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BRAIN RESEARCH

Research Report

Oral supplementation with docosahexaenoic acid and uridine-5′-monophosphate increases dendritic spine density in adult gerbil hippocampus

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ARTICLE INFO

Article history:
Accepted 29 August 2007
Available online 21 September 2007

Keywords:
Docosahexaenoic acid
Uridine
Membrane synthesis
Spine formation
Synaptogenesis
Phosphatides

ABSTRACT

Docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid, is an essential component of membrane phosphatides and has been implicated in cognitive functions. Low levels of circulating or brain DHA are associated with various neurocognitive disorders including Alzheimer's disease (AD), while laboratory animals, including animal models of AD, can exhibit improved cognitive ability with a diet enriched in DHA. Various cellular mechanisms have been proposed for DHA's behavioral effects, including increases in cellular membrane fluidity, promotion of neurite extension and inhibition of apoptosis. However, there is little direct evidence that DHA affects synaptic structure in living animals. Here we show that oral supplementation with DHA substantially increases the number of dendritic spines in adult gerbil hippocampus, particularly when animals are co-supplemented with a uridine source, uridine-5′-monophosphate (UMP), which increases brain levels of the rate-limiting phosphatide precursor CTP. The increase in dendritic spines (>30%) is accompanied by parallel increases in membrane phosphatides and in pre- and post-synaptic proteins within the hippocampus. Hence, oral DHA may promote neuronal membrane synthesis to increase the number of synapses, particularly when co-administered with UMP. Our findings provide a possible explanation for the effects of DHA on behavior and also suggest a strategy to treat cognitive disorders resulting from synapse loss.

1. Introduction

Nutritional supplementation with essential omega-3 fatty acids including docosahexaenoic acid (DHA) is increasingly practiced (Marszalek and Lodish, 2005). DHA, a 22-carbon compound with six double bonds, is synthesized from its precursor α-linolenic acid, which cannot be synthesized in mammals (mammals lack the desaturase enzyme that forms the double bond at the third carbon from its methyl terminal) (Marszalek and Lodish, 2005). Since conversion from α-linolenic acid to DHA is very slow, DHA must also be obtained from dietary sources, for example fatty fish (Marszalek and Lodish, 2005). Like other fatty acids, DHA is a precursor of diacylglycerol (DAG) (Marszalek and Lodish, 2005), an essential and sometimes rate-limiting precursor for the phosphatides in cellular membranes (Araki and Wurtman, 1997). Clinical

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doi:10.1016/j.brainres.2007.08.089

studies suggest that DHA can maintain or enhance cellular functions in various physiological systems including the nervous system (Marszalek and Lodish, 2005).

The mechanisms by which DHA affects brain functions or behaviors are poorly understood. The fatty acid readily crosses the blood-brain barrier (Spector, 2001; Hashimoto et al., 2002) and then cellular membranes, whereupon it is acylated and can be incorporated into DAG (Marszalek and Lodish, 2005). The DHA-containing DAG is preferentially utilized for membrane phosphatide synthesis (Marszalek and Lodish, 2005). Low levels of circulating or brain DHA are reportedly associated with neurodegenerative or psychiatric disorders, such as Alzheimer's disease (Soderberg et al., 1991; Schaefer et al., 2006), depression (Logan, 2004; Appleton et al., 2006) and schizophrenia (Assies et al., 2001; Reddy et al., 2004). Behavioral studies in laboratory rodents suggest that DHA is involved in learning and memory (Yoshida et al., 1997; Gamoh et al., 1999; Moriguchi et al., 2000; Calon et al., 2004; Hashimoto et al., 2002, 2006). Animals given a diet deficient in omega-3 fatty acids exhibit learning impairments (Yoshida et al., 1997; Moriguchi et al., 2000), while those receiving supplemental DHA show improved learning (Gamoh et al., 1999; Calon et al., 2004; Hashimoto et al., 2002, 2006). Various cellular mechanisms have been proposed as mediating DHA's neurobehavioral effects, for example increasing cell membrane fluidity (Hashimoto et al., 2006), promoting neurite extension (Ikemoto et al., 1997; Calderon and Kim, 2004; Marszalek et al., 2004, 2005; Darios and Davletov, 2006), inhibiting apoptosis (Hashimoto et al., 2002; Calon et al., 2004) and increasing synthesis of the phosphatides in synaptic membranes (Wurtman et al., 2006). However, there is little direct evidence that DHA affects synaptic structures in intact animals.

Uridine-5′-monophosphate (UMP) is a precursor of circulating uridine (Cansev et al., 2005). Like DHA, uridine readily crosses the blood-brain barrier and enters brain cells (Cansev, 2006). It is then phosphorylated by uridine–cytidine kinases to form uridine triphosphate (UTP), which can be further transformed by CTP synthetase to cytidine triphosphate (CTP), the usual rate-limiting precursor in phosphatide biosynthesis (Ross et al., 1997). CTP, for example, can combine with phosphocholine to form cytidine-5′-diphosphocholine (CDP–choline) (Kennedy and Weiss, 1956), which then combines with DAG to yield phosphatidylcholine (PC), the major phosphatide in neuronal membranes (Sastry, 1985). Uridine promotes neurite outgrowth in PC-12 cells treated with nerve growth factor (Pooler et al., 2005) and neurotransmitter release from the rat striatum in vivo (Wang et al., 2005, 2007). Moreover, when given with DHA, uridine increases membrane phosphatides and synaptic proteins in the adult gerbil brain (Wurtman et al., 2006). These results suggest that uridine as well as DHA can enhance phosphatide synthesis in neuronal or synaptic membranes.

Here we examined the effects of supplemental DHA and uridine on the number of dendritic spines in brains of living animals. Dendritic spines are small membranous protrusions extending from dendrites, which compartmentalize postsynaptic responses. They mediate most excitatory connections and are thought to reflect the number of excitatory synapses in the central nervous system (Matus, 2000; Hering and Sheng, 2001; Nimchinsky et al., 2002; Yuste and Bonhoeffer, 2004). We show that oral supplementation with DHA increases the number of dendritic spines in adult gerbil hippocampus, particularly when animals are also supplemented with UMP, a uridine source. Oral DHA may thus increase the number of brain synapses, particularly when co-administered with UMP. We have also examined the ability of arachidonic acid, an omega-6 fatty acid, to increase hippocampal dendritic spines. Unlike DHA, this fatty acid has been shown (Cansev and Wurtman, 2007) not to affect brain levels of phosphatides or synaptic proteins.

2. Results

2.1. *Oral supplementation with DHA increases dendritic spine density in adult gerbil hippocampus*

To examine whether DHA affects the number of dendritic spines, we treated adult gerbils with the fatty acid daily for 4 weeks, in oral doses of 0, 50, 100 or 300 mg/kg. The spine density increased significantly in the primary apical dendrites of CA1 pyramidal neurons after the animals received either the 100 or 300 mg/kg/day doses (Fig. 1). The increases were 12% ($p=0.04$) with the 100 mg/kg/day dose and 18% ($p<0.001$) with the 300 mg/kg/day dose.

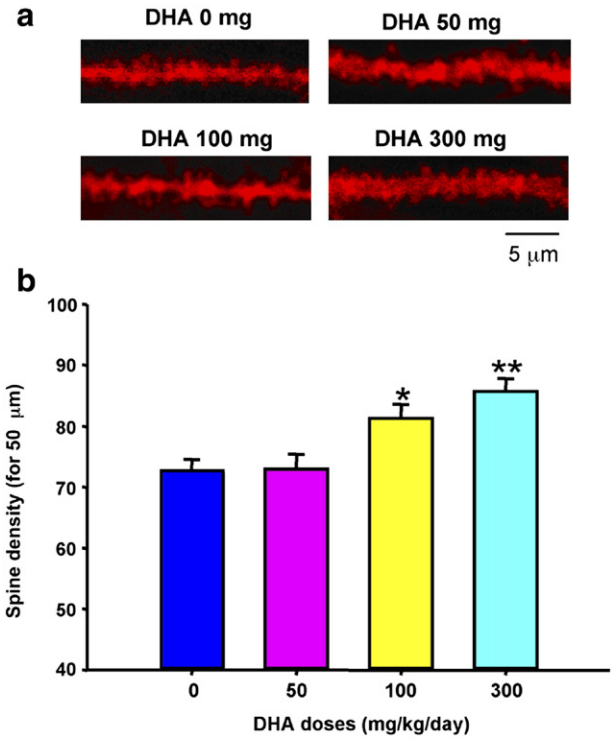

Fig. 1 – Oral supplementation with DHA increases dendritic spine density in adult gerbil hippocampus. Eight animals were randomly divided into 4 groups and were supplemented with 0, 50, 100 or 300 mg/kg of DHA daily for 4 weeks. (A) Primary apical dendrites of CA1 pyramidal neurons. (B) Animals supplemented with 100 or 300 mg/kg/day showed increased spine density: a 12% increase after the 100 mg/kg/day dose (*$p=0.04$) and an 18% increase after the 300 mg/kg/day dose ($p<0.001$ vs. 0 mg/kg/day). $n=16$–20 neurons from 2 animals per group. One-way ANOVA followed by Tukey's test.**