesizing membrane phosphatides via the Kennedy cycle [18].

Oral DHA has now also been shown to promote the synthesis of synaptic membranes, elevating the levels, per brain cell, of both the phosphatides and the specific pre- and postsynaptic proteins that characterize these membranes [19]. Docosahexaenoic acid also increases the numbers of dendritic spines [20], and probably synapses, on hippocampal neurons, particularly on excitatory glutamatergic synapses. These effects, described below, can also be produced by eicosapentaenoic acid (EPA), another ω-3 fatty acid, but not by the ω-6 fatty acid AA [21]. They are considerably amplified if animals also receive 2 compounds that, with DHA, are present in mother's milk or infant formulas, that is, uridine [19], a circulating pyrimidine that gives rise in brain to uridine triphosphate (UTP) and cytidine triphosphate (CTP) [22,23], and choline. It is thus possible that DHA affects cognition principally by promoting neurotransmission and that it does so by increasing the numbers of certain synapses.

2. DHA and uridine increase phosphatide and synaptic protein levels in gerbil and rat brains

Three circulating compounds are essential precursors in the synthesis of PC, the major phosphatide in neuronal membranes [1], as well as of phosphatidylethanolamine (PE), and, indirectly, by base exchange, of phosphatidylserine (PS): DHA; a uridine source; and a choline source. Each of these precursors is able to limit the overall rate of PC synthesis because its levels in brain are insufficient to saturate the brain enzymes that catalyze its utilization; moreover, the effects of giving all 3 together tend to be substantially greater than the summed effects of giving each alone. Uridine may also promote membrane synthesis via UTP, which activates P2Y receptors that promote neurite outgrowth [24]; and DHA's effects may, as described above, also involve additional sites of action besides neuronal phosphatide synthesis. Perhaps surprisingly, when the 3 precursors are administered chronically, not only do brain levels of phosphatides—a lipid moiety—rise but also those of various pre- and postsynaptic proteins [19]; moreover, structural changes occur—an increase in the number of dendritic spines, and thus synapses, on hippocampal neurons [20].

The utilization of DHA, uridine, and choline to form phosphatides such as PC and PE is mediated by the enzymes of cytidylyldiphosphate (CDP)-choline cycle or Kennedy cycle [25]. Phosphatidylserine, the other main structural phosphatide, is formed by exchanging a serine molecule for the choline in PC or the ethanolamine in PE. Phosphatidylinositol (PI) synthesis also uses diacylglycerol (DAG) and uridine but different biosynthetic enzymes.

In the CDP-choline cycle, first choline is phosphorylated to phosphocholine by the enzyme choline kinase (CK); then CTP-phosphocholine cytidyl transferase (CT) transfers a cytidylylmonophosphate moiety from CTP to the phosphorus of phosphocholine, yielding cytidylyldiphosphocholine (also known as CDP-choline, or citicoline). Much of the CTP that the human brain uses for this reaction derives from circulating uridine; hence, brain CTP levels vary with plasma uridine concentrations [22]. The third and last reaction, catalyzed by CDP-choline:1,2-diacylglycerol choline phosphotransferase (CPT), bonds the phosphocholine of CDP-choline to the hydroxyl group on the 3-carbon of DAG, yielding PC. There are many types of DAG in the brain, differing in their 2 fatty acid constituents. Diacylglycerol molecules that contain DHA are preferentially used for phosphatide synthesis [18]. Once the new phosphatide molecule has been formed, this DHA can be removed by phospholipase A_2 and replaced by a different fatty acid, which need not be polyunsaturated [1]. Hence, giving DHA can increase total membrane phosphatide levels without necessarily increasing steady-state membrane DHA contents. All 3 of the PC precursors must be obtained by brain entirely or in large part from the circulation; all 3 readily cross the blood-brain barrier [23,26,27] and are metabolized by low-affinity brain enzymes to form PC.

Thus, choline administration increases brain phosphocholine levels in rats [28] and humans [29] because choline kinase's Km for choline (2.6 mmol/L) is much higher than usual brain choline levels (30-60 μmol/L). Generally, the second, CT-catalyzed reaction is most rate-limiting in PC synthesis, either because not all of the CT enzyme is fully activated by being attached to a cellular membrane or because local CTP concentrations are insufficient to saturate the CT [30]. Thus, when brain CTP levels are increased by giving animals uridine [22], CTP's circulating precursor in human blood [31], PC synthesis is accelerated [19]. The activity of CPT, the third enzyme, and the extent to which it is saturated with DAG, can also control the overall rate of PC synthesis, as has been demonstrated in, for example, permeabilized HeLa cells exposed to glycerol-3-phosphate and acyl-CoA [32], or in PC12 cells extending neurites after exposure to the nerve growth factor [24]. In PC-12 cells, nerve growth factor increased DAG levels 5-fold, CPT activity by 70%, and the incorporation of choline into PC by 2-fold [33].

If rodents are given a standard diet supplemented with choline and uridine (as its monophosphate, UMP) and also, by gavage, DHA, brain PC synthesis rapidly increases [22], and absolute levels of PC per cell (DNA) or per milligram of protein increase substantially (eg, by 40%-50% after several weeks of daily treatment [19]). This treatment also increases the levels of each of the other principal membrane phosphatides, as well as of particular proteins [19-21] known to be localized within presynaptic and postsynaptic membranes (eg, synapsin-1, PSD-95, and syntaxin-3, but not a ubiquitously distributed brain protein, β-tubulin). Moreover, it promotes the formation of dendritic spines by excitatory glutamatergic neurons in adult gerbil hippocampus [20] and improves hippocampus-dependent cognitive behaviors in gerbils and rats (cf, reference [34]) (eg, in aged animals or those reared in a socially deprived environment). Providing supplemental DHA or UMP alone can also increase brain phosphatide levels and those of some of the synaptic proteins, but by considerably less than when all 3 precursors are presented.

3. Synaptic phosphatides or proteins are not increased by ω-6 fatty acid AA

In experiments designed to compare the effects on brain phosphatide levels of administering each of the 3 polyunsaturated fatty acids in brain, the ω-3 fatty acids DHA and EPA, and the ω-6 fatty acid AA, animals received by gavage one of the fatty acids daily for 4 weeks and consumed a choline-containing diet that did or did not also contain UMP. Giving DHA without uridine increased PC, PI, PE, and PS levels significantly, by 18%, 20%, 22%, and 28% respectively, throughout the brain (eg, in cortex, striatum, hippocampus, brain stem, and cerebellum). Giving EPA also increased brain PE, PS, and PI levels significantly, by 21%, 24%, and 27%, respectively [21]. In contrast, AA administration failed to affect brain levels of any of the phosphatides. Consuming the UMP-supplemented diet alone increased brain PS and PC levels significantly and enhanced the effects of DHA or EPA on all of the phosphatides. In contrast, when UMP was given with AA, its effects were no greater than when it was given alone.

Giving the gerbils DHA (or EPA) alone also increased brain levels of all pre- or postsynaptic proteins examined, eg, syntaxin-3, the postsynaptic density protein PSD-95, synapsin-1, actin, the metabotropic glutamate receptor 1, but failed to affect brain levels of the ubiquitous protein β-tubulin. Giving UMP enhanced these increases in synaptic proteins. Arachidonic acid failed either to affect levels of any of the proteins or to increase the effects of giving UMP alone.

The mechanism that allows the ω-3 fatty acids DHA or EPA, given with UMP, to produce substantial increases in pre- and postsynaptic proteins may involve expressing the genes for these proteins: in a preliminary study, hippocampi of gerbils receiving DHA plus UMP for 4 weeks were found to contain elevated levels of the mRNA for the metabotropic-1 glutamate receptor (Cansev, Wurtman, unpublished observations), a protein located within dendritic spines; levels of this protein, assayed by Western blots, also increased.

Mechanisms that could underlie the differential effects of ω-3 and ω-6 PUFAs on synaptic membrane synthesis might include, among others, different efficacies for their uptakes into brain or their acylation; different half-lives in the circulation; different affinities for enzymes that control their incorporation into DAG and phosphatides; differences in the rates at which the PUFAs are removed from phosphatides by deacylation; the differential activation of genes encoding proteins that affect membrane synthesis (cf, reference [35]); or the ability of AA but not DHA to be incorporated into phospholipids by the acetylation of 1-acyl-2-lyso-glycerophospholipids and not solely via the Kennedy cycle [36].

4. DHA increases hippocampal dendritic spines

Most dendritic spines, the small membranous protrusions extending from postsynaptic dendrites in neurons, eventually form synapses with presynaptic axon terminals. These structures compartmentalize postsynaptic responses, and their numbers are thought to reflect the density of excitatory synapses within regions of the central nervous system, for example, the glutamatergic hippocampal synapses [37] that participate in learning and memory. Gerbils that received daily doses of DHA for 4 weeks (100 or 300 mg/kg, by gavage) exhibited increased dendritic spine density (ie, the number of spines per length of dendrite) in CA1 pyramidal neurons; the increases were 12% ($P = .04$) with the 100 mg/kg per day dose, and 18% ($P < .001$) with the 300 mg/kg per day dose. These effects were amplified when gerbils received both DHA (300 mg/kg per day, by gavage, as above) and UMP (0.5%, via the standard choline-containing diet) for 4 weeks, DHA supplementation alone increasing spine density by 19% ($P < .004$) and administration of both precursors doing so by 36%. (Giving UMP alone did not affect dendritic spine density significantly; however, it did increase spine density when all dendritic protrusions were included for statistical analysis, including the filopodia, which are precursor forms of dendritic spines [38,39].) The effect on dendritic spine density of giving DHA with UMP was already apparent after 1 week of treatment and continued for as long as animals were treated (4 weeks). Giving DHA with uridine promoted cognitive behaviors in aged rats or animals reared in a socially deprived environment (cf, reference [34,40]).

5. Effects of DHA on brain during early development

If pregnant rats receive—during the 10 days before parturition and for the initial 20 days of lactation—daily doses of DHA by gavage and supplemental uridine (as UMP) via their diets, brains of their offspring exhibit neurochemical changes similar to those described above in adult animals: DHA alone produces small (20%-30%) increases in each of the major phosphatides, whereas giving DHA plus UMP produces greater (50% or more) increases, per cell and per brain. The largest changes occur in PI, the precursor of the second messengers inositol triphosphate and diacylglycerol, its levels more than doubling in animals whose mothers receive DHA plus UMP. Most of these increases result from supplementation during the postnatal period because brains of offspring obtained and assayed at birth did not exhibit significant changes. Treating the mothers with DHA plus

UMP also elevates brain levels of pre- and postsynaptic proteins (eg, PSD95, synapsin-1, the metabotropic GluR-1 receptor, syntaxin-3) in the infant rat and the numbers of hippocampal dendritic spines. Hence, this treatment may affect the number of brain synapses formed during development. No data on how long these increases persist nor on their possible functional or behavioral consequences are available at present. However, it should be noted that, in other studies, brains of rats whose mothers received supplemental choline during embryonic days 11 to 17 exhibited, postnatally (days 15-34), increased expression of genes for brain proteins thought to be related to cognitive function (eg, calcium-calmodulin–dependent protein kinase, insulin-like growth factor), and such animals have been shown to manifest lifelong improvements in memory performance [41].

Human breast milk contains both DHA and AA, the levels of which depend on the mother's diet: among women consuming cod liver oil, the DHA/AA ratio is about 4:1, whereas among those who instead consume corn oil, this ratio is only 1:1. Breast milk also contains choline and uridine, as such and as UMP, UDP-glucose, and UTP, but also as cytidine, cytidylylmonophosphate, CTP—all converted to uridine in the human liver [31]—and as RNA, which is readily broken down to uridine in the newborn's intestine [42,43] Commercial infant formulas also include these compounds; however, their uridine contents are not, in general, as great as those a breast-fed infant would obtain from the uridine- or cytidine-containing compounds (including RNA) in mothers' milk. Whether or not providing additional DHA uridine or choline would improve brain development in normal infants, or facilitate recovery from neonatal brain injury remains to be determined.

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