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Pure docosahexaenoic acid can improve depression behaviors and affect HPA axis in mice

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Abstract. – OBJECTIVE: Aim of this study was to identify whether docosahexaenoic acid (DHA) has a vital antidepressant by hypothalamic-pituitary-adrenal axis (HPA axis) or not.

METHODS: Mice were divided into 2 groups: control group and DHA dietary group. DHA dietary group was treated with DHA dietary everyday for consecutive 50 days. The forced swimming test and tail suspension test were conducted. Hypothalamic and erythrocyte fatty acids and monoamine neurotransmitters levels in hypothalamus were assayed; corticosterone, adrenocorticotropic hormone and corticotropin-releasing factor in serum, hypothalamus and pituitary were assayed, respectively.

RESULTS: (1) In the forced swimming test, DHA dietary significantly decreased immobility time, whereas swimming time and climbing time were increased. In tail suspension test DHA dietary significantly shortened immobility time. (2) DHA dietary increased the ration of n-3/n-6 (polyunsaturated fatty acids) and DHA level in hypothalamic and erythrocyte fatty acids; (3) DHA dietary significantly increased 5-hydroxytryptamine, 5-hydroxyindoleacetic acid and dopamine levels in hypothalamus; (4) DHA dietary significantly decreased serum corticosterone level by 20.23% and serum adrenocorticotropic hormone level by 25.13%; significantly increased serum corticotropin releasing factor level by 21.92%. Besides, DHA dietary decreased arginine vasopressin level by 20.11% in hypothalamus, by 23.76% in pituitary tissues, respectively; (5) DHA dietary decreased corticotropin-releasing factor levels by 30.83% in hypothalamus, by 29.75% in pituitary tissues, respectively. In hypothalamus, DHA dietary decreased significantly adrenocorticotropic hormone level by 19.14%, but insignificantly decreased adrenocorticotropic hormone level in pituitary.

CONCLUSIONS: DHA shows an antidepressant property. Moreover, DHA has multiple effects on depression including the monoamine neurotransmitter systems, red blood cell membranes and HPA axis.

Key Words:
Docosahexaenoic acid, Depression, Monoamine neurotransmitter, Hypothalamic-pituitary-adrenal axis, Fatty acids.

Introduction

Depression, as a widespread incapacitating psychiatric disease, imposes a substantial health burden on society. The mechanism of depression is complex. Monoamine neurotransmitters such as 5-hydroxytryptamine, noradrenaline and dopamine in the central nervous system play a key role in depression[1]. Clinical and experiment evidences suggest that alterations of monoamine neurotransmitters are associated with several therapeutic mechanisms underlying potential favorable activity of the antidepressant drugs[2,3]. Additionally, the endocrine stress response in mammals is mediated by the hypothalamic-pituitary-adrenal (HPA) axis which is involved in depression[4,5]. HPA axis activity is governed by the secretion of adrenocorticotrophic hormone-releasing factor and vasopressin from the hypothalamus, which in turn activate the secretion of adrenocorticotrophic hormone from the pituitary, which finally stimulates the adrenal cortex secretion of the glucocorticoids (cortisol in humans and corticosterone in rodents). Glucocorticoids (corticosterone in rodents), then, interact with their receptors in multiple target tissues including the HPA axis, where they are responsible for feedback inhibition both on adrenocorticotrophic hormone-releasing factor and vasopressin from the hypothalamus and directly on secretion of adrenocorticotrophic hormone-releasing factor from pituitary corticotropes[6]. It has been reported that therapeutic effects of antidepressants were by means of acting on the HPA axis in depressive patients[7]. Thus, the restoration of a normal functional status of HPA axis may be critically involved in the therapeutic intervention of depression.

N-3 polyunsaturated fatty acids (PUFAs) supplementation may be beneficial to depression. Human studies have indicated that N-3 PUFAs may act as a defense against the onset of depres-

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sion[8]. Docosahexaenoic acid (DHA, 22:6n-3), the major PUFA in the neuronal membrane, is involved in the regulation of various biological functions, and its dietary administration has beneficial effects on the central nervous system[9]. Previous studies have showed that mildly depressed subjects had significantly lower levels of DHA in adipose tissue samples when compared with non-depressed subjects. Whereas, low levels of the DHA can predict low levels of cerebrospinal fluid 5-hydroxyindolacetic acid, the major metabolite of serotonin, which is known to be protective against the depression[10].

Judged from above statement, it seems that DHA has a certain effect on depression. However, despite encouraging evidence for the antidepressant efficacy of pure eicosapentaenoic acid and eicosapentaenoic acid-DHA mixtures[11,12]; it is still unclear whether pure DHA has an effective antidepressant or not. To the best of our knowledge, few studies investigated the effect of pure DHA on depression. Therefore, we performed this experiment to study the effect of pure DHA on depression.

We hypothesize that DHA as antidepressant may play an important role in HPA axis. To further assess the potential role of reduced levels of brain DHA in depression, we examine that DHA enriched diet impacts several neurobiological systems related depression. HPA axis was focused on our experiment. The forced swimming test and tail suspension test were performed on a rodent model for assessment of antidepressant efficacy. This research is useful for understanding the preventive and therapeutic significance of pure DHA on depression, which is a hot and novel view of the nutrition field.

Materials and Methods

Animals Treatment

Male Kunming mice (24 ± 2 g), aged 3 months, were purchased from Animal Experimental Center of Guangxi (Nanning, China). The mice were housed in a controlled environment with temperature maintained at 23 ± 2°C and humidity at 42 ± 2% under a 12:12 h light/dark cycle, with free access to water and food. The control components of diet were based on the American Institute of Nutrition-76 (AIN-76) and with minor modifications[13].

After a week of adaptation, mice were divided into two experiment groups as follows: control group (n=10) and DHA dietary group (n=10). DHA from cod liver oil was purchased from Sigma-Aldrich (Sigma Chemical Co., St. Louis, MO, USA), and was mixed into diet. Control group received normal laboratory food with added saturated fatty acids (soybean oil) to ensure isocaloric intake, as previously reported[14]. DHA sufficient diet was prepared for three or four days and daily allowances were stored in airtight containers at –80°C. The composition of control diet and DHA diets was showed in Table I, and the comparison of fatty acids in diet was showed in Table II. There were no any differences in animal body weight between control group and DHA dietary group. The animals were treated according to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals and the Animal Ethics Committee of the Guangxi University.

Behavioral Experiments

Forced Swimming Test

The forced swimming test was performed 2 times. One was performed for ruling out an effect

Table I. Composition of control and DHA diets.

Fatty acids	Control diet (g/kg)	DHA diet (g/kg)
Casein	200	200
L-Cystine	12	12
Corn starch	442	442
Maltodextrin	24	24
Sucrose	200	200
Cellulose	50	50
Mineral mix	35	35
Vitamin mix	12	12
Choline bitartrate	2	2
Cholesterol	30	30
TBHQ (antioxidant)	0.02	0.02
Soybean oil	70	–
DHA	–	70

Note: TBHQ: 2,5-di-(*t*-butyl)-1,4-benzohydroquinone. The salt mixture contained the following (mg/g): calcium phosphate diabasic, 500; sodium chloride, 74; potassium sulfate, 52; potassium citrate monohydrate, 20; magnesium oxide, 24; manganese carbonate, 3.5; ferric citrate, 6; zinc carbonate, 1.6; cupric carbonate, 0.3; potassium iodate, 0.01; sodium selenite, 0.01 and chromium potassium sulfate, 0.55. The vitamin mixture contained the following (mg/g): thiamin hydrochloride, 0.6; riboflavin, 0.6; pyridoxine hydrochloride, 0.7; nicotinic acid, 3; calcium pantothenate, 1.6; D-biotin, 0.05; cyanocobalamin, 0.001; retinyl palmitate, 1.6; DL-α-tocopherol acetate, 20; cholecalciferol, 0.25 and menaquinone, 0.005.

Table II. The comparison of fatty acids in diet (total fatty acids is about 9.4% in diet).

Fatty acids	Control diet (%)	DHA diet (%)
SFAs	**17.1**	**12.1**
C16:0	9.5	8.4
C18:0	5.2	2.5
C20:0	2.4	1.2
MUFAs	**49.3**	**44.9**
C18:1n-9	37.6	40.1
C18:1n-7	5.8	2.5
C20:1n-9	5.9	2.3
Total n – 3 PUFAs	**17.9**	**26.5**
C18:3	15.6	6.9
C20:5	2.3	1.2
C22:6	-	18.4
Total n – 6 PUFAs	**14.7**	**18.7**
C18:2	14.7	18.7
C20:4	–	–

Note: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. The unit was expressed as percentage.

of muscular capacity of the animal; another was performed after DHA dietary consecutive 50 days.

The forced swimming test employed was similar to that described elsewhere[15]. Briefly, mice had a swimming-stress session for 15 min pretest, 24 h before being individually placed in glass cylinders (height: 25 cm; diameter: 10 cm; containing 10 cm of water temperature at 24 ± 1°C) for 5 min test. One of the following behaviors: (1) immobility, defined as a state in which a mouse ceased struggling and remained floating motionless in the water, making only small movements necessary to keep its head above water; (2) swimming, active motions made by animals that result in movements within the pool (i.e. moving around the jar and diving); (3) climbing, defined as strong movements executed with forepaws in and out of the water usually against the walls[16,17]. All animals were forced to swim for 5 min, and the duration of immobility was observed and measured during the final 4 min interval of the 5-min testing period.

Additional Experiment

Given hormones are so sensitive and the mice behaviors are difference performances in two groups, we conducted an additional experiment to rule out the effect of behavior experiments on hormones changes. The experimental protocol was the same as above but the mice were decapitated before the behavioral experiments. Therefore, mice weren't conducted behavior experiments. We assayed hormones in the serum.

Tail Suspension Test

The tail suspension test, a model developed to assess antidepressant activity of drugs, was carried out according to a modified procedure, previously described[18]. Mice were suspended on a horizontal bar by the tail by using adhesive scotch tape for a duration of total 6 minutes. Mice were not considered immobile unless they hung passively and completely motionless.

Hypothalamus and Erythrocyte Lipids Assay

Measurement of Hypothalamus Lipid

At the end of behavioral test, the mice were decapitated in 1 hours, the hypothalamus were removed and weighted, and then frozen at –80°C for subsequent biochemical analysis. Hypothalamus were homogenized and extracted with distilled water and chloroform/methanol (2:1, v/v) which contained 0.02% butylated hydroxytoluene (w/v) by using a method modified from Bligh and Dyer[19]. After a centrifuged procedure at 1500 g for 10 min, the substratum was transferred to the test tube for vacuum drying. Dried crude lipids were weighted before proceeding to the phospholipids separation. The crude lipids were then dissolved in 200 l of chloroform and applied into an Amino disposable extraction column (Bakerbond spe™ Amino, Baker, Phillipsburg, NJ, USA) for phospholipids separation[20].

The fatty acid in individual sample of the hypothalamus was analyzed by gas chromatography. The detailed step-by-step procedures were applied according to the laboratory practice manual, as briefly described below. Extracted phospholipids were placed into 16 × 150 mm test tubes with Teflon-lined screw caps, and dissolved with 1 mL of 14% boron trifluoride methanol (BF3-methanol, Sigma), for fatty acid methylation reaction. Fatty acid methyl esters were analyzed by capillary gas chromatography (Shimadzu Scientific Instruments Inc., Columbia MD, USA). The column was a DB-23 (123-2332): 30 m length, I.D. 0.32 mm wide bore, film thickness of 0.25 M.

The gas chromatography conditions include: column temperature ramping by holding at 120°C

for 1 min followed by an increase of 5°C/min from 120 to 240°C. The temperature of the injector and flame ionization detector was 250°C. A split (8:1) injection mode was used. The carrier gas was helium with a column flow rate of 2.5 ml/min. Fatty acid identification was determined using retention times of authenticated fatty acid methyl ester standards (Matreya LLC Inc., Pleasant Gap, PA, USA). Analysis of fatty acid methyl esters is based on areas calculated with Shimadzu Class VP 4.3 software (Shimadzu Scientific Instruments Inc., Columbia, MD, USA). Fatty acid profiles were identified according to the retention time of appropriate standard fatty acid methyl esters. Researchers who participated in the laboratory were blind to the information of coded samples.

Measurement of Erythrocyte Lipid

Whole blood sample was centrifuged at 4000 g for 10 min in order to separate into red blood cells. Total fatty acid composition was determined by using the saponification and methylation methods originally described by Metcalfe et al[21]. Erythrocyte samples were placed in a 20 ml glass vial into which 4 ml of 0.5 N methanol sodium hydroxide was added, and the sample heated at 80°C for 5 min. Following a 10 min cooling period, 3 ml of boron trifluoride in methanol was added to methylate the sample. After an additional 5 min of heating in the water bath (80°C), the sample vial was allowed to cool, and 2 ml of a saturated solution (6.2 M) of sodium chloride and 10 ml of hexane was added. The samples were then mixed by vortex for 1 min. The hexane fraction was then transferred into a 20 ml vial containing 10 mg of sodium sulfate to dry the sample. The hexane solution was then removed for gas chromatography analysis. The conditions were the same as above statement.

Measurement of the Levels of 5-hydroxytryptamine, 5-hydroxyindoleacetic Acid, Noradrenalin and Dopamine in Hypothalamus

5-hydroxytryptamine, 5-hydroxyindoleacetic acid, noradrenaline and dopamine levels in hypothalamus were measured as described previously[23], using high-performance liquid chromatography with electrochemical detection, with minor modifications. Briefly, each frozen tissue sample was homogenized by ultrasonication in 200 µl of 0.4 M perchloric acid (solution A). The homogenate was kept on ice for 1 h and then centrifuged at 12,000 g (4°C) for 20 min, and the pellet was discarded. An aliquot of 160 µl of supernatant was added to 80 l of solution B (containing 0.2 M potassium citrate, 0.3 M di-potassium hydrogen phosphate and 0.2 M EDTA). The mixture was kept on ice for 1 h and then centrifuged at 12,000 g (4°C) for 20 min again. 20 l of the resultant supernatant was directly injected into an ESA liquid chromatography system equipped with a reversed-phase C_{18} column (150 × 4.6 mm I.D., 5 µm) and an electrochemical detector (ESA CoulArray, Chelmstord, MA, USA). The detector potential was set at 50, 100, 200, 300, 400, 500 mV, respectively. The mobile phase consisted of 125 mM citric acid-sodium citrate (pH 4.3), 0.1 mM EDTA, 1.2 mM sodium octanesulfonate and 16% methanol, the flow rate was 1.0 ml/min. Unit was expressed in terms of ng/g of tissue.

Measurement of Hormones

Measurement of Hormones in Serum

Following behavioral testing, blood samples were collected from the jugular vein and transferred to ice-chilled tubes containing EDTA and plain serum. Plasma and serum were separated by centrifugation at 4°C for 15 min and stored at −80°C until assayed.

The level of serum corticosterone was determined by using an enzyme immunoassay (magnetic solid phase) kits (Beijing Bio-Ekon Biotechnology Company Limited, Beijing, PR China). The variation coefficients of intra-assay and inter-assay were less than 7.1% and 10.3%, respectively.

The level of serum adrenocorticotropic hormone was measured in duplicate by immunoradiometric assay (Allegro HS-adrenocorticotropic hormone; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The variation coefficients ranged from 6.9 to 8.9% in intra-assay and from 1.1 to 3.0% in inter-assay.

The level of serum corticotrophin releasing factor was measured by utilizing a commercially available radio-immunoassay kit (Technique Center of Radioimmunity of Navy in Beijing, PR China). The sensitivity of the assay was 0.2 ng/ml. The variation coefficients of both intra-assay and inter-assay were less than 8%.

Measurement of Hormones in Hypothalamus and Pituitary

The hypothalamus and pituitary were removed and weighed, and then were frozen at −80°C for subsequent biochemical analysis.

They were instantaneously placed in liquid nitrogen and stored at −80°C until biochemical measurements, when they were homogenized in 10 volumes of ice-cold phosphate buffer (0.1 M, pH 7.4) containing 140 mM KCl and 1 Mm EDTA.

The method for measurement of adrenocorticotropic hormone and corticotrophin releasing factor is the same as in serum.

Measurement of arginine vasopressin contents in hypothalamus and pituitary: the concentration of arginine vaspressin was determined by specific rabbit anti-rat arginine vasopressin serum[33]. The effective dilution of the antiserum was 1:60,000. The peptide was labeled 125 Iodine (Amersham Pharmacia, Buckinghamshire, UK) using the chloramines-T method and iodinated peptide was purified by Sephadex G-50. The sensitivity of the assay for the arginine vasopressin was 1.0 pg/tube with variation coefficients of intra-assay and inter-assay being less than 3.6% and 6.0%, respectively[24].

Statistical Analysis

Data are expressed as means ± standard error of the mean (SEM). The data were analyzed by one-way ANOVA using SPSS program, version 10.0 (SPSS Inc., Chicago, IL, USA). Significance was set at $^{a}p < 0.05$ and $^{aa}p < 0.01$ v control group.

Results

The Effects of DHA on Mice Behavioral Activities In Forced Swimming Test and Tail Suspension Test

As shown in Table III, the result showed DHA dietary can significantly reduce immobility time in the forced swimming test, in the tail suspension test, DHA dietary can remarkably decrease immobility time, when compared with control group, respectively.

The Effects of DHA on Fatty Acids of Hypothalamus and Erythrocyte

Fatty acid changes in hypothalamus were illustrated in Table IV. The level of DHA was significantly increased in DHA dietary group. It is apparent that the percentage of total n-3 polyunsaturated fatty acids was increased, whereas the percentage of total n-6 polyunsaturated fatty acids was decreased in DHA dietary group, when compared with control group. DHA dietary group increased the ration of n-3/n-6 (PUFAs) from 1.12 times in control group to 1.31 times in DHA dietary group.

Table III. The effect of DHA on the forced swimming test and tail suspension test.

Group	Control group	DHA dietary group
Forced swimming test		
Immobility time (s)	75.26 ± 7.18	45.48 ± 4.29^{aa}
Swimming time (s)	133.29 ± 10.58	146.98 ± 8.56^{aa}
Climbing time (s)	93.59 ± 9.98	105.04 ± 8.73^{aa}
Tail suspension test		
Immobility time (s)	167.59 ± 7.68	108.26 ± 8.69^{aa}

Note: The unit was expressed in terms of second (s). The data are presented as the mean ± SEM n = 6. Significantly different (for $^{a}p < 0.05$, $^{aa}p < 0.01$), compared with the control group.

Fatty acid changes in erythrocyte were summarized in Table V. The erythrocyte DHA level in the DHA fatty acid group was distinctly higher than that in the control group. Additionally, we

Table IV. The effect of DHA on the level of fatty acid in hypothalamus tissue.

Group	Control group	DHA dietary group
SFAs	**42.53 ± 4.02**	**42.03 ± 4.19**
C14:0	1.40 ± 0.13	2.53 ± 0.32^{aa}
C16:0	18.79 ± 1.42	19.24 ± 1.67
C18:0	22.34 ± 2.17	20.26 ± 1.94
MUFAs	**24.48 ± 2.59**	**23.47 ± 3.26**
C16:1	2.73 ± 0.21	2.76 ± 0.18
C18:1	20.81 ± 1.49	19.94 ± 2.16
C21:1	0.94 ± 0.13	0.77 ± 0.17^{aa}
Total n – 3 PUFAs	**15.91 ± 1.64**	**18.76 ± 2.64^{aa}**
C18:3	0.43 ± 0.12	0.40 ± 0.13
C20:5	1.44 ± 0.15	1.57 ± 0.25
C22:5	1.08 ± 0.12	1.48 ± 0.18^{aa}
C22:6	12.96 ± 2.47	15.31 ± 1.73^{aa}
Total n – 6 PUFAs	**15.44 ± 1.86**	**14.32 ± 1.75**
C18:2	1.21 ± 0.11	1.28 ± 0.13
C20:4	11.56 ± 1.44	9.64 ± 1.69^{aa}
C22:4	1.69 ± 0.16	2.84 ± 0.21
C22:5	0.98 ± 0.23	0.56 ± 0.11^{a}
UK	1.57 ± 0.14	1.42 ± 0.16
∑n-3/∑n-6	1.03 ± 0.19	1.31 ± 0.12^{aa}
DHA/AA	1.12 ± 0.14	1.31 ± 0.13^{aa}

Note: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. UK: refer to do not know what specific substances. The unit was expressed as percentage. The data are presented as the mean ± SEM n = 6. Significantly different (for $^{a}p < 0.05$, $^{aa}p < 0.01$), compared with the control group.

Table V. The effect of DHA on the level of fatty acid in erythrocyte.

Group	Control group	DHA dietary group
SFAs	**42.79 ± 4.27**	**43.89 ± 4.07**
C14:00	2.52 ± 0.19	2.75 ± 0.34
C16:00	28.09 ± 2.74	27.69 ± 3.26
C18:00	12.18 ± 1.29	13.45 ± 1.55
MUFAs	16.96 ± 2.32	16.27 ± 2.47
C16:1	1.46 ± 0.16	1.81 ± 0.22[a]
C18:1	13.47 ± 1.63	12.57 ± 1.78
C21:1	2.03 ± 0.15	1.89 ± 0.21[a]
Total n – 3 PUFAs	**6.19 ± 0.53**	**9.69 ± 1.73[aa]**
C18:3	0.21 ± 0.11	0.68 ± 0.15[aa]
C20:5	0.55 ± 0.13	1.64 ± 0.14[aa]
C22:5	1.90 ± 0.27	1.89 ± 0.29
C22:6	3.53 ± 0.24	5.48 ± 0.54[aa]
Total n – 6 PUFAs	**32.97 ± 3.87**	**27.79 ± 3.16[aa]**
C18:2	12.42 ± 2.18	11.59 ± 2.44
C20:3	2.52 ± 0.21	1.60 ± 0.21[aa]
C20:4	14.87 ± 1.63	12.43 ± 1.38[aa]
C22:4	3.16 ± 0.32	2.17 ± 0.24[aa]
UK	**1.09 ± 0.11**	**2.36 ± 0.37[aa]**
∑n-3/∑n-6	0.18 ± 0.16	0.34 ± 0.17[aa]
DHA/AA	1.17 ± 0.12	2.25± 0.21[aa]

Note: SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. UK: refer to do not know what specific substances. The unit was expressed as percentage. The data are presented as the mean ± SEM n = 6. Significantly different (for $^{a}p < 0.05$, $^{aa}p < 0.01$), compared with the control group.

found that there was significantly decreased in the percentage of arachidonic acid of erythrocyte in DHA-supplemented group. The ration of n-3/n-6 (PUFAs) was increased from 1.17 times in control group to 2.25 times in DHA treated group.

The Effects of DHA on 5-hydroxytryptamine, 5-hydroxyindoleacetic Acid, Noradrenaline and Dopamine in Hypothalamus Tissue

As shown in the Table VI, the data revealed DHA dietary significantly increased 5-hydroxytryptamine, 5-hydroxyindoleacetic acid and dopamine levels in hypothalamus; however, there was no significant alteration of noradrenaline level in DHA dietary group, when compared with control group.

The Effects of DHA Corticosterone, Adrenocorticotropic Hormone and Corticotrophin Releasing Factor Levels in Serum

As was shown in Table VII, DHA dietary attenuated the levels of corticosterone and adrenocorticotropic hormone in serum. The level of corticosterone was significantly decreased by 20.23%; serum adrenocorticotropic hormone level was notably decreased by 25.13%, while corticotrophin releasing factor level was significantly increased by 21.92% in DHA dietary group, respectively, when compared with control group.

Our additional experiment indicated that although the mice have not been conducted the behavior experiment, the level of adrenocorticotropic hormone was significantly decreased. Corticotrophin releasing factor was significantly increased in DHA dietary group. However, there were not significant difference between DHA dietary group and control group in the level of corticosterone.

The Effects of DHA on Corticotrophin Releasing Factor, Adrenocorticotropic Hormone and Arginine Vasopressin Levels In Hypothalamus And Pituitary Tissues

The effects of DHA supplementation on corticotrophin releasing factor, adrenocorticotropic hormone and arginine vasopressin levels in hypothalamus and pituitary were shown in Table VIII, compared with control group, arginine vasopressin level was decreased by 20.11% in hypothalamus, by 23.76% in pituitary tissues, respectively. Corticotropin-releasing factor level was decreased by 30.83% in hypothalamus, by 29.75% in pituitary tissues, respectively in DHA dietary group.

Table VI. The effect of DHA on 5-hydroxytryptamine 5-hydroxyindoleacetic acid, noradrenalin and dopamine levels in hypothalamu tissues.

Monoamine neurotransmitter	Control group	DHA dietary group
5-hydroxytryptamine	1385.45 ± 74.46	1748.95 ± 52.65[a]
5-hydroxyindoleacetic acid	633.13 ± 56.53	867.48 ± 54.68[aa]
Noradrenalin	357.64 ± 38.73	289.56 ± 33.76[aa]
Dopamine	231.63 ± 33.49	298.16 ± 36.47[a]

Note: The data are presented as the mean ±SEM n = 10. Significantly different (for $^{a}p < 0.05$, $^{aa}p < 0.01$), compared with the control group. The units were expressed as ng/g.

Table VII. Effect of DHA on corticosterone, corticotropin-releasing factor and adrenocorticotropic hormone levels in serum of mice.

Hormones	Control group	DHA dietary group
No behavior experiments		
Corticosterone	112. 73 ± 11.85	104.21 ± 10.04
Adrenocorticotropic hormone	119.59 ± 12.67	82.11 ± 7.91^{aa}
Corticotropin-releasing factor	10.07 ± 2.84	13.54 ± 2.16^{aa}
Performed behavior experiments		
Corticosterone	156. 16 ± 21. 64	124.57 ± 12.42^{aa}
Adrenocorticotropic hormone	149. 74 ± 14.38	112.11 ± 8.28^{aa}
Corticotropin-releasing factor	12.23 ± 2.39	16.13 ± 2. 81^{aa}

Note: The corticosterone units was expressed as μg/ml, the adrenocorticotropic hormone unite was expressed as pg/ml, the corticotropin-releasing factor unit was expressed as ng/ml. The data are presented as the mean ± SEM n = 10. Significantly different (for $^{a}p < 0.05$, $^{aa}p < 0.01$), compared with the control group.

In hypothalamus, the level of adrenocorticotropic hormone decreased significantly by 19.14% in DHA dietary group. However, in pituitary, it was observed that the level of adrenocorticotropic hormone decreased insignificantly in DHA dietary group.

Discussion

DHA is very important to brain function, our previous reports have showed DHA can improve learning and memory ability[25,26]. Other reports have showed erythrocyte and adipose DHA levels were 33-36% lower in depressives than controls group and it is correlated with the serious depression[27,28]; however, there have no any convicting evidences to show what a role pure DHA plays in depression. Thus, we performed this study. Judged from Table I and II, the diets used were mainly different in DHA content. We minimize the effect of other N-3 PUFAs and maximize the effect of DHA.

The forced swimming test was proposed as a model to test for antidepressant activity by Porsolt et al[15], it is sensitive and selective to antidepressant drugs. Forced swimming test resembles depression in humans to some extent. Furthermore, the forced swimming test reveals the participation of 5-hydroxytryptamine or noradrenaline neurotransmission in the antidepressant-like effect of various compounds, and the behaviors of swimming denote 5-hydroxytryptamine participation, and noradrenaline is involved in forced swimming test[16]. Major depression is characterized by disorders in noradrenaline and 5-hydroxytryptamine neurotransmission[29,30].

In this study, we evaluated the effect of DHA on depression by forced swimming test. The results showed that immobility time was significantly decreased; swimming and climbing time was significantly increased. Tail suspension test also showed immobility time was shortened. The data presented also demonstrated that DHA dietary significantly increased dopamine, noradrenaline and 5-hydroxyindoleacetic acid levels in hypothalamus. DHA has similar effect in hippocampus[26]. Our experiment showed DHA may be play an important role in neurotransmission of depressant-like effects in mice.

The HPA axis plays a key role in the physiological response to various stressful situations. Deregulation of HPA axis is the most common and consistently reported as symptom of depression[31]. The corticotrophin releasing factor and arginine vasopressin are key regulators of the HPA axis and act synergistically to induce the synthesis and release of adrenocorticotropic hormone from the pituitary, which ultimately results in the secretion of cortisol from the adrenal cor-

Table VIII. Effect of DHA on arginine vasopressin, adrenocorticotropic hormone, corticotropin-releasing factor, and levels of hypothalamus and pituitary of mice.

Hormones	Control group	DHA dietary group
In hypothalamus		
Arginine vasopressin	50.78 ± 7.63	40. 57 ± 5. 27^{aa}
Adrenocorticotropic hormone	132.45 ± 12.48	107.1 ± 12.45^{aa}
Corticotropin-releasing factor	36.76 ± 3.78	25.43 ± 3.92^{aa}
In pituitary		
Arginine vsopressin	285.17 ± 30.61	217. 42 ± 30. 42^{aa}
Adrenocorticotropic hormone	149.86 ± 15.86	136.39 ± 13.54
Corticotropin-releasing factor	33.50 ± 3.64	23.76 ± 3.27^{aa}

Note: The unites of arginine vsopressin and adrenocorticotropic hormone were expressed as pg/ml, the unit of corticotropin-releasing factor was expressed as ng/ml.
The data are presented as the mean ± SEM n = 10. Significantly different (for $^{a}p < 0.05$, $^{aa}p < 0.01$), compared with the control group.

tex. Corticotrophin releasing factor and arginine vasopressin have also been strongly implicated in depressive symptom[32]. Hormones in serum were assessed after the behavioral testing. However, the direct effects of DHA diet on hormone changes are confounded by the behavioral testing because that the two groups showed different amounts of struggling. Given hormones are so sensitive, we conducted an experiment to rule out the effect of behavioral experiments. The mice weren't conducted behavior experiments and hormones in serum were assessed. Our experiment results show that the supplement of DHA can decrease the levels of corticosterone, adrenocorticotropic hormone and arginine vasopressin and increase the level of corticotrophin releasing factor. The data support the hypothesis that DHA antidepressant acts on, at least in part, regulating corticotrophin releasing factor, arginine vasopressin, adrenocorticotropic hormone and corticosterone levels, thus normalizing the HPA axis hyperactivity.

The relationship between dietary N-3 PUFAs, red blood cell membrane N-3 PUFAs and severity of depression supports the possible therapeutic use of N-3 polyunsaturated fatty acids in the treatment of depression[11]. We assayed fatty acids in erythrocytes. The results of the present study showed DHA dietary group increased the ration of n-3/n-6 (PUFAs) in DHA treated group mice erythrocytes.

Our observations indicate that DHA contributes to normalize HPA axis response to stress, and is concordant with a role for reduced brain DHA in the etiologies of depressive illnesses. The major finding of this study provides direct evidence that pure DHA shows an antidepressant property rather than the mix of PUFAs. Furthermore, DHA has many multiple effects on depression including the monoamine neurotransmitter systems, red blood cell membranes and HPA axis. This finding suggests the mechanism of DHA improving depression behavior is complex. Many researches are still required to uncover the mechanism of DHA on depression.

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