BioFactors

The antianxiety-like effect of astaxanthin extracted from *Paracoccus carotinifaciens*

*Yasushi Nishioka,[1#] Atsushi Oyagi,[1#] Kazuhiro Tsuruma,[1] Masamitsu Shimazawa,[1] Takashi Ishibashi,[2] Hideaki Hara[1]**

[1]Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, Gifu, Japan

[2]Biotechnology Business Section, Special and Performance Chemicals Department II, Chemical Division, Nippon Oil Corporation, Tokyo, Japan

Abstract.

Astaxanthin is a red carotenoid pigment and is widely found in living organisms. Astaxanthin has a potent antioxidative ability and has been reported as having various biological effects on the central nerve system, such as a protective effect against ischemia/reperfusion injury and improvement in cognitive function. In this study, to investigate the effects of astaxanthin on anxiety and depression, we performed some behavioral trials including the elevated plus maze test, hole-board test, forced swim test, and tail suspension test. Astaxanthin (100 and 300 mg/kg/day for 10 days, p.o.) significantly increased the time spent in open arms in the elevated plus maze test and increased the head-dipping count and duration in the hole-board test. On the other hand, astaxanthin (10, 100, 300, and 500 mg/kg/day for 10 days, p.o.) did not change the immobility time in the forced swim test or the tail suspension test. In conclusion, in mice, astaxanthin exerted anxiolytic-like effects, but not antidepressant-like effects.

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E-mail: hidehara@gifu-pu.ac.jp

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1. Introduction

Astaxanthin is a red carotenoid pigment and is widely found in living organisms. Astaxanthin is only produced by algae, plants, and a limited number of fungi and bacteria. It acts as a quencher and a scavenger of active oxygen species [1] and has the ability to be located either inside the phospholipid membrane or at the membrane surface [2] and to cross the blood–brain barrier in rodents [3]. In the central nervous system (CNS), astaxanthin has been reported to protect against ischemia/reperfusion-induced neurodegeneration in rats [4], improves mice memory in the Morris water maze test [5], and affects cognitive functioning in humans [6].

Anxiety and depression disorders are some of the most common psychiatric conditions. The etiology is complicated and not precisely understood, but multifactorial changes in neurotransmitter levels involving heredity, alteration neuroendocrine function, and some psychosocial factors are believed to be concerned [7]. The biological study of psychiatric disorders benefits from standardized animal behavioral tests and predictive validities of antianxiety and antidepressant are determined. The elevated plus maze test and hole-board test were developed to evaluate the antianxiety-like effects of agents [8,9]. The forced swim test and tail suspension test were developed to evaluate the antidepressant-like effects of agents [10,11].

Previous reports have shown that oxidative stress is associated with depression, anxiety, and related psychiatric disorders. Treatment of mice with L-buthionine-(*S*,*R*)-sulfoximine (BSO), an inducer of oxidative stress, causes anxiety-like behavior through the NADPH oxidase pathway [12]. Another report has also suggested a linear and significant relationship between the intracellular redox status of peripheral blood granulocytes and the different parameters of anxiety-related behavior [13]. Although astaxanthin has the ability to cross the blood–brain barrier and has a beneficial effect on the CNS, the effects of astaxanthin on anxiety and depression have not been reported. In this study, we investigated the effects of astaxanthin on anxiety and depression in mice models.

*Address for correspondence: Hideaki Hara, PhD, Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu 501-1196, Japan. Tel.: +81-58-230-8126; Fax: +81-58-230-8126; E-mail: hidehara@gifu-pu.ac.jp.
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2. Materials and methods

2.1. Animals

Four-week-old male ICR mice, weighing 17–19 g, were used in this study. The animals were obtained from colonies of

specific pathogen-free ICR mice maintained by Japan SLC (Shizuoka, Japan). All procedures relating to animal care and treatment conformed to the animal care guidelines of the Animal Experiment Committee of Gifu Pharmaceutical University. All efforts were made to minimize both suffering and the number of animals used. The animals were housed at 24°C ± 2°C under a 12 h light-dark cycle (lights on from 8:00 to 20:00) and had *ad libitum* access to food and water. Behavioral experiments were performed between 10:00 and 18:00.

2.2. Administration of astaxanthin

Astaxanthin, extracted from *Paracoccus carotinifaciens* and composed of 67.1% astaxanthin, was obtained from Nippon Oil Corporation (Tokyo, Japan) and suspended in olive oil (Wako Pure Chemicals, Osaka, Japan). In evaluation of antianxiety-like behaviors, astaxanthin was orally administrated using a sonde by gavage at the doses of 100 and 300 mg/kg/day for 10 days; 1 h after the last administration, the animals were subjected to the experiment. In evaluation of antidepressant-like behaviors, astaxanthin was orally administrated using a sonde by gavage at the doses of 10, 100, 300, and 500 mg/kg/day for 10 days; 1 h after the last administration, the animals were subjected to the experiment.

2.3. Elevated plus maze test

An elevated plus maze test apparatus was made up of two open arms (length 30 cm × width 5 cm), two closed arms of the same size with semi-transparent wall (height 15 cm), and a central platform (length 5 cm × width 5 cm). These arms and the central platform were elevated 50 cm above the floor. As a positive standard, mice were intraperitoneally administered diazepam (1 mg/kg, suspended in 0.5% carboxymethylcellulose solution) (Wako Pure Chemicals). One hour after astaxanthin or 30 min after diazepam administration, each mouse was placed on the central platform, facing one of the open arms. During a 10 min test session, the time spent in the open arms, the time spent in closed arms, frequency to open arms, and frequency to closed arm were recorded using EthoVision XT (Noldus, Wageningen, Netherlands).

2.4. Hole-board test

The hole-board test was performed following the method of a previous report [14]. As a positive standard, the mice were intraperitoneally administered diazepam (1 mg/kg, suspended in 0.5% carboxymethylcellulose solution). One hour after astaxanthin or 30 min after diazepam administration, each mouse was placed in the center of the open field apparatus (length 30 cm × width 30 cm × height 16 cm) with four equidistant holes, 2 cm in diameter, in the floor. Head-dip duration and counts were manually recorded for a 5 min test session.

2.5. Forced swim test

The forced swim test was performed following the method of a previous report [10]. As a positive standard, mice were intraperitoneally administered imipramine hydrochloride (20 mg/kg, dissolved in saline) (Wako Pure Chemicals). One hour after astaxanthin or 30 min after imipramine administration, mice were placed in a glass cylinder (diameter 10 cm) filled with 10 cm of water (22°C ± 1°C). Over a period of 6 min, only the last 5 min of immobility time was measured. Mice were judged to be immobile when they remained floating passively in the water, making only small movements to keep their heads above the water.

2.6. Tail suspension test

The tail suspension test was performed following the method of a previous report [11]. As a positive standard, mice were intraperitoneally administered imipramine hydrochloride (20 mg/kg, dissolved in saline). One hour after astaxanthin or 30 min after imipramine administration, mouse tail was suspended 50 cm above the floor using adhesive tape, and their behavior was recorded for 7 min. Immobility time was manually measured for the last 6 min of the test.

2.7. Statistical analysis

Data are presented as the means ± S.E.M. Statistical comparisons were made by a Student's *t*-test or a one-way ANOVA, followed by Dunnett's test using Statview version 5.0 (SAS Institute, Cary, NC), with $P < 0.05$ being considered to indicate a statistical significance.

3. Results

3.1. Elevated plus maze test

In the elevated plus maze test, the aversion that mice show for the two open arms is attenuated by anxiolytics [8]. Therefore, the time spent in the open arm is considered to be an indicator for anxiety. Diazepam, which affects the GABA-benzodiazepine receptor, shows an anxiolytic effect and is used as a standard drug for anxiety. In this study, diazepam (1 mg/kg, i.p.)-treated mice spent a longer amount of time in the open arms than the control mice (Fig. 1a). The time that the astaxanthin (100 and 300 mg/kg/day)-treated mice spent in the open arms was significantly longer than that of the control mice (Fig. 1a). We also measured the other parameters in elevated plus maze test. Open/closed arm ratio of time spend in open arms (Fig. 1b) and frequency to open arms (Fig. 1c) were significantly increased in astaxanthin (100 mg/kg/day)-treated group than those of the control mice. Open/closed arms ratio of frequency was also increased in astaxanthin (300 mg/kg/day)-treated group than that of the control mice (Fig. 1d).

We have investigated the general levels of motility in open field test. No significant differences were observed in the locomotor activity between each group [Control: 70.5 ± 4.88 (meter) (mean ± S.E.M, $n = 20$), astaxanthin 100 mg/kg: 62.7 ± 3.17 ($n = 12$), astaxanthin 300 mg/kg: 55.9 ± 3.73 ($n = 10$)].

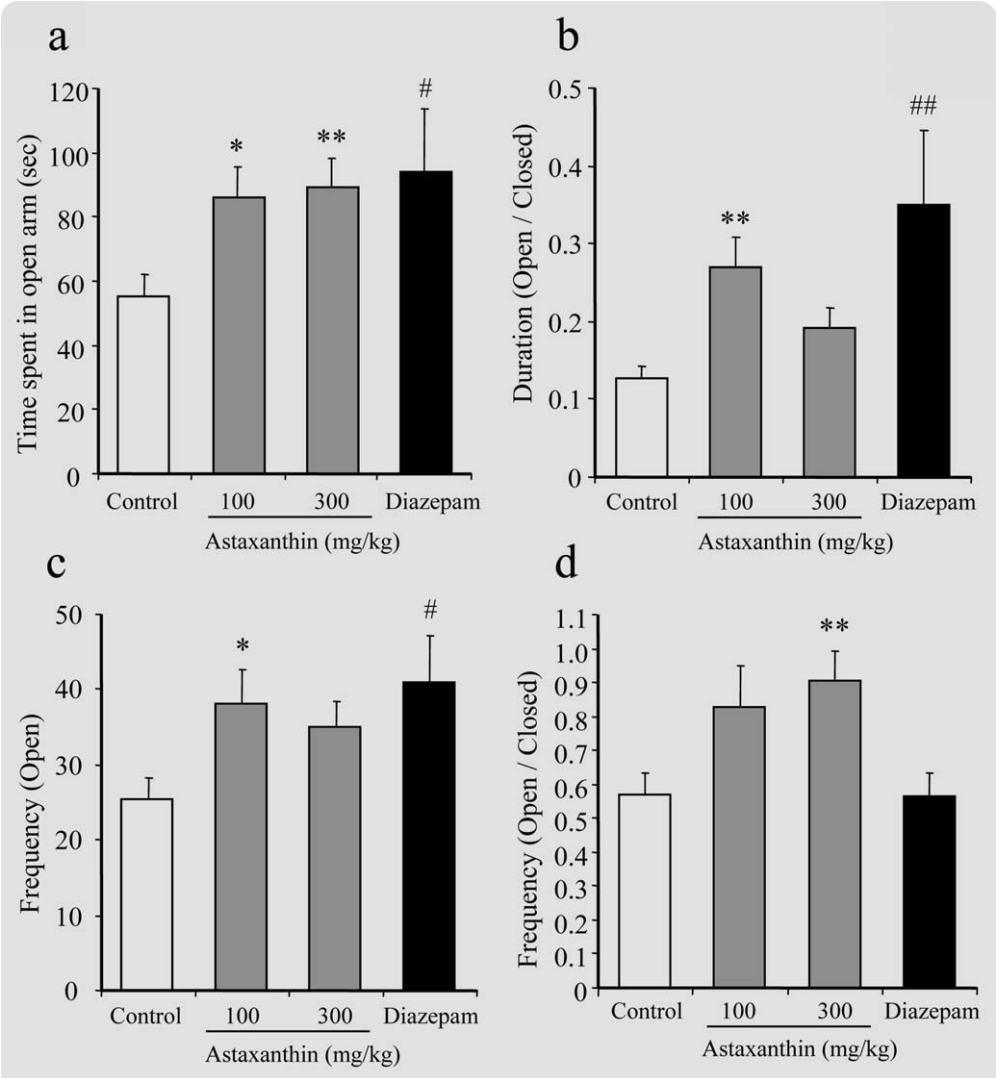

Fig. 1. Effects of astaxanthin on animal behavior in the elevated plus maze test. The graph shows the time that astaxanthin (100 and 300 mg/kg/day for 10 days, p.o.)-, vehicle (olive oil, p.o.)-, and diazepam (1.0 mg/kg, i.p.)-treated mice spent in open arms (a), open/closed arm ratio of time spend in open arm (b), frequency to open arm (c), and open/closed arm ratio of frequency (d). Each column and bar represents mean ± S.E.M. *: $P < 0.05$, **: $P < 0.01$, versus control, Dunnet's test. #: $P < 0.05$, ##: $P < 0.01$, versus control, Student's t-test. Control ($n = 27$), astaxanthin (100 or 300 mg/kg/day) ($n = 11$ or 21), and diazepam ($n = 7$).

3.2. Hole-board test

In the hole-board test, head dipping is considered to be an indicator of anxiety [9], and an anxiolytic state in mice may be reflected by an increase in head-dipping behavior [15]. The head-dipping duration and head-dipping counts of the astaxanthin (100 mg/kg/day and 300 mg/kg/day)-treated mice were significantly longer and larger than the control mice (Figs. 2a and 2b). Diazepam was also used as a standard drug for anxiety. Both parameters in the diazepam (1 mg/kg, i.p.)-treated mice were also significantly longer and larger than that of the control mice (Figs. 2a and 2b).

3.3. Forced swim test

In the forced swim test, antidepressant-like behavior is modeled as decreased immobility [10]. Imipramine has an inhibiting effect of noradrenaline reuptake and acts as an antidepressant. Imipramine is known to reduce immobility time in the forced swim test, and thus, we used imipramine as a standard drug (positive control) for depression. The immobility time of the imipramine (20 mg/kg, i.p.)-treated mice was shorter than that of the control mice (Figs. 3a and 3b), as previously reported. However, no significant changes were observed between the astaxanthin (10, 100, 300, and 500 mg/kg/day)-treated and control mice (Figs. 3a and 3b).

3.4. Tail suspension test

The tail suspension test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail will develop an immobile posture. In this test, the immobility time of the imipramine (20 mg/kg, i.p.)-treated mice was shorter than that of the control mice

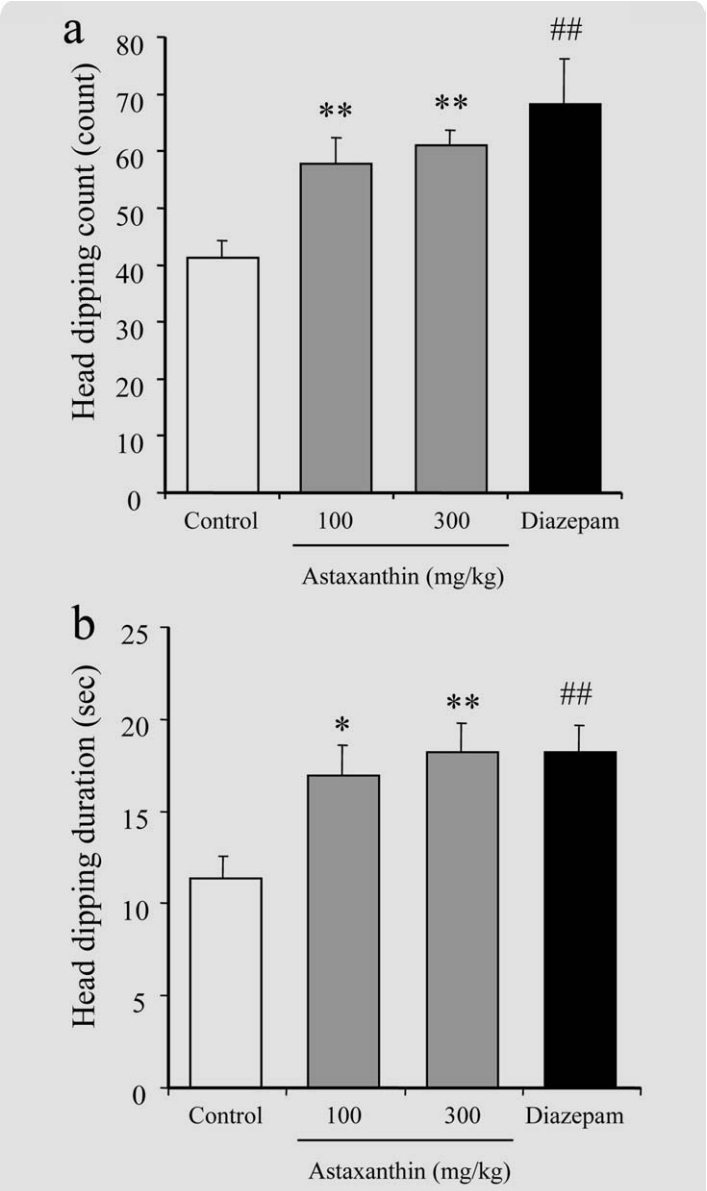

Fig. 2. Effects of astaxanthin on animal behavior in the hole-board test. The graph shows the duration (a) and count (b) that astaxanthin (100 and 300 mg/kg/day for 10 days, p.o.)-, vehicle (olive oil, p.o.)-, and diazepam (1.0 mg/kg, i.p.)-treated mice dipped on the holes. Each column and bar represents mean ± S.E.M. *: $P < 0.05$, **: $P < 0.01$ versus control Dunnet's test. ##: $P < 0.01$, versus control, Student's *t*-test. Control ($n = 10$), astaxanthin (100 and 300 mg/kg/day) ($n = 10$), and diazepam ($n = 4$).

(Figs. 3c and 3d). However, no significant changes were observed between the astaxanthin (10, 100, 300, and 500 mg/kg/day)-treated and control mice (Figs. 3c and 3d).

4. Discussion

In this study, we examined the effects of astaxanthin on anxiety and depression in mice behavioral models. Astaxanthin showed antianxiety-like effects on mice behavior in the elevated plus maze test and hole-board test. Recent studies suggest that astaxanthin may play a beneficial role in oxidative stress-induced neural cell death in retina [16] and brain [17]. Regarding the effects on higher brain function, astaxanthin has been reported to protect against ischemia/reperfusion-induced neurodegeneration [4], improve mice memory [5], and have an effect on cognitive function [6]. Previous report also demonstrated the antagonistic effect of astaxanthin against the ethanol-induced facilitation of cortical spreading depression [18], which is an electrophysiological phenomenon characterized by a wave of excitation followed by inhibition and has been implicated in disorders of neurovascular regulation such as stroke, head trauma, and migraine. This result indicates that astaxanthin may affect on the neuronal and glial depolarization and neurovascular regulation in CNS. However, a study on the effects of astaxanthin on anxiety and depression has not been reported yet. Therefore, this study is the first report to prove the benefit of astaxanthin on anxiety.

Astaxanthin showed no significant change on mice behavior in the forced swim test or tail suspension test. These results indicate that there are some different etiologies between anxiety and depression. Indeed, the mechanisms of the standard drugs that we used in this study were different. The diazepam causes the GABA benzodiazepine receptor to induce an anxiolytic effect, whereas imipramine causes some neurotransmitter systems and reuptake of neurotransmitters to induce antidepressant effects. These etiological differences may be associated with the different actions of astaxanthin on anxiety and depression.

In this study, we used relatively high doses (100 and 300 mg/kg) of astaxanthin. High concentrations of carotenoids were reported to exert a pro-oxidant effect [19]. On the other hand, astaxanthin has been classified as a "pure antioxidant," unlike certain other carotenoids such as β-carotene, lutein, and lycopene that may show "pro-oxidative" characteristics under certain conditions [20]. From this reason, astaxanthin may be less likely to become pro-oxidant.

The precise molecular mechanism of astaxanthin on anxiety has not been determined in this study. Previous reports have shown that oxidative stress is associated with depression, anxiety, and related psychiatric disorders. Treatment of mice with BSO, an inducer of oxidative stress, causes anxiety-like behavior through the NADPH oxidase pathway [12]. In fact, a phosphodiesterase-2 inhibitor reversed the oxidative stress-induced anxiety through an increase in cyclic guanosine monophosphate signaling [12]. Thus, phosphodiesterase-2 may be a novel pharmacological target for treatment of anxiety in neuropsychiatric and neurodegenerative disorders that involve oxidative stress. Another report has also suggested a linear and significant relationship between the intracellular redox status of peripheral blood granulocytes and the different parameters of anxiety-related behavior [13].

It has been suggested that carotenoids localize not only in the cell membrane but also in lipid rafts [21]. Carotenoids were reported to inhibit the antigen-induced

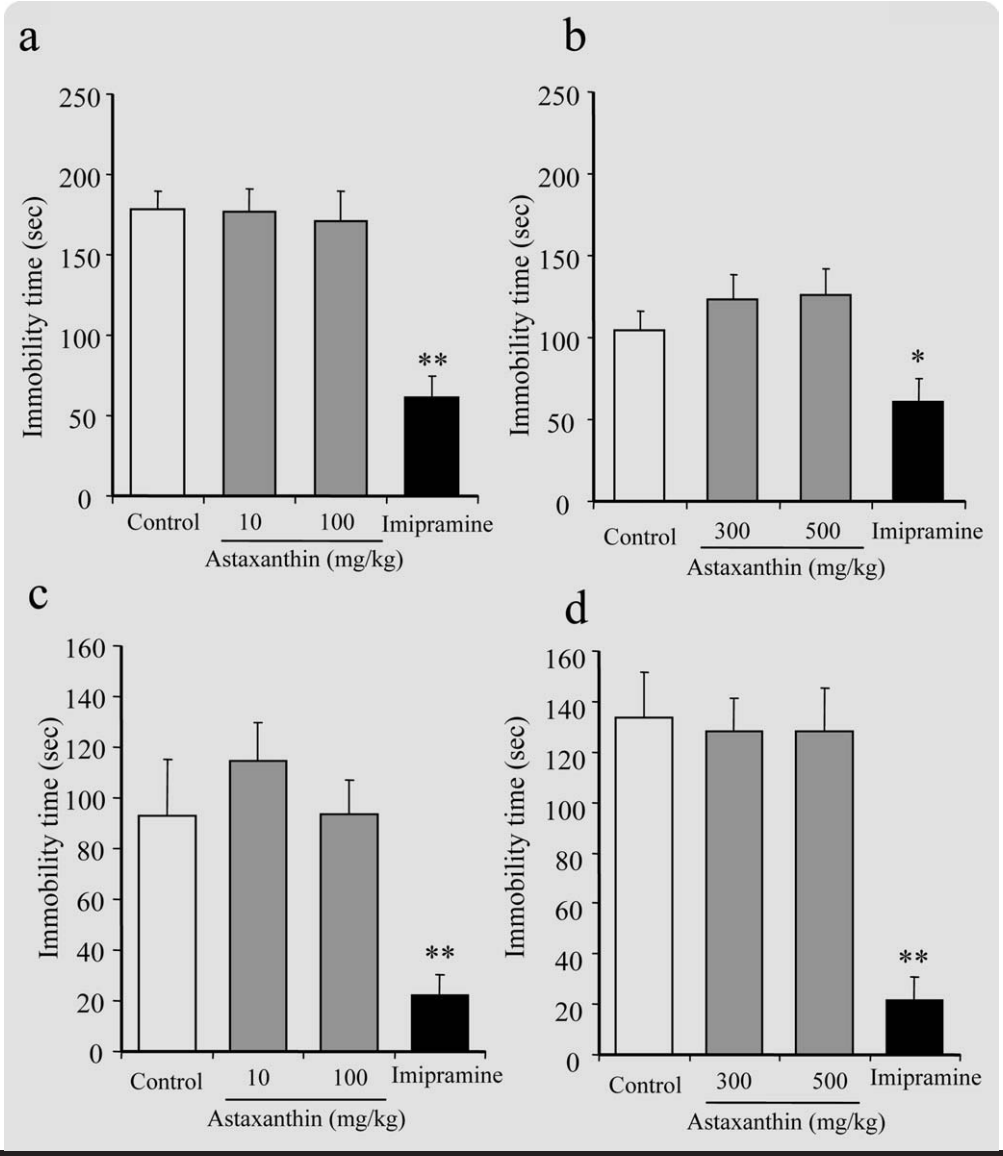

Fig. 3. Effects of astaxanthin on animal behavior in the forced swim test. The graph shows immobility time of control (olive oil, p.o.)-, astaxanthin (10 and 100 mg/kg/day for 10 days, p.o.)- (a), astaxanthin (300 and 500 mg/kg/day for 10 days, p.o.)- (b), and imipramine (20 mg/kg, i.p.)-treated mice in the forced swim test. Control (n =14), astaxanthin (10, 100, 300, or 500 mg/kg/day) (n = 10–14), and imipramine (n = 10). Effects of astaxanthin on animal behavior in the tail suspension test. The graph shows immobility time of control (olive oil, p.o.)- mice, astaxanthin (10 and 100 mg/kg/day for 10 days, p.o.)- (c), astaxanthin (300 and 500 mg/kg/day for 10 days, p.o.)- (d), and imipramine (20 mg/kg, i.p.)-treated mice. Each column and bar represents mean ± S.E.M. **: $P < 0.01$, *: $P < 0.05$, versus control, Student's t-test. Control (n = 9 or 10), astaxanthin (10, 100, 300, or 500 mg/kg/day) (n = 9–11), and imipramine (n = 8 or 9).

translocation of high-affinity IgE receptors (FcεRI) to lipid rafts and modulate the function of lipid rafts. Modulation of lipid rafts may be one of the important molecular mechanisms for various physiological functions of carotenoids [21]. The molecular biological mechanism of anxiety in lipid rafts were reported [22]. Neural cell adhesion molecule (NCAM)-deficit mice show increased anxiety and aggression [23]. Action of NCAM on inward rectifying K^+ (Kir3) channel via lipid rafts may be important for the aggression and anxiety condition of NCAM-deficit mice [22]. We consider that the alternative behaviors, such as anxiety and depression, are remotely related to oxidative stress or the functions of lipid rafts, which may be affected by astaxanthin activity to some degree.

In conclusion, these findings indicate that astaxanthin may be useful on anxiety-related psychiatric diseases.

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