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DIETARY EFFECTS OF DOCOSAHEXAENOIC ACID ON SPATIAL COGNITION LEARNING ABILITY IN RATS FED N-3 FATTY ACID-ADEQUATE DIET

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Twenty-two 5-week-old male Wistar rats fed an n-3 fatty acid-adequate diet were supplemented with docosahexaenoic acid (DHA) administered perorally for 14 weeks. Six weeks after the start of the administration, the rats were for 6 weeks trained to acquire a reward at the end of each of 4 arms of an 8-arm radial maze. The retention test interposed with a delayed task (20-min interval after acquiring the first 2 rewards) was then given to each rat to determine spatial working memory retention. DHA administration reduced the number of working memory errors after the 20-min interval in the retention test and tended to induce an increase in the DHA/arachidonic acid ratio in the hippocampus, indicating that chronic administration of DHA to rats fed an n-3 fatty acid-adequate diet is conducive to the improvement of working memory-related learning ability.

Key Words: n-3 fatty acid-adequate diet, docosahexaenoic acid, spatial working memory, rats.

INTRODUCTION

Docosahexaenoic acid (DHA; 22:6n-3) is one of the main structural lipids in the mammalian brain and is highly concentrated in the adult mammalian nervous system (1). It is also essential for normal neurological development and vision (2) and may, consequently, be requisite for memory formation. The precursor of DHA, α-linolenic acid (18:3n-3), cannot be synthesized *de novo* by mammals, and is therefore considered an essential fatty acid. Long-term deficiency of α-linolenic acid leads to changes in brain fatty acid composition, especially to a decrease in DHA, with a loss of learning ability (3, 4). More interestingly, the DHA level in the hippocampus has been found to be extremely low in patients with Alzheimer's disease (AD), compared with that in brain samples from age-matched human controls (5). Epidemiologic studies also show a relation between sources of dietary fish oil and AD. Intake of DHA, one of the main fatty acids in fish oil, has been associated with reduced risk of AD (6). We have made several studies toward clarifying the dietary effects of DHA on brain function and brain diseases, especially AD, by using rats fed a fish oil-deficient diet through three generations. Chronic administration of DHA enhanced reference memory of young (7) and old (8) third generation inbred rats, and dietary DHA administration protected against (9) and ameliorated (10) the impairment of learning ability in AD model rats. On the other hand, it is important to examine the effects of DHA supplementation of normal diets on cognition tests in studies of formula comparisons in humans, because fish consumption in Japan is higher than in the West. Only a few studies, however, on animals fed an n-3 fatty acid-adequate diet have examined the effects of DHA supplementation on performance in cognition learning ability. Here, we studied the effects of dietary DHA on the learning ability of rats fed an n-3 fatty acid-adequate diet and examined

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whether it improved their spatial cognition learning ability.

MATERIALS AND METHODS

The rats were provided for and killed in accordance with the procedures outlined in the Guidelines for Animal Experimentation of Shimane Medical University (Shimane, Japan), complied from the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. The animals were housed in a room under controlled temperature (23 ± 2℃), relative humidity (50 ± 10%) and light-dark cycles (light: 8:00 to 20:00; dark: 20:00 to 08:00).

Twenty-two male Wistar rats (5 weeks old, 60 to 70 g, Jcl: Wistar), purchased from Clear Japan (Osaka, Japan), were randomly divided into two groups: a DHA group (n=12) was orally fed DHA-95E (300 mg/kg/day; an ethyl-ester all-cis-4,7,10,13, 16,19-docosahexaenoate with a purity of over 95%; Harima Chemicals, Inc., Tokyo, Japan) gently emulsified in a 5% gum Arabic solution in ice-cold water before administration; a control group (n=10) was fed an equal volume of only the vehicle. All rats were provided with an n-3 fatty acid-adequate diet (MF pellet, Oriental Yeast Co., Ltd., Osaka, Japan) and water *ad libitum* for 14 weeks.

Learning-related behavior was assessed using an eight-arm radial maze (Toyo Sangyo Co., Toyama, Japan) as described (3, 4). Briefly, six weeks after the start of DHA administration, the rats were trained to acquire a reward (food-pellet) at the end of each of 4 arms of an 8-arm radial maze. The performance involved two parameters of memory function: reference memory error (RME), entry into unbaited arms; and working memory error (WME), repeated entry into arms that had already been visited, and obtaining the rewards within one trial. Each rat was given two daily trials, six days a week for a total of six weeks, and 2 weeks thereafter subjected to the retention test which included a 20-min interval between the rat's acquiring the first two rewards and then acquiring the remaining two rewards. The animal was returned to its cage during the interval.

After completing the behavioral test, the rats were anesthetized with sodium pentobarbital (65 mg/kg, intraperitoneal), their blood was collected from the inferior vena cava with heparinized syringes, and the cerebral cortex and hippocampus were separated. The brain tissues were stored, until the assay, at -80℃ after flash freezing with liquid N_2.

The fatty acid profiles of plasma and brain tissues were determined by the one-step analysis of Lepage and Roy (11) using gas chromatography as described (9, 10). Protein concentrations were estimated by the method of Lowry et al. (12).

All data are expressed as means ±SE. Behavioral data were analyzed by a 2-factor (group and block) randomized block factor ANOVA, and the between-group differences by one-way ANOVA. ANOVA was followed by Fisher's PLSD for post hoc comparisons. Correlation was determined by simple regression analysis. Statistical programs GB-STAT™ 6.5.4 (Dynamic Microsystems. Inc., Silver Spring, MD, USA) and StatView® 4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA) were used. A $P < 0.05$ was considered statistically significant.

RESULTS

The effect of the chronic administration of DHA, during 6 weeks before the retention test, on working and reference memory-related learning ability is shown in Figure 1(A). The score is expressed as the mean number of WMEs and RMEs for each group of n-3 Adq rats, with data averaged over blocks of six trials. Randomized 2-factor (block and group) ANOVA was conducted based on the scores. The analysis revealed a significant main effect of blocks ($P < 0.0001$), but no significant main effect of groups ($P = 0.533$), on the number of WMEs. Also, no significant main effect of block x group interaction ($P = 0.811$) was observed. Similarly, the analysis revealed a significant main effect of blocks of trials ($P < 0.001$), but no significant main effect of groups ($P = 0.337$) or of block x group interaction ($P = 0.303$), on the number of RMEs (data not shown). These results indicate that DHA administration did not affect working and reference memory-related learning ability before the retention test in n-3 Adq rats.

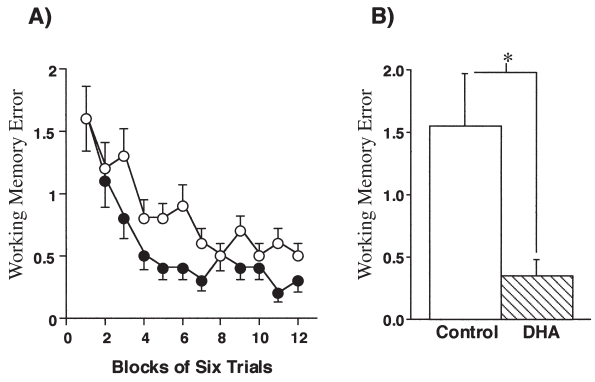

Fig. 1. Effect of chronic administration of DHA on the number of WMEs of rats in the radial maze task.
Fig. 1(A) The number of WMEs during the 5 weeks before the retention test. (○) control (n=10): (●) DHA administered at 300 mg/kg/day (n=12). Each value represents the number of WMEs made until the rat acquired all the rewards; mean ± SE in each block of six trials.
Fig. 1(B) The number of WMEs made when acquiring the last two rewards after the 20-min interval between trials 2 and 3. (□)control (n=10): (▨) DHA administered at 300 mg/kg/day (n=12). Vertical bars represent the SE *P = 0.0027.

The effect of chronic DHA administration on working memory-related learning ability in the retention test is shown in Figure 1(B). No statistically significant difference was observed in the number of the WMEs between the vehicle- and DHA-fed n-3 Adq rats in the course of acquiring the first 2 rewards before the 20-min interval (data not shown). The interval of 20 min interposed between acquiring the second and third rewards effected a significant decrease in the number of WMEs in the DHA-fed n-3 Adq rats (P = 0.0027) compared with the decrease in the vehicle-fed n-3 Adq rats. No effect of chronic administration of DHA was observed on the number of RMEs before or after the 20-min interval (data not shown). These results suggest that control rats were less retentive of spatial memory than the DHA-administered n-3 Adq rats.

Plasma fatty acid profiles of vehicle- and DHA-fed n-3 Adq rats are shown in Table 1. Chronic administration of DHA significantly increased the levels of EPA and DHA and the ratio of n-3 fatty acids/total fatty acids, and significantly decreased the level of arachidonic acid (AA) in the plasma of n-3 Adq rats.

Brain fatty acid profiles of vehicle- and DHA-fed n-3 Adq rats are shown in Table 2. The contents of AA and DHA in both regions of n-3 Adq rats were not affected by the chronic administration of DHA. The ratio of DHA/AA and that of n-3 fatty acids/total fatty acids in the hippocampus showed a tendency to increase by 8.9 % (P = 0.10) and by 12.9 % (P = 0.10), respectively, by the administration. The ratio of both DHA/AA and n-3 fatty acids/total fatty acids in the cerebral cortex was not affected by the administration. Regression analysis of the DHA/AA ratio in the cerebral cortex and the hippocampus and the n-3 fatty acids/total fatty acids ratio in this study revealed a highly significant positive correlation (r=0.835, P=0.0312; r=0.891, P<0.0001; respectively).

DISCUSSION

This study investigated the effects of the chronic administration of DHA on brain fatty acid profiles and on spatial memory-related behavior of rats fed a fish-oil adequate diet. Chronic administration of DHA (300 mg/kg/day) to young rats fed an n-3

Table 1. Plasma fatty acid profiles in vehicle and DHA rats fed n-3 fatty acid-adequate diet

	PA (μg/mL)	SA (μg/mL)	OA (μg/mL)	LA (μg/mL)	LNA (μg/mL)	AA (μg/mL)	EPA (μg/mL)	DHA (μg/mL)	n-3/T-FFA (mol%)
Vehicle (n=10)	413 ±34	161 ±6	260 ±35	503 ±49	17.3 ±2.2	341 ±12	7.95 ±0.76	67.7 ±3.7	5.52 ±0.20
DHA (n=12)	408 ±42	158 ±10	241 ±33	533 ±52	15.2 ±2.6	250* ±22	29.2* ±2.9	160* ±13	11.5* ±0.3

Values are means ± SE; *P < 0.05, vehicle group vs. DHA group.

PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; LNA, linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-3, n-3 fatty acid; T-FFA, total fatty acid; Vehicle, rats administered 5% Arabic solution orally; DHA, rats administered DHA (300 mg/kg/day) orally.

Table 2. Brain fatty acid profiles in vehicle and DHA rats fed n-3 fatty acid-adequate diet

	Cerebral cortex				Hippocampus			
	AA (μg/mg protein)	DHA (μg/mg protein)	DHA/AA	n-3/T-FFA (mol%)	AA (μg/mg protein)	DHA (μg/mg protein)	DHA/AA	n-3/T-FFA (mol%)
Vehicle (n=10)	30.2 ±0.6	48.6 ±1.3	1.49 ±0.04	20.6 ±0.3	32.0 ±1.4	42.6 ±1.6	1.23 ±0.02	17.1 ±0.4
DHA (n=12)	29.3 ±1.2	47.2 ±1.3	1.52 ±0.08	20.5 ±0.6	30.1 ±0.9	43.1 ±1.0	1.34 ±0.06	19.3 ±0.42

Values are means ± SE; *P < 0.05, vehicle group vs. DHA group.

AA, arachidonic acid; DHA, docosahexaenoic acid; n-3, n-3 fatty acid; T-FFA, total fatty acid; Vehicle, rats administered 5% gum Arabic solution orally; DHA, rats administered DHA (300 mg/kg/day) orally.

fatty acid-deficient diet for 12 weeks causes a significant increase in the content of DHA and in the ratio of DHA/AA in the cerebral cortex and the hippocampus (7). In this study, the administration of DHA to n-3 Adq rats for 14 weeks slightly, but not significantly, increased the ratio of DHA/AA in the hippocampus, but did not affect the hippocampal content of DHA. The DHA/AA ratio in the hippocampus showed a strong correlation with the ratio of n-3 fatty acids/total fatty acids. Thus, the enhancing effect of dietary DHA administration on the hippocampal DHA/AA ratio may be affected by the ratio of n-3 fatty acids/total fatty acids in brain tissues.

The present results of the radial maze task were not consistent with the results (13) demonstrating that dietary supplementation of DHA did not affect performance in cognitive behavioral tests in the Morris water-maze, despite both the increased DHA and the decreased AA levels in forebrain membrane phospholipids of rats fed an n-3 adequate diet. We have previously demonstrated improved performance in cognitive behavioral tests through radial-maze tasks by rats fed an n-3 fatty acid-deficient diet and administered dietary DHA. It is difficult to differentiate between the effects of the two mazes on the performance of rats in cognitive behavioral tests. The Morris water maze is most commonly used in learning and memory tests in rodent studies. It is, however, well known that placed in a water pool, the animal easily develops stomach ulcers, suggesting that the rat is more susceptible to stress in the Morris water maze than in the radial maze. Consequently, the radial-maze tasks may be more fitting than the Morris water maze for studying the performance of rats in cognitive behavioral tests.

The hippocampus and the cerebral cortex are the key structures of memory function (14, 15, 16). Hippocampal damage is generally known to disrupt radial arm maze performance, especially in relation to working memory (but not to reference memory) (17). Hippocampal lesions induce impairment of spatial discrimination; therefore, the hippocampus plays an important role in spatial information processing (18). Evaluation of the role of the hippocampus in working memory-related spatial cognition learning ability of rats is possible with a retention test in radial maze tasks (19). In this study, chronic administration of DHA to n-3 Adq rats reduced the number of WMEs after the 20-min interval in the retention test, suggesting DHA-induced improvement in working memory-related learning ability.

The DHA/AA ratio of the rat hippocampus is inversely related to the reference memory-related learning ability in radial maze tasks (7), and the increased DHA/AA ratio in the hippocampus protects against (9) and ameliorates (10) the impairment of learning ability in AD model rats. The forebrain ischemia-induced cognitive deficiency is associated with a reduced DHA/AA ratio (20). Taking these findings into account, we suggest that the dietary administration of DHA to n-3 Adq rats is conducive to improvement in spatial cognition, at least in working memory-related learning ability, by the association of an increase in the ratio of DHA/AA in the hippocampus. Therefore, DHA may be an effective means of improving cognition learning ability, even of subjects fed an n-3 fatty acid-adequate diet.

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