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Research Report

Some subtypes of endocannabinoid/endovanilloid receptors mediate docosahexaenoic acid-induced enhanced spatial memory in rats

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ABSTRACT

ω-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) enhances cognitive functions; however, the underlying molecular mechanism remains unclear. Compelling evidence suggests that the endocannabinoid/endovanilloid systems play a pivotal role in regulating cognitive function. Thus, to correlate the effect of DHA on cognitive performance with the expression of endocannabinoid and endovanilloid receptors, we supplemented the diet of rats with DHA and performed *in vitro* experiments that focused on the endocannabinoid/endovanilloid receptors. We found that *in vivo* supplementation with an appropriate dose of DHA (150 or 300 mg/kg/d) significantly improved learning and memory but that a higher intake (600 mg/kg/d) increased the risk of memory impairment. In addition, we found that some subtypes of endocannabinoid/endovanilloid receptors (cannabinoid [CB] and transient receptor potential vanilloid [TRPV] receptors) were regulated *in vitro* by different concentrations of DHA in primary hippocampal neuron culture medium. Real-time polymerase chain reaction and western blot analysis showed that expression of both CB1 and TRPV1 was upregulated in a dose-dependent manner and reached a maximum level at 30 μmol/L (CB1) and 60 μmol/L (TRPV1) DHA. However, TRPV2 expression was downregulated in a dose-dependent fashion, and the peak of TRPV2 suppression was observed at 60 μmol/L. The dose-dependent effects of DHA on the expression of these receptors were well correlated with DHA's effect on spatial memory. Meanwhile, CB2, TRPV3, and TRPV4 expressions were not altered at diverse concentrations of DHA. We concluded that some subtypes of endocannabinoid/endovanilloid receptors might be involved in enhanced spatial memory induced by DHA supplementation.

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Abbreviations: CB, cannabinoid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; M-MLV, Moloney murine leukemia virus; mRNA, messenger RNA; PCR, polymerase chain reaction; TRPV, transient receptor potential vanilloid

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1. Introduction

ω-3 polyunsaturated fatty acid docosahexaenoic acid (DHA, 22:6n-3), a component of the phospholipid structure of cellular membranes in the brain, is essential for normal neuronal function (Fedorova and Salem, 2006). Recently, DHA has gained increasing attention for its influence on cognitive functions. DHA modulates neurotransmitter release, gene expression, membrane-bound enzyme and ion channel activity, and synaptic plasticity (Horrocks and Farooqui, 2004). DHA accumulates primarily in the brain during brain development in the perinatal period (Green et al., 1999) and during aging (Delion et al., 1997; Favreliere et al., 2003) and in neurodegenerative disease such as Alzheimer's disease (Cole and Frautschy, 2006; Soderberg et al., 1991) DHA levels in the brain decrease. Epidemiological and clinical studies have indicated a link between high DHA intake and a decreased risk of cognitive decline and dementia in old age (Carrie et al., 2009). Long-term administration of DHA in a model of Alzheimer disease prevented β-amyloid deposition and impairment of cognitive functions (Hashimoto et al., 2002; Lim et al., 2005). Additionally, mental disorders such as depression and schizophrenia have been associated with low DHA levels, and supplementation with DHA has been shown to ameliorate the symptoms of these disorders (Colangelo et al., 2009; Horrocks and Farooqui, 2004; Rees et al., 2009).

The endocannabinoid system – a group of neuromodulatory lipids and their receptors, including anandamide, 2-arachidonoylglycerol, and cannabinoid (CB) receptors – is involved in cognition regulation. Endocannabinoids bind not only to CB receptors but also potentially to transient receptor potential vanilloid (TRPV) receptors. Anandamide has been shown to be an endogenous agonist of TRPV1 channels (Zygmunt et al., 1999). TRPV2 is an ionotropic CB receptor that is activated by cannabidiol (Qin et al., 2008). Anatomical, electrophysiological, and neurochemical evidence support the role of the endocannabinoid/endovanilloid systems in cognition. CB1 receptors are expressed at high concentrations in the hippocampus and other forebrain areas associated with memory. Moreover, CB1 and TRPV1 receptors were found to be coexpressed in mouse brain (Cristino et al., 2006). Endocannabinoids modulate glutamatergic (Sullivan, 2000), cholinergic (Gifford et al., 2000), and γ-aminobutyric acidergic (Katona et al., 2000; Wilson and Nicoll, 2001) pathways. Behavioral data also strongly support the involvement of endocannabinoids in cognition. Inhibition of endocannabinoid-degrading enzymes, namely fatty acid amide hydrolase, accelerates memory acquisition during the execution of a spatial memory task (Varvel et al., 2007). Activation of CB1 receptors facilitates the extinction of contextual fear memory (Pamplona et al., 2006). Exposure to CBs during adolescence increases not only the risk of memory impairment but also the risk of mental disorders such as schizophrenia and depression (Horder et al., 2009; Parolaro, 2010).

In summary, both DHA and the endocannabinoid/endovanilloid systems are closely related to cognitive function. Although DHA plays a pivotal role in cognition, the cellular and molecular mechanisms remain to be elucidated. Recently, it was reported that docosahexaenoyl ethanolamide, a derivative of DHA, acts as an endocannabinoid not only in its ability to activate CB1 and CB2 receptors but also in its susceptibility to metabolism by fatty acid amide hydrolase (Brown et al., 2010). Moreover, the endocannabinoid system and DHA are closely involved in the lipid-eicosanoid pathway. Endocannabinoids such as anandamide (derived from ω-6 polyunsaturated fatty acid arachidonic acid) act as eicosanoid signal molecules. DHA is a precursor of eicosanoids that serve as lipid mediators, exert anti-inflammatory effects, and have the ability to inhibit the formation of ω-6 polyunsaturated fatty acid-derived eicosanoids (Wall et al., 2010).

In this study, we attempted to correlate the effect of DHA on cognitive performance with the expression of endocannabinoid/endovanilloid receptors. Very little is known about the roles of TRPV2, TRPV3, and TRPV4, which we used as controls, in cognitive function in the central nervous system.

2. Results

2.1. *Spatial learning and memory performance*

Spatial learning and memory performance were evaluated using the Morris water maze. Statistical data of swimming speed on the pretest day showed no difference between the control group and the groups dosed with 150 mg/kg, 300 mg/kg, and 600 mg/kg DHA per day, indicating that the swimming ability of the rats before the tests was similar in all groups (data not shown). The escape latencies decreased over the course of learning and memory tests. The escape latencies in the groups dosed with either 150 mg/kg or 300 mg/kg DHA were significantly shorter than those in the control group by the fifth day of the acquisition phase ($n=10$, $P<0.05$, Fig. 1). Moreover, the latency in the 300 mg/kg group was significantly shorter than that in the 150 mg/kg group, indicating a dose-dependent effect. However, the latency in the 600 mg/kg group was reversed ($n=10$, $P<0.05$, Fig. 1).

The probe trial was carried out on the sixth day. Rats dosed with 150 mg/kg or 300 mg/kg DHA had longer searching times and more annulus crossings in the target quadrant than did the control group. Particularly, the 150 mg/kg group had significantly more annulus crossings than did the control group ($n=10$, $P<0.05$, Fig. 2B). Both the target quadrant time ratio and the number of annulus crossings in the 300 mg/kg group increased significantly ($n=10$, $P<0.05$, Figs. 2A and B). The number of correct annulus crossings was significantly greater in the 300 mg/kg group than in the 150 mg/kg group, again indicating a dose-dependent effect. In addition, rats dosed with 600 mg/kg DHA spent significantly less time and performed fewer crossings in the target quadrant than did the control group, indicating that too much DHA can impair memory function ($n=10$, $P<0.05$, Figs. 2A and B). These results suggest that appropriate dietary DHA supplementation enhances spatial memory performance. On the other hand, excess intake is associated with spatial memory impairment.

2.2. *Transcript expression of CB and TRPV subtypes*

We determined expression of the CB1, CB2, and TRPV1–4 subtypes using real-time polymerase chain reaction (PCR). *CB1*