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Effect of long-term administration of arachidonic acid on n-3 fatty acid deficient mice

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ABSTRACT

The effect of long-term oral administration of arachidonic acid (ARA, 240 mg/kg/day) on brain function was assessed for mice maintained on an n-3 fatty acid adequate or deficient diet. The administration of ARA for 13 weeks resulted in an elevation of spontaneous motor activity, or the tendency thereof, in both the n-3 fatty acid adequate and deficient groups. However, the n-3 fatty acid deficient mice that were administered with ARA revealed marked deterioration in motor function in a motor coordination test. In the experiment to investigate changes over time, the motor activity of the ARA-administered group continued to increase mildly in n-3 deficient mice, although that of the control group showed a decrease involving habituation for both diet groups from the second week. The fatty acid composition of the brain at the end of the behavioral experiments indicated an increase in the levels of ARA and other n-6 fatty acids, as well as a decrease in the levels of docosahexaenoic acid. These results suggest that long-term administration of ARA causes an increase of futile spontaneous motor activity and the diminution of motor function by aggravation of n-3 fatty acid deficiency.

1. Introduction

About 60% of the brain (dry weight) is composed of lipids, of which phospholipids are primarily represented. Docosahexaenoic acid (22:6n-3, DHA) and arachidonic acid (20:4n-6, ARA) are the main polyunsaturated fatty acid components (PUFAs) of the phospholipids in the brain, and the correct balance of these fatty acids is crucial for maintaining brain function. The most represented n-6 fatty acid in the brain is ARA, which accounts for around 10% of the total fatty acid composition. The amount of DHA as n-3 fatty acid is around 15% of the total fatty acid in the brain [1]. Several studies have reported that n-3 fatty acid deficiency causes poor cognitive performance and abnormal emotional behavior, linked to the decline of brain DHA, including symptoms such as cognitive dysfunction, aggressive and anxiety [2–8]. Research into the brain function of adult animals, demonstrated that old-aged rats and Alzheimer's disease model rats recovered spatial cognitive learning ability by the administration of DHA, and increased the ratio of DHA/ARA. The levels of lipid peroxide and oxygen species were also found to have decreased in the brain [9,10].

On the other hand, ARA is also an important fatty acid in the development of the fetus and in early childhood. The plasma ARA level in preterm infants was positively correlated with not only the development of brain but also with increase of body weight or height [11]. However, there has been little research of the effects of chronic supplementation with ARA on brain function of adult or aged animals [12,13]. Therefore, the details of period and amount required for administration of ARA are still not clear. Recently excessive intake of ARA in adults has led to concerns of negative effects on health such as accelerated allergies, cardiovascular disease and cancer promotion [14–17].

In this study, we assessed the effects and behavioral changes over time of long-term administration of ARA on motor activity and motor coordination in mice, which raised on either an n-3 fatty acid deficient (n-3 Def) diet or an n-3 fatty acid adequate (n-3 Adq) diet.

2. Materials and methods

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Azabu University.

Abbreviations: ARA, arachidonic acid, 20:4n-6; DHA, docosahexaenoic acid, 22:6n-3; n-3 Def, n-3 fatty acid deficiency; n-3 Adq, n-3 fatty acid adequacy

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2.1. Animals and experimental diets

Two types of diets based on the AIN-93G diet containing 5% lipids (Oriental Yeast Co., Ltd., Tokyo, Japan) were prepared: the n-3 deficient (Def) diet (14.6% linoleic acid; 0.3% α-linolenic acid) and the n-3 adequate (Adq) diet (14.6% linoleic acid; 2.5% α-linolenic acid, Table 1) [18]. Male offspring of female CD-1 mice, which were reared from 3 weeks old (Charles River Japan, Inc. Yokohama, Japan) and raised on either type of diet, were used in this study. To diminish maternal effects, the experimental groups comprised individuals from different litters.

2.2. Study design

Animals were housed in an artificially regulated environment at 23 ± 3 °C, 55 ± 10% humidity, under a 12-h light/dark cycle (lights on between 07:00 and 19:00). Animals aged 12 or 4 months, reared under n-3 Def or n-3 Adq conditions, were subjected to daily oral administration of ARA oil (Lot. 10050701, CABIO Bioengineering, Wuhan, Hubei, China). The ARA dosage was set at 240 mg/kg/day. Control groups were given mixed oil as the control oil (2:1:1 of lard: soy oil: rapeseed oil) to equalize to the amount of fatty acids of ARA oil. This study consisted of two experiments. In the first experiment, mice were subjected to spontaneous motor activity and motor coordination tests after ARA oil administration for 13 weeks. In the following experiment, 4-month-old mice were administered ARA oil for 5 weeks in order to observe behavioral changes over time. Motor activity of these animals was measured every day for an initial week and once a week after that. Whole brains were collected at the end of each experiment and analyzed for fatty acid composition.

2.3. Behavioral experiments

2.3.1. Motor activity test

Motor activity was measured using cages ($19 \times 30 \times 13$ cm^3) equipped with wireless running wheels (Wireless Low Profit Running Wheel, ENV-044 wheel and SOF-860 software, Neuro-science Co., Ltd., Tokyo, Japan). Mice were assessed individually by recording the number of wheel rotations over 30 min at the same time of day (08:00–11:00).

2.3.2. Motor coordination test (Rota-Rod test)

The motor coordination test was carried out using a Rota-Rod setup (Rota-Rod Treadmill, ENV-575M, Neuro-science Co., Ltd., Tokyo, Japan). The mice were allowed a trial run of 5 min on the rotor set at 20 rpm on the day preceding the measurement. On the following day, the motor coordination abilities of the mice were assessed on the rotor, with the rotational speed set to increase to 40 rpm from 4 rpm over 5 min. The duration for which the mice could walk on the rotor, up to a limit of 300 s, was recorded [19].

2.4. Fatty acid analysis

2.4.1. Lipid extraction and methylation

A half brain (approximately 250 mg) was homogenized with 6 ml methanol:hexane (4:1) solvent containing 50 μg/ml butylhydroxytoluene and an additional 60 μg docosatrienoic acid methyl ester (22:3n-3 methyl ester) as an internal standard. After homogenizing, 200 μl acetylchloride was added to each 2 ml homogenate and the air in the sample tubes was replaced by nitrogen. Samples were then mixed and heated for 1 h at 100 °C for lipid extraction and methylation. Sample tubes were then cooled rapidly, shaken with 5 ml 6% potassium carbonate solution, and centrifuged at 2200*g* at 4 °C for 15 min. The hexane layer (supernatant) was transferred to a microvial for gas chromatography. The percentage of each fatty acid was calculated from the results of gas chromatography [20,21]. Fatty acid analysis was also carried out for the oils given to the mice (Table 2). The final quantity given to the mice was determined from the total fatty acid and ARA contents calculated using the internal standard (22:3n-3 methyl ester).

2.4.2. Gas chromatography

Fatty acid methyl esters were analyzed on an Agilent 7890A gas chromatograph (Agilent Technologies Ltd., California, USA) equipped with a split injector, an Agilent 7693 ALS automatic liquid sampler, and detected using a flame ionization detector (FID). The instrument was controlled and the data were collected by using the Agilent ChemStation software (Rev. B.04.01.SP1, Agilent Technologies Ltd., California, USA). The GC column was a DB-FFAP 15 m × 0.10 mm i.d. with 0.10-μm film thickness (J&W Scientific from Agilent Technologies Ltd., California, USA). The detector and the injector temperature were set at 250 °C. The oven temperature program initiated at 150 °C with a 0.25-min hold, and then ramped at 35 °C/min to

Table 1
Composition of experimental diets.

	Amount (g/100 g diet)	
	n-3 Def.	n-3 Adq.
Casein, vitamin free	20	20
Carbohydrate:	65	65
Cornstarch	25	25
Sucrose	10	10
Glucose	20	20
Dextrose	5	5
Maltose	5	5
Cellulose	5	5
Mineral–salt mix	3.5	3.5
Vitamin mix	1	1
L-Cystine	0.3	0.3
Choline bitartrate	0.25	0.25
TBHQ	0.002	0.002
Fat	5	5
Hydrogenated coconut oil	4.05	3.88
Safflower oil	0.95	0.89
Flaxseed oil	None	0.24
Fatty acid composition (% of total fatty acids)		
Saturates	78.1	75.1
Monounsaturates	4.7	5.4
18:2n-6	14.6	14.6
18:3n-3	0.25	2.5
n-6/n-3	58.5	5.8

The two experimental diets, an n-3 fatty acid dequate diet (n-3 Adq) and an n-3 fatty acid deficient diet (n-3 Def), were based on the AIN-93 formulation with several modifications to obtain the extremely low basal level of n-3 fatty acid required in this study.

Table 2
Fatty acid composition of evaluated oils (% of total fatty acids).

Fatty acids	Control oil	ARA oil
Total Sat.	**26.47 ± 0.04**	**26.89 ± 0.003**
Total Mono.	**44.79 ± 0.08**	**6.46 ± 0.01**
18:2n-6	22.26 ± 0.03	9.38 ± 0.01
18:3n-6	0.03 ± 0.01	2.40 ± 0.004
20:2n-6	0.20 ± 0.01	0.47 ± 0.002
20:3n-6	ND	3.85 ± 0.01
20:4n-6	0.05 ± 0.002	45.11 ± 0.04
Total n-6 FAs	**22.54 ± 0.01**	**61.53 ± 0.05**
18:3n-3	3.84 ± 0.01	0.06 ± 0.003
20:5n-3	0.10 ± 0.001	0.52 ± 0.002
Total n-3 FAs	**3.94 ± 0.01**	**0.58 ± 0.004**
ARA conc. (mg/100 μl)	0.043 ± 0.002	37.5 ± 0.2
Fatty acid conc. (mg/100 μl)	82.6 ± 0.2	83.2 ± 0.4

Mean ± SEM, ND: not detected.

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