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Dietary Docosahexaenoic Acid Increases Cerebral Acetylcholine Levels and Improves Passive Avoidance Performance in Stroke-Prone Spontaneously Hypertensive Rats

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MINAMI, M., S. KIMURA, T. ENDO, N. HAMAUE, M. HIRAFUJI, H. TOGASHI, M. MATSUMOTO, M. YOSHIOKA, H. SAITO, S. WATANABE, T. KOBAYASHI AND H. OKUYAMA. *Dietary docosahexaenoic acid increases cerebral acetylcholine levels and improves passive avoidance performance in stroke-prone spontaneously hypertensive rats.* PHARMACOL BIOCHEM BEHAV 58(4) 1123–1129, 1997.—We have recently shown that inferior performance in passive avoidance task is accompanied with decreased hippocampal choline (Ch) in stroke-prone spontaneously hypertensive rats (SHRSP) compared with normotensive control Wistar–Kyoto rats (WKY). We also reported that dietary docosahexaenoic acid (DHA) suppresses the development of hypertension and stroke-related behavioral changes, resulting in the prolongation of the life span of SHRSP. In this study, we examined the effect of dietary DHA on the cerebral acetylcholine (ACh) levels and learning performance in passive avoidance tasks in SHRSP. The arachidonic acid decreased and the DHA increased in plasma lipids dose dependently with dietary DHA treatments, which decreased the systolic blood pressure in SHRSP. Dietary DHA significantly restored the significantly inferior learning performance in passive avoidance response observed in control SHRSP (DHA 0%). Furthermore, the hippocampal ACh levels were correlated positively with the total response latency in passive avoidance tasks. These results suggest that cholinergic dysfunction in the brain of control SHRSP is responsible, at least in part, for the impaired learning ability and the dietary DHA ameliorates this performance failure. © 1997 Elsevier Science Inc.

Docosahexaenoic acid (DHA) n-6/n-3 ratio Brain ACh level Learning ability Passive avoidance task stroke-prone spontaneously hypertensive rats (SHRSP)

THE SHRSP strain is considered as a possible animal model for vascular dementia because some symptoms as well as the lethal course of stroke in SHRSP coincide well with those of patients with cerebrovascular lesions (38,41). This strain of rats develops the highest blood pressure among the common rat strains and dies frequently of cerebral bleeding or cerebral infarction. We have reported that, at the onset of cerebral stroke, the SHRSP strain exhibits behavioral anomalies including increased ambulatory activity and disrupted circadian rhythms, which may correspond to the delirium-state ob-

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served in patients with vascular dementia (29). In addition, we have observed behavioral anomalies in the passive avoidance task in SHRSP (45).

Functional abnormalities such as decreased membrane fluidity and increased Ca^{2+} permeability have been observed in cell membranes of SHR and SHRSP (12,21,30,48,55). Dietary marine oils rich in n-3 fatty acids, eicosapentaenoic acid [EPA, (20): 5n-3] and docosahexaenoic acid [DHA, (22): 6n-3] have beneficial effects on cardiovascular and cerebrovascular disorders (13,42,55). We previously demonstrated that dietary DHA significantly suppresses the age-dependent increase in the systolic blood pressure of SHRSP in a dose-dependent manner (22). Umemura et al. (49) showed that dietary DHA produced antithrombotic effects and caused a reduction in the size of ischemic cerebral lesions in a midcerebral artery-thrombosis model (rat).

Essential fatty acid (n-6 and n-3) deficiency during the gestational period is reported to induce irreversible damage to brain function, and supplementation after this period does not allow rats to recover from their decreased learning ability (32). The decreased learning ability induced by n-3 deficiency in the presence of n-6 fatty acid, contrarily, was reversed by supplementation with n-3 after weaning (39). The behavioral changes induced by n-3 deficiency could be due to changes in neurotransmitters or synaptic junctions. Decreased dopamine content was reported in n-3–deficient rat brain (10), although we have so far been unsuccessful to confirm the result (Tosaki et al., unpublished observation). n-3 deficiency has also been reported to decrease serotonin receptor density (11), dopamine receptor density (10), and synaptic vesicle density (58). Thus, dietary supplementation with n-3 fatty acids appears to exert pleiotropic effects on animal physiology.

A disturbance in the central cholinergic systems is reported in patients with vascular dementia (52) as well as in the normal aging human and in patients with Alzheimer's disease (2,40). We have previously indicated the possibility that central cholinergic dysfunction might characterize pathophysiological state of SHRSP (46,47). Namely, we demonstrated that the decrease in the choline (Ch) levels in SHRSP was observed in all cerebral regions (47); cerebrospinal fluid levels of Ch and acetylcholine (ACh) were significantly lower in SHRSP than in WKY (46); behavioral impairment in the passive avoidance task was observed in SHRSP with positive correlations between the hippocampal ACh levels and the latency of the passive avoidance response (47). Furthermore, we previously reported that decreased DHA in the brain was associated with the inferior learning ability of the SHR in the brightness-discrimination learning test (53). These observations led us to examine whether dietary DHA could ameliorate cholinergic dysfunction and behavioral performance in SHRSP.

METHOD

Animals and Feeding

The original SHRSP and normotensive control WKY were donated by the late professor Kozo Okamoto, Department of Pathology, Kinki University School of Medicine, Osaka, Japan. Six-week-old male rats were subjected to a 12-h light and dark alternation cycle (lights on 1900 to 0700). Food and water were given ad lib. Illumination was provided by fluorescent light (100 lx). Room temperature was maintained at 22 ± 2°C throughout the experiment. After being measured for body weight and blood pressure, rats were divided into three groups with approximately equal mean body weights and systolic blood pressures (Table 1). Rats were randomly assigned to groups received 1% DHA, 5% DHA, or to control WKY (DHA 0%) and control SHRSP (DHA 0%) groups without DHA administration. One series of SHRSP was used for the measurements of systolic blood pressure and lipids. Another batch of SHRSP was used for the passive avoidance test and for the measurment of ACh. To examine the effects of DHA, a semipurified diet (Clea Japan Co. Ltd., Tokyo) was used, which consisted of 24.5% milk casein, 46.5% corn starch, 5.0% cellulose, 10.0% sucrose, 1.0% vitamin mixture, 7.0% mineral mixture (Ca 1.19 g, P 1.13 g, Mg 0.39 g, K 1.09 g, Na 0.26 g, Mn 10.87 mg, Fe 35.4 mg, Cu 0.79 mg, Zn 6.48 mg, Co 0.13 mg, I 45.5 μg/100 g diet), and 6% safflower oil. For this basal diet, DHA (DHA ethyl ester, 98% pure, Harima Chemicals Co. Ltd., Japan) was supplemented at 0, 1, or 5% (w/w). The fatty acid composition of the basal diet was 16: 0 (8.6% of

TABLE 1

EFFECTS OF DOCOSAHEXAENOIC ACID ON BODY WEIGHT, TOTAL PLASMA PROTEIN, AND SYSTOLIC BLOOD PRESSURE

	Body Weight (g)	Total Plasma Protein (g/dl)	Systolic Blood Pressure (mmHg)	
			Tail-Cuff Method	Direct Method
WKY (DHA 0%) (n = 6)				
6 weeks old	107.2 ± 4.0		119.0 ± 2.7	
20 weeks old	302.9 ± 14.6	5.5 ± 0.1	127.7 ± 3.3‡	125.0 ± 2.4‡
SHRSP (DHA 0%) (n = 9)				
6 weeks old	102.6 ± 5.15		120.2 ± 2.2	
20 weeks old	287.0 ± 18.9	5.5 ± 0.1	202.9 ± 5.7	206.1 ± 10.9
SHRSP (DHA 1%) (n = 10)				
6 weeks old	103.7 ± 4.0		117.2 ± 1.6	
20 weeks old	291.6 ± 14.6	5.7 ± 0.1	167.8 ± 7.1*	172.1 ± 7.6*
SHRSP (DHA 5%) (n = 11)				
6 weeks old	101.8 ± 3.8		119.3 ± 1.4	
20 weeks old	296.9 ± 12.5	5.5 ± 0.1	149.8 ± 1.4†	148.9 ± 3.2†

Means ± SEM.

*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. SHRSP (DHA 0%). SHRSP = stroke-prone spontaneously hypertensive rats, WKY = Wistar–Kyoto rats, DHA = docosahexaenoic acid.

TABLE 2
EFFECTS OF DIETARY DOCOSAHEXAENOIC ACID ON THE PLASMA FATTY ACIDS IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

	SHRSP (DHA 0%) (n = 9)	SHRSP (DHA 1%) (n = 10)	SHRSP (DHA 5%) (n = 11)
AA (% of total fatty acids)	35.00 ± 2.09	21.17 ± 2.12†	7.47 ± 0.58‡
EPA (% of total fatty acids)	0	1.39 ± 0.05*	4.53 ± 0.33‡
DHA (% of total fatty acids)	2.73 ± 0.51	11.28 ± 0.27‡	18.42 ± 0.91‡
n-3 (% of total fatty acids)	3.14 ± 0.62	13.34 ± 0.66‡	23.17 ± 0.94‡
n-6 (% of total fatty acids)	54.01 ± 1.22	45.10 ± 0.92‡	34.88 ± 0.68‡
n-6/n-3	17.14 ± 0.01	3.38 ± 0.02‡	1.50 ± 0.04‡
Total fatty acids (mg/ml)	1.66 ± 0.71	1.32 ± 0.24	0.87 ± 0.14

Means ± SEM.
*$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$ vs. DHA 0%. EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.

the total fatty acids), 18: 0 (2.2%), 18: 1n-9 (10.4%), 18: 2-n-6 (78%), and 18: 3n-3 (0.05%). Fatty acids were designated by carbon chain: the number of double bonds and the position of the first double bond numbered from the methyl terminus as n-9, n-6, or n-3 (42). Diets with peroxide values below 30 mEq/kg were served throughout the experiments.

Blood Pressure Measurement

The plethysmographic tail-cuff method (Natsume Co. Ltd., Tokyo, Japan: KN-0090) was used as a noninvasive measurement of blood pressure and heart rate. The blood pressure and heart rates of SHRSP were determined before (6 weeks of age) and after (20 weeks of age) the DHA treatment. To avoid unreliable blood pressure measurements due to the tail-cuff method, we determined blood pressure at several settings for every measurement in habituated animals and neglected the first few settings from the calculation. At the end of the experiments, we also confirmed the effects of DHA treatment on blood pressure using a direct measurement via a cannula inserted into the femoral artery of the rat anesthetized with α-chloralose (50 mg/kg, IP) and urethane (500 mg/kg, IP) (Table 1).

Lipid Analysis

Tissue and plasma samples were obtained from SHRSP at 20 weeks of age after 14 weeks of DHA treatment. At the end of the experiments, SHRSP were anesthetized with intraperitoneally administered α-chloralose (50 mg/kg) and urethane (500 mg/kg). A polyethylene cannula was inserted into the femoral artery and blood was withdrawn for lipid analysis. Plasma was obtained by centrifugation of the blood samples with ethylenediamine tetraacetic acid (EDTA) supplemented as an anticoagulant. Plasma samples were kept frozen at −80°C until lipid analysis. Total lipids were extracted from the plasma by the method of Bligh and Dyer (5). Fatty acids were converted to methyl esters by treatment with 5% HCl in methanol and then analyzed by gas liquid chromatography (GLC) using a capillary column (DB-225, J & W Scientific, Folsom, CA) essentially as described previously (53). Heptadecanoic acid was added as an internal standard. Protein concentration was determined by the method of Lowry et al. (27).

Passive Avoidance Response

Rats were tested using an one-way step-through type of passive avoidance apparatus divided into light and dark cham-

TABLE 3
EFFECTS OF DOCOSAHEXAENOIC ACID ON THE CEREBRAL LEVELS OF ARACHIDONIC ACID AND DOCOSAHEXAENOIC ACID IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

	SHRSP (DHA 0%) (n = 9)	SHRSP (DHA 1%) (n = 10)	SHRSP (DHA 5%) (n = 11)
Cortex			
AA (μg/mg protein)	9.94 ± 0.07	8.55 ± 0.12	7.39 ± 0.16*
DHA (μg/mg protein)	11.32 ± 0.21	15.75 ± 0.19*	17.17 ± 0.20*
Hippocampus			
AA (μg/mg protein)	11.33 ± 0.19	10.30 ± 0.42	8.63 ± 0.09*
DHA (μg/mg protein)	9.90 ± 0.16	13.60 ± 0.13*	15.79 ± 0.20*

Means ± SEM.
*$p < 0.001$ vs. DHA 0%. AA = arachidonic acid, DHA = docosahexaenoic acid. No significant amount of EPA was detected in brain regions.

bers as described previously (47). Acquisition trials were carried out 24 h before the retention trials. Each animal received a foot shock (75 V, 0.2 ms) for 3 s upon entering the dark chamber. This trial was repeated until the rats eventually remained in the light chamber for more than 300 s. In the retention trials, rats were placed in the light chamber and the time taken to enter the dark chamber, the response latency, was measured up to a maximum of 600 s. The retention trials were performed 1, 2, 3, 4, and 7 days after the acquisition trials. Passive avoidance test were performed in the dark phase (1300–1600). The passive avoidance performance was evaluated by the response latency in each retention trial and by the area under the retention curve for seven days after acquisition trials.

Acethylcholine (ACh) and Choline (Ch) Determination

After the passive avoidance test, rats were killed by microwave irradiation (5 kW for 1.5 s), brains were removed, and right hemispheres were dissected into seven regions (cortex, cerebellum, midbrain, hippocampus, hypothalamus, medulla oblongata, and striatum) according to the method of Glowinski and Ivelsen (17) to measure ACh and Ch contents. The tissues were stored at −80°C until they were assayed. Extraction of the ACh and Ch tissue was carried out with aliquots of 0.2 N perchloric acid including ethylhomocholine (EHC) as an internal standard. The homogenates were centrifuged for 10 min (10,000 r.p.m., 2°C). The supernatant, which was neutralized with 0.2 N potassium hydrogen carbonate, was filtrated (0.22 mm Millipore filter, Bedford, MA) and injected into a high-performance liquid chromatography (HPLC) (Eicom, Japan) system connected with an immobilized enzyme reactor and an electrochemical detector (ECD) (Eicom, Japan) as reported previously (28).

Statistical Analysis

Values were expressed as the means ± SEM. The Student's *t*-test was used to analyze difference between two groups. When more than two groups were compared, the significance of the difference among groups was evaluated by ANOVA and, where applicable, was followed by Turkey's test. The Bonferroni adjustment was used for testing at two points (51). $p < 0.05$ was considered to be significant.

RESULTS

Effect of Dietary DHA on Blood Pressure

The systolic blood pressure of the control SHRSP (DHA 0%) increased progressively from 120.2 ± 2.2 mmHg at 6 weeks of age to 202.9 ± 5.7 mmHg at 20 weeks (Table 1). On the other hand, the systolic blood pressure of 1% DHA-treated SHRSP was 117.2 ± 1.6 mmHg at 6 weeks of age and then rose to 167.8 ± 7.1 mmHg at 20 weeks. Systolic blood pressure of 5% DHA-treated SHRSP increased from 119.3 ± 1.4 mmHg at 6 weeks of age to 149.8 ± 1.4 mmHg at 20 weeks ($n = 10$). A significant difference in systolic blood pressures was noted between the control SHRSP (DHA 0%) and the DHA-treated SHRSP at 20 weeks of age. The systolic blood pressure of SHRSP (DHA 0%) at 20 weeks of age was significantly higher than that of WKY (DHA 0%). Dietary DHA significantly suppressed the increase in systolic blood pressure of SHRSP in a dose-dependent manner (Table 1). As shown in Table 1, the body weight and plasma total protein of SHRSP (DHA 0%) were not significantly different from those of WKY (DHA 0%) or DHA-treated SHRSP.

Effects of Dietary DHA on Plasma and Brain Fatty Acids

DHA supplementation resulted in significant increases in plasma DHA and EPA, a possible retroconversion product of DHA (Table 2). Dietary DHA significantly decreased the plasma arachidonic acid (AA) levels and the plasma n-6/n-3 ratios compared with those of control SHRSP (DHA 0%) in a dose-dependent manner. DHA treatment decreased the plasma total fatty acids from 1.66 ± 0.71 at 0% DHA to 1.32 ± 0.24 at 1% DHA and to 0.87 ± 0.14 mg/ml plasma at 5% DHA at 20 weeks of age. The DHA treatment-induced changes in plasma lipid n-6/n-3 ratio correlated positively with the systolic blood pressure ($r = 0.7756$, $p < 0.01$).

Brain cortex total fatty acid content was not affected by the diets. Reflecting the dietary fatty acid compositions—the basal diet with a high linoleate (18: 2n-6) content vs. the DHA supplemented diets—DHA treatment significantly decreased the cortex AA levels and increased DHA levels (Table 3). Similar changes in fatty acid composition were observed in the hippocampus. It should be noted that dietary DHA-induced changes in brain fatty acid composition were much less than in plasma lipids.

Effect of DHA on Passive Avoidance Response in SHRSP

There was no significant difference in the response latency and the frequency of foot shocks needed to acquire the passive avoidance task (acquisition performance) between SHRSP and WKY (data not shown). As shown in Fig. 1, control SHRSP (DHA 0%) showed a significantly inferior memory in passive avoidance test. The overall difference evaluated by the repeated-measure ANOVA was statistically significant between the control SHRSP (DHA 0%) and WKY (DHA 9%). At 24 h after the acquisition trial, the response latency of the control SHRSP (DHA 0%) was 413.1 ± 83.0, which was significantly shorter than that in WKY (DHA 0%) (595.3 ± 4.8 s, $n = 8$, $p < 0.05$). The shortened response latency continued during the successive retention trials for 7 days in the control SHRSP (DHA 0%). Dietary DHA ameliorated the performance failure of SHRSP. The performance of DHA-treated

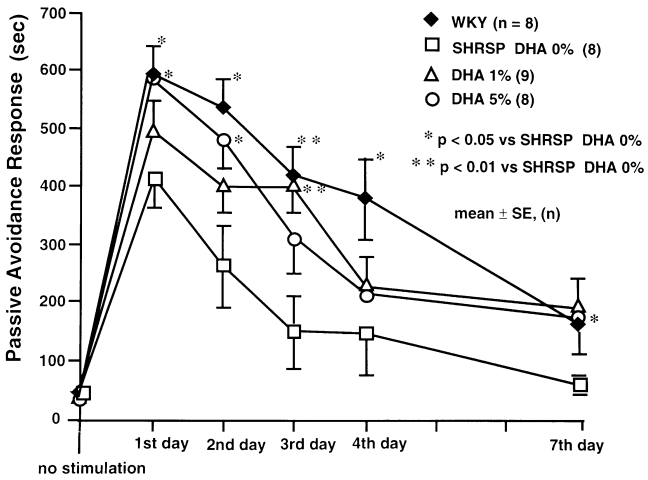

FIG. 1. Response latency in the passive avoidance task in SHRSP and WKY. Rats were subjected to an one-way step-through type of passive avoidance apparatus. The retention trials were carried out 1, 2, 3, 4, and 7 days after the acquisition trials (75 v, 3 s) and the response latency was measured up to a maximum of 600 s.

TABLE 4

EFFECTS OF DOCOSAHEXAENOIC ACID ON THE CEREBRAL LEVELS OF CHOLINE ACETYLCHOLINE IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

	SHRSP (DHA 0%) (n = 8)	SHRSP (DHA 1%) (n = 6)	SHRSP (DHA 5%) (n = 5)	WKY (DHA 0%) (n = 8)
Cortex				
Ch (pmol/mg protein)	164.92 ± 1.31	219.85 ± 15.5*	187.78 ± 10.2	236.37 ± 27.1*
ACh (pmol/mg protein)	185.39 ± 15.0	254.68 ± 17.2*	255.50 ± 22.0*	233.10 ± 14.6*
Hippocampus				
Ch (pmol/mg protein)	165.98 ± 14.8	209.80 ± 13.7	224.56 ± 21.9*	283.54 ± 18.1†
ACh (pmol/mg protein)	163.42 ± 23.8	249.03 ± 8.2	262.05 ± 18.5*	258.29 ± 16.9*

Means ± SEM.
*$p < 0.01$, †$p < 0.001$ vs. SHRSP (DHA 0%). DHA = docosahexaenoic acid, Ch = choline, ACh = acetylcholine.

SHRSP was superior to that of control SHRSP (DHA 0%). The response latency of SHRSP measured before acquisition trials (during the nonstimulated period) was almost the same (44.8 ± 7.9 s, n = 8) as that in sex- and age-matched WKY (39.6 ± 5.8 s, n = 8), indicated that the difference in response latencies in passive avoidance performance is not due to the difference in behavioral activities.

The ACh and Ch Contents in Brain Regions

As shown in Table 4, Ch and ACh levels of SHRSP (DHA 0%) in the cortex and hippocampus were significantly lower than those of WKY (DHA 0%). The cortex Ch and ACh levels in SHRSP showed either statistically significant increase or a tendency to increase with dietary DHA treatment. In SHRSP, hippocampal Ch and ACh levels also increased significantly with DHA treatment, whereas the Ch and ACh levels of the medulla oblongata were unchanged (data not shown).

The Correlation Between the Cerebral ACh Levels and Passive Avoidance Performance

As shown in Fig. 2, the hippocampal ACh level positively correlated with the total response latency for days 1, 2, 3, 4, and 7 (r = 0.753, n = 27, $p < 0.01$). No statistically significant correlation was observed between the response latency and Ch levels in any regions examined nor in ACh levels of the cortex.

DISCUSSION

DHA supplementation decreased systolic blood pressure, which was associated with decreased plasma and brain AA and increased plasma and brain DHA in SHRSP. The significantly inferior learning performance in control SHRSP compared with WKY was also significantly ameliorated by dietary DHA. Concomitantly, DHA increased the cerebral Ch and ACh levels in SHRSP.

It has become apparent that a deficiency in DHA is associated with a loss of discriminative learning ability (15,35,53). Patients with Alzheimer's disease (44) show extremely low levels of DHA in their brains. The activity of dietary DHA to restore the decreased learning ability of rats was reported by Fujimoto et al. (15). Yokota (57) reported that both the DHA level in brain and learning ability decreased in rats that were deficient in n-3 polyunsaturated fatty acids. Decreased n-3 fatty acids in diets were shown to accelerate the aging process by decreasing longevity, discrimination-learning ability and memory in aged rats (54).

It is well known that activation of the *N*-methyl-D-asparate (NMDA) receptor an absolute requirement for the induction of long-term potentiation (LTP) in the hippocampal region and neocortex (3,8,20,36). Nishikawa et al. (36) reported that DHA potentiated the NMDA-induced response under voltage-clamp conditions in the rat, raising the possibility that DHA may play a role in LTP, at least in the process involving the activation of NMDA receptors. Astrocytes release DHA and AA into the extracellular fluid (31), which may potentiate the NMDA response (36), although we have no evidence to indicate that free DHA is formed in vivo in amounts large enough to stimulate the receptors affecting the synaptic transmission.

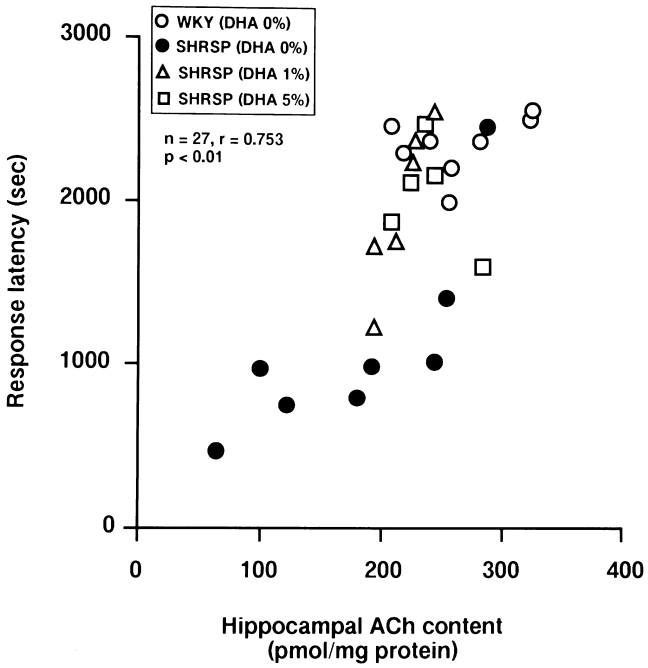

FIG. 2. Correlation between hippocampal Ach levels and total response latency in passive avoidance task in DHA-treated SHRSP (DHA 0, 1, 5%) and control WKY (DHA 0%).

Our present findings appear to confirm those presented by Togashi et al. (47), that the central cholinergic dysfunctions characterize the pathophysiology of SHRSP. Brain cholinergic systems are generally thought to be critical for memory function and disturbance of the central cholinergic system has been shown in patients with vascular dementia as well as in those with senile dementia of the Alzheimer's type (7,9, 16,52). In this study, we demonstrated that dietary DHA improved increasing cerebral Ch and ACh levels along with the performance impairment of passive avoidance task in SHRSP. Improved hemodynamic properties induced by DHA would also be favorable for the maintenance of brain functions (19). It remains to be clarified whether the change in the central ACh levels are a direct consequence of DHA in the diet or whether it is an indirect effect mediated by diet-induced alteration of the blood pressure. It is possible that neurotransmitters are involved in the observed effects of DHA on learning ability, although no definitive data have been reported.

The beneficial effects of DHA and n-3 fatty acids on hypertension, cerebral bleeding, or cerebral infarction appear to be associated mainly with decreased AA and its metabolites. DHA supplementation resulted in a marked decrease in the plasma AA levels of SHRSP: a 50% decrease with 1% DHA supplementation and a 90% decrease with 5% DHA. A decrease in the AA levels in tissue phospholipids is known to decrease the vasoconstrictor thromboxane A_2 (TXA_2) to vasodilator prostaglandin I_2 (PGI_2) ratio (1,14,26). DHA may inhibit TXA_2 production via the cyclooxygenase pathway (18). The existence of an enzyme system which converts DHA to EPA has been reported (34). Numerous studies show that dietary (n-3) fatty acids modified the tissue lipid composition and changed the prostanoid synthesis (6,23,37,43,56). The incorporation of AA into phospholipids (33) and the formation of eicosanoids from AA are competitively inhibited by (n-3) fatty acids (23,25,50). EPA is a relatively poor substrate for cyclooxygenases and the vasoconstrictive activity of TXA_3 derived from EPA is much less than that of TXA_2 from AA (13). Thus, dietary n-3 fatty acids may ameliorate eicosanoid-mediated diseases such as atherosclerosis and inflammatory diseases by reducing the tissue AA content and by inhibiting eicosanoid synthesis (24).

Long-term feedings of DHA suppressed behavioral failure and renal dysfunction, leading to a prolongation of the life span of SHRSP (22), which is probably caused by the suppression by n-3 fatty acids of the development of hypertension, renal function, cerebral bleeding, or cerebral infarction as well as on brain functions. Functional abnormalities in cell membranes might originate from a change in the lipid structure of the membrane as suggested by Bing et al. (4).

Alternatively, it is likely that dietary DHA prevented the developed cerebral lesions caused by prolonged hypertension in SHRSP and resulted in restoring the cerebral ACh and Ch levels and behavioral performance in passive avoidance task.

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