

# The impact of taurine- and beta-alanine-supplemented diets on behavioral and neurochemical parameters in mice: antidepressant versus anxiolytic-like effects

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**Abstract** Taurine, a substrate of taurine transporter, has functions as a neuromodulator and antioxidant and beta-alanine, a taurine transporter inhibitor, has a role as a neurotransmitter in the brain, and they were expected to be involved in depression-like behavior and antidepressant treatment. These facts aroused our interest in new capabilities of taurine and beta-alanine. Thus, to investigate the effects of chronic ingestion of taurine- (22.5 mmol/kg diet) supplemented diet and beta-alanine- (22.5 mmol/kg diet) supplemented diet under acute stressful conditions, behavioral changes and brain metabolites were compared with mice fed a control diet. In the open field test, no significant difference was observed in locomotor activity among groups. In the elevated plus-maze test, however, significant increases in the percentage of time spent and entries in the open arms were observed in the beta-alanine-supplemented diet fed group compared to both controls and animals fed with taurine-supplemented diet. Moreover, a significant decrease in the duration of immobility was observed in the taurine-supplemented diet group in the forced swimming test compared to both controls and animals fed with beta-alanine-supplemented diet. Taurine-supplemented diet increased taurine and L-arginine concentrations in the hypothalamus. In contrast, beta-alanine-supplemented diet decreased the concentration of 5-hydroxyindoleacetic acid, a major metabolite of serotonin, in the hypothalamus. Beta-alanine-supplemented diet also increased carnosine (beta-alanyl-L-histidine) concentration in the cerebral cortex and hypothalamus, and

brain-derived neurotrophic factor concentration in the hippocampus. These results suggested that taurine-supplemented diet had an antidepressant-like effect and beta-alanine-supplemented diet had an anxiolytic-like effect.

**Keywords** Taurine · Beta-alanine · Elevated plus-maze test · Forced swimming test · Amino acids · Brain-derived neurotrophic factor

## Introduction

Depression is a common mental disorder, and unipolar major depression is expected to be one of the main causes of disability-adjusted life years in 2020 by the World Health Organization (Murray and Lopez 1997). To treat this disorder, antidepressants have been usually used which is related to the monoaminergic neuron system. However, antidepressive effects of nutrients have not been clarified yet. To identify such nutrients may raise the possibility of treating or preventing depression by dietary regimens.

Taurine, 2-aminoethylsulfonic acid, is one of the most abundant free amino acids in the central nervous system, including the glial cell in the hypothalamus (Hussy et al. 2000). Although taurine is not a constituent of any structural mammalian protein, it has various important physiological roles as an antioxidant, osmoregulator, membrane stabilizer, neurotransmitter and so on. Especially, by acting as an inhibitory neurotransmitter through gamma-aminobutyric acid (GABA) and glycine (Gly) receptors, it has been reported that taurine has an anxiolytic-like effect in mice and rat (Chen et al. 2004; Kong et al. 2006). Taurine also has a neuroprotective effect from the excitotoxicity induced by excitatory amino acids, and this function was assumed to be effective against some disturbances

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in the brain function such as epilepsy, ischemia, and hypoxia (Saransaari and Oja 2000).

In our previous study, taurine concentration in the cerebral cortex was increased by forced swimming, which is often regarded as an animal model of depression, and beta-alanine concentration in the hypothalamus was increased by imipramine treatment, which is one of the major antidepressants (Murakami et al. 2009). Taurine was also suggested to be released in the hypothalamic supra-optic nucleus and in the medio-lateral septum by forced swimming (Engelmann et al. 2002; Singewald et al. 1999).

Beta-alanine has also been suggested to act as an inhibitory neurotransmitter, as it was reported to act on GABA and Gly receptors (Wu et al. 1993). However, effects of beta-alanine on emotional behavior tests such as the elevated plus-maze test or forced swimming test are unknown.

These two amino acids were increased under forced swimming or antidepressant treatment as mentioned above, and were also suggested to have some effective functions in the brain. The relationship between taurine and beta-alanine is: taurine is recognized as a substrate of the taurine transporter and beta-alanine is a taurine transporter inhibitor since they have the beta-amino acid group. They are antagonistic to each other at the blood–brain barrier due to sharing the same transporter (Takeuchi et al. 2000). Accordingly, it aroused our interest in whether taurine and beta-alanine have new capabilities and whether both amino acids produce similar behavioral effects after forcing the animals to eat the diet supplemented by the respective amino acid.

Both monoamines and amino acids are principal neurotransmitters; especially noradrenaline (NA) and 5-hydroxytryptamine (5-HT), which are thought to be deeply involved in the aetiology of depression and anxiety. Indeed, antidepressants like tricyclic antidepressants or selective serotonin reuptake inhibitors, which were able to enhance the monoaminergic neurotransmission, were found to be effective in the treatment of depression. In the elevated plus-maze test, it was also reported that buspirone, a 5-HT receptor agonist, showed an anxiolytic-like effect (Cao and Rodgers 1997). With respect to amino acids, GABA and its receptor agonists had an anxiolytic- and antidepressant-like effect (Aley and Kulkarni 1989; Chen et al. 2004). Further, L-tryptophan, and L-arginine (L-Arg) were also reported to have an antidepressant-like effect in the forced swimming test (Wong and Ong 2001; Inan et al. 2004). These reports suggested that not only monoamines, but also amino acids, are involved in anxiety and depression.

Brain-derived neurotrophic factor (BDNF), a secretory protein in the neurotrophin family, has been linked to depression and anxiety. Postmortem brain samples from

depressed patients showed lower BDNF concentration than those from patients receiving antidepressant treatments, and antidepressant treatments have been shown to restore brain BDNF concentration to the normal range (Castrén 2004; Chen et al. 2001). Moreover, reduced blood BDNF concentration in depressed patients has also been observed and, again, antidepressant treatments normalized it (Karege et al. 2005; Shimizu et al. 2003).

The aim of the present study was to examine whether chronic ingestion of taurine- or beta-alanine-supplemented diet attenuate the behaviors under the stressful conditions such as the open field test, the elevated plus-maze test, and the forced swimming test. In addition, monoamines, amino acids and BDNF concentrations in the brain were also analyzed to investigate potential targets of the amino acid supplemented diets.

## Materials and methods

### Animals

Three-week-old male ICR mice, purchased from SLC Japan, Inc. (Hamamatu, Japan), were used. Mice were housed 2 per cage under a light/dark cycle (lights on at 0800 hours, lights off at 2000 hours) at room temperature of  $23 \pm 1^\circ\text{C}$ , and had ad libitum access to food and water. This study was performed according to the guidance for Animal Experiments in Faculty of Agriculture and in the Graduate Course of Kyushu University and the Act on Welfare and Management of Animals (No. 105) and Standards relating to the care and management, etc. of experimental animals (No. 6) of the Government of Japan.

### Experimental procedure

After 1 week of acclimation with normal powder diet (MF; Oriental Yeast, Tokyo, Japan), mice were divided into three groups; control group, taurine group, and beta-alanine group. The control group was fed normal powder diet. The taurine and beta-alanine groups were fed with the powder diet containing 22.5 mmol taurine or beta-alanine per kg diet, respectively. Diets were given ad libitum until the end of the experiment. All mice were included in all behavioral tests. Mice were tested during the light period and were kept in a closed room at constant temperature ( $23 \pm 1^\circ\text{C}$ ). We conducted the open field test on the 26th day of the feeding trial, the elevated plus-maze test on the 29th day, and the forced swimming test on the 32nd day. Each test was recorded on a video recording system for analysis. On the 36th day, mice were sacrificed by cervical dislocation, and the cerebral cortex, hypothalamus and hippocampus were immediately dissected and weighed.

The samples were frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  until analysis.

#### Open field test

The locomotor activity in a novel environment was recorded employing the open field test. Briefly, animals were individually transferred to an open field area from the home cage. The arena was circular (diameter 60 cm and height 35 cm), and made of black takiflex. The test was begun by placing the animals at the center of the arena. The behavior of the animals was then observed for 5 min under dim light (100 lux). After each test, the field was cleaned with an ethanol–water solution. The distance of the path was examined as the locomotor activity and was automatically analyzed with a computer-based video tracking system (AXIS-90, Neuroscience, Inc., Japan).

#### Elevated plus-maze test

The elevated plus-maze consisted of a black acrylic cross of two closed arms ( $27.5 \times 5 \times 20$  cm) and two open arms ( $27.5 \times 5$  cm) raised 60 cm above the floor. The open and closed arms were connected by a central platform ( $5 \times 5$  cm). For each test, the mouse was placed in the center of the cross facing an open arm and was allowed to explore the maze for 5 min. After each test, the arms and a central platform were cleaned with an ethanol–water solution. In this test, anxiolytic compounds selectively increase the percentage of time spent and/or entries in the open arms, in contrast, anxiogenic compounds selectively decrease the percentage of time spent and/or entries in the open arms. Each trial was for 5 min under dim light (70 lux). An entry was counted when all four paws of the mouse entered an open or closed arm.

#### Forced swimming test

The experiment was carried out as previously described (Porsolt et al. 1977). Briefly, mice of three groups were individually placed for 6 min in a plastic bottle (22 cm high and 9.7 cm in diameter) containing water 16 cm deep maintained at  $24\text{--}26^{\circ}\text{C}$ . A mouse was judged to be immobile when it remained floating in the water, making only small movements to keep its head above water. The total duration of immobility was recorded by video camera during the final 4 min after a 2 min habituation period. In each test, fresh water was used.

#### Analysis of monoamines

The concentrations of monoamines and their metabolites were analyzed by high-performance liquid chromatography

(HPLC). The tissue samples of the cerebral cortex and hypothalamus were homogenized in ice-cold 0.2 M perchloric acid solution containing 0.01 mM ethylenediaminetetraacetic acid disodium salt dihydrate and left for deproteinization in ice. After 30 min, the mixtures were centrifuged at  $20,000 \times g$  for 15 min at  $0^{\circ}\text{C}$ . After centrifugation, the pH of the supernatant was adjusted to approximately 3.0 by adding 1 M sodium acetate. The supernatant was then centrifuged with a centrifuge-filtration unit (Ultra Free C3-GV Millipore, Bedford, MA, USA) at  $10,000 \times g$  for 5 min at  $0^{\circ}\text{C}$ . A 30  $\mu\text{l}$  portion of filtrate was applied to an HPLC system (Eicom, Kyoto, Japan) with a  $150 \times 2.1$  mm octadecyl silane (ODS) column (SC-5ODS, Eicom) and an electrochemical detector (ECD-300, Eicom, Kyoto, Japan) at an applied potential of +0.75 V versus Ag/AgCl reference analytical electrode. Changes in electric current (nA) were recorded in a computer using an interface system (Power Chrom ver 2.3.2.j; AD Instruments, Tokyo, Japan). The mobile phase consisted of 0.1 M aceto-citric acid buffer (pH 3.5), methanol, 0.46 M sodium 1-octane sulfonate, and 0.015 mM disodium ethylenediaminetetraacetic acid (830:170:1.9:1) at a flow rate of 0.2 ml/min. The concentrations of monoamines and metabolites including, NA, 5-HT, NA metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG), and 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined, and their concentrations in the brain were calculated.

#### Analysis of free amino acids

The concentrations of free amino acids were also analyzed by HPLC. The tissue samples of the cerebral cortex and hypothalamus were homogenized, deproteinized, and centrifuged by same process as for the analysis of monoamines. After centrifugation, the resultant supernatants were then adjusted to pH 7 with 1 M sodium hydroxide. Each sample (20  $\mu\text{l}$ ) was then completely dried under reduced pressure. Dried residues were dissolved in 10  $\mu\text{l}$  of 1 M sodium acetate–methanol–triethylamine (2:2:1) solution, re-dried, and dissolved in 20  $\mu\text{l}$  of derivatization solution [methanol–water–triethylamine–phenylisothiocyanate (7:1:1:1)]. After 20 min at room temperature, phenylisothiocyanate was allowed to react with the amino groups and the samples were dried again and then dissolved in 100  $\mu\text{l}$  of Pico-Tag Diluent (Waters, Milford, USA). These diluted samples were filtered through a 0.45-mm filter (Millipore, Bedford, USA). The same method was applied to standard solutions prepared by diluting a commercially available L-amino acid solution (type AN II and type B; Wako, Osaka, Japan) with distilled water. These derivatized samples were applied to a Waters HPLC system (Pico-Tag free amino acid separation column ( $3.9 \text{ mm} \times 300 \text{ mm}$ ), Alliance 2690 separation

module, 2487 dual-wavelength UV detector, and Millennium 32 chromatography manager; Waters, Milford, USA). They were equilibrated with buffer A [70 mM sodium acetate (pH 6.45 with 10% acetic acid)–acetonitrile (975:25)] and eluted with a linear gradient of buffer B [water–acetonitrile–methanol (40:45:15)] (0, 3, 6, 9, 40, and 100%) at a flow rate of 1 ml/min at 46°C. The absorbance at 254 nm was applied to determine concentrations of free amino acids. Triethylamine and sodium acetate trihydrate were purchased from Wako (Osaka, Japan), while other drugs for which no manufacturer is noted were purchased from Sigma (St Louis, USA).

### Analysis of BDNF

The concentration of BDNF was analyzed by enzyme-linked immunosorbent assay (ELISA). The tissue samples of the hippocampus were homogenized in 200 µl of lysis buffer [137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% nonidet P-40 (NP-40), 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/ml aprotinin, 1 µg/ml leupeptin, and 0.5 mM sodium vanadate (Sigma-Aldrich Inc., USA)] and then centrifuged at  $16,000\times g$  for 30 min at 4°C. Fifty microliters of the supernatant was moved to another tube, and diluted with 200 µl of Dulbecco's phosphate buffered saline. Samples were further treated with 1 M HCl to decrease the pH to 2.0–3.0, incubated at room temperature for 15 min, and then neutralized with 1 M NaOH to a pH value around 7.6. The acid treatment procedure increases the detectable amounts of endogenous neurotrophic factors because it promotes the dissociation of ligands from receptors or binding protein.

To measure the amount of BDNF, Promega BDNF Emax ImmunoAssay System was employed (Promega Co., Madison, WI, USA). Ninety-six-well polystyrene plates (Nunc-Immuno<sup>TM</sup> Maxisorp Plates, Denmark) were coated with anti-BDNF monoclonal antibody (mAb) (diluted 1:1,000 in carbonate coating buffer, 25 mM sodium carbonate, pH 9.7). Plates were washed once with Tris-buffered saline Tween 20 (TBST) wash buffer (20 mM Tris–HCl (pH 7.6), 150 mM NaCl and 0.05% (v/v) Tween 20) then blocked using 200 µl Promega 1× Block and Sample buffer for 1 h at room temperature. After washing with TBST buffer, 100 µl of the samples were added to the blocked wells, incubated with shaking (<500 rpm) at room temperature for 2 h, then washed five times with TBST buffer. One hundred microliters of chicken anti-Human BDNF polyclonal antibody (pAb) diluted 1:500 in 1× Block and Sample buffer was added to each well and incubated with shaking at room

temperature for 2 h. Plates were washed again five times using TBST wash buffer. One hundred microliters of rabbit anti-chicken IgY horseradish peroxidase conjugate diluted 1:200 in 1× Block and Sample buffer was next added to each well and incubated with shaking at room temperature for 1 h. Plates were emptied again and washed five times using TBST wash buffer. Finally, plates were developed using 100 µl Promega 3,3',5,5'-tetramethylbenzidine one solution and the reaction was stopped using 100 µl 1 M HCl. Absorbance was measured at 450 nm.

### Statistical analysis

All analyses were conducted using a one-way analysis of variance. When significant ( $P < 0.05$ ) effects were detected, comparisons between means were carried out using the Tukey–Kramer test. These analyses were performed with StatView (version 5, SAS Institute Cary, United States, SAS 1998). Outlying data were eliminated by Thompson's test criterion for outlying observations ( $P < 0.05$ ).

## Results

Dietary condition did not affect the body weight and food intake of mice (data not shown).

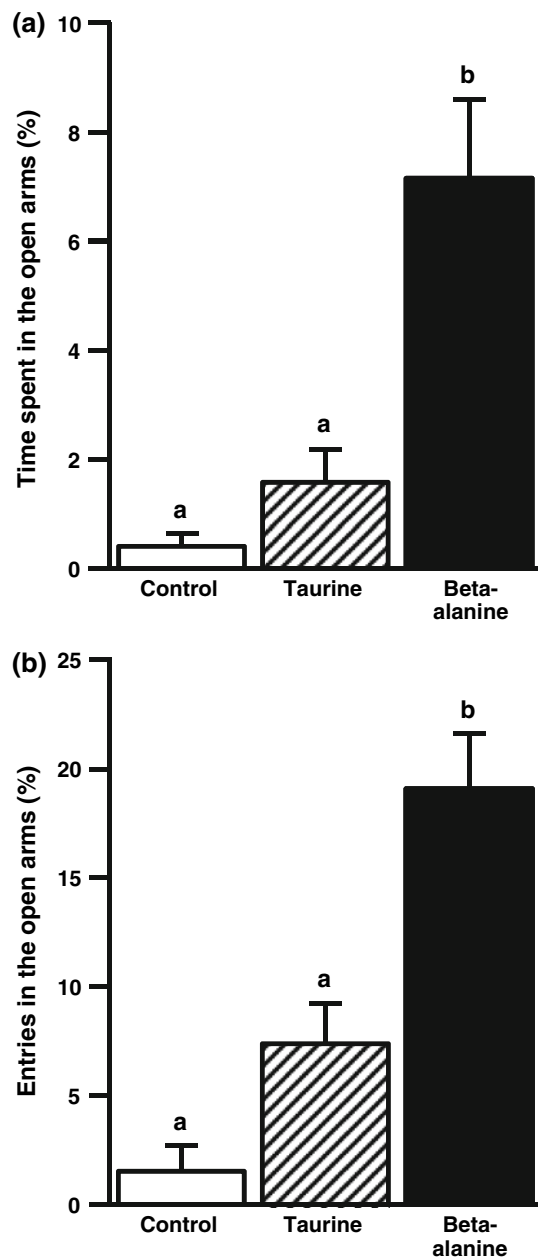
### Locomotor activity in the open field test

Neither taurine ( $33.5 \pm 1.6$ ) nor beta-alanine ( $29.9 \pm 2.6$ ) group significantly changed the distance (m) of pass compared to the control group ( $31.4 \pm 2.6$ ) ( $F(2,24) = 0.632$ ,  $P = 0.5402$ ).

### Percentage of time spent and entries in the open arms in the elevated plus-maze test

The effect of taurine- or beta-alanine-supplemented diet on the percentage of time spent and entries in the open arms in the elevated plus-maze test for 5 min is shown in Fig. 1. Significant increases of the percentage of time spent ( $F(2,25) = 14.382$ ,  $P < 0.0001$ ) and entries ( $F(2,24) = 21.427$ ,  $P < 0.0001$ ) in the open arms were observed in the beta-alanine group compared to the other two groups.

The number of entries in the closed arms was: control group ( $7.11 \pm 1.3$ ), taurine group ( $10.9 \pm 1.1$ ), and beta-alanine group ( $10.6 \pm 0.93$ ). This parameter was usually regarded as a measure of locomotor activity. There were significant differences by ANOVA ( $F(2,24) = 3.562$ ,

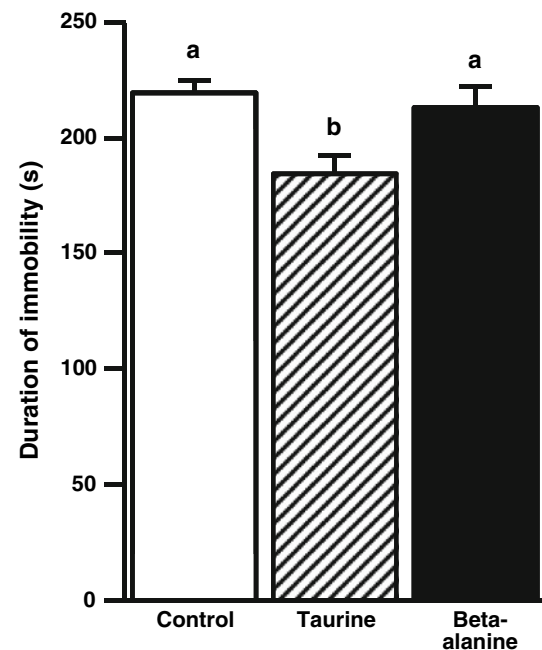


**Fig. 1** The effects of taurine- and beta-alanine-supplemented diets on the percentage of time spent (**a**) and the entries (**b**) in the open arms in the elevated plus-maze test. Data express mean + SEM of 9–10 mice per group. Groups with different letters are significantly different ( $P < 0.05$ )

$P = 0.455$ ); however, no significant differences was shown among three groups by Tukey–Kramer’s test.

#### Duration of immobility in the forced swimming test

The effect of taurine- or beta-alanine-supplemented diet on the duration of immobility in the forced swimming test is



**Fig. 2** The effects of taurine- and beta-alanine-supplemented diets on the duration of immobility in the forced swimming test. Data express mean + SEM of 9–10 mice per group. Groups with different letters are significantly different ( $P < 0.05$ )

shown in Fig. 2. Taurine group significantly reduced the duration of immobility compared to the other two groups ( $F(2,25) = 6.785$ ,  $P = 0.0044$ ).

#### Monoamine concentrations in the cerebral cortex and hypothalamus

The concentrations of each monoamine and its metabolite in the cerebral cortex and hypothalamus are shown in Table 1. In the cerebral cortex, taurine- or beta-alanine-supplemented diet did not change the monoamine concentrations. In contrast, 5-HIAA concentration was significantly decreased by beta-alanine-supplemented diet in the hypothalamus ( $F(2,24) = 4.518$ ,  $P = 0.0216$ ).

#### Amino acid concentrations in the cerebral cortex and hypothalamus

The concentrations of free amino acids in the cerebral cortex and hypothalamus are shown in Table 2. By taurine-supplemented diet, hypothalamic taurine ( $F(2,24) = 7.462$ ,  $P = 0.0030$ ) and L-Arg ( $F(2,23) = 16.351$ ,  $P < 0.0001$ ) concentrations were increased compared to the other two groups. On the other hand, increased carnosine concentration was found in the cerebral cortex ( $F(2,24) = 12.673$ ,  $P = 0.0002$ ) and hypothalamus ( $F(2,24) = 27.713$ ,  $P < 0.0001$ ) in the beta-alanine group compared to the other two groups. These results might not be affected by



**Table 1** The effects of taurine- and beta-alanine-supplemented diets on the concentrations of monoamines and their metabolites in the cerebral cortex and hypothalamus

	NA	MHPG	5-HT	5-HIAA
Cerebral cortex				
Control	27.6 ± 1.3	6.92 ± 0.72	52.4 ± 1.9	14.0 ± 0.4
Taurine	22.5 ± 1.6	6.32 ± 0.58	50.7 ± 1.9	14.4 ± 0.2
Beta-alanine	23.3 ± 1.9	8.31 ± 0.43	48.8 ± 2.3	13.4 ± 0.4
Hypothalamus				
Control	194 ± 6	ND	142 ± 7	71.4 ± 3.6 <sup>a</sup>
Taurine	204 ± 10	ND	154 ± 7	70.9 ± 2.3 <sup>a</sup>
Beta-alanine	190 ± 9	ND	143 ± 5	61.3 ± 1.7 <sup>b</sup>

The numbers of samples used for analysis were 8–10. Values are means  $\mu\text{mol/kg}$  wet tissue  $\pm$  SEM

NA noradrenaline, MHPG 3-methoxy-4-hydroxyphenylglycol, 5-HT serotonin, 5-HIAA 5-hydroxyindoleacetic acid, ND not detectable

Groups with different letters are significantly different ( $P < 0.05$ )

plasma amino acid concentrations because the alterations of some brain amino acid concentrations were not in accordance with those of the plasma. Plasma taurine concentration was significantly increased in taurine group ( $F(2,25) = 121.763$ ,  $P < 0.0001$ ): control group ( $162 \pm 5$ ), taurine group ( $388 \pm 19$ ), and beta-alanine group ( $141 \pm 8$ ), and this result was in accordance with the hypothalamus. Plasma beta-alanine concentration was increased in beta-alanine group ( $F(2,24) = 6.061$ ,  $P = 0.0074$ ): control group ( $2.31 \pm 0.18$ ), taurine group ( $2.90 \pm 0.24$ ), and beta-alanine group ( $5.25 \pm 1.05$ ). However, no significant difference was shown in beta-alanine concentration of the cerebral cortex or hypothalamus. Therefore, the plasma amino acids concentrations might not affect the tissue measurement.

## BDNF concentration in the hippocampus

The concentration of BDNF in the hippocampus is shown in Fig. 3. The beta-alanine group had increased BDNF concentration compared to the other two groups ( $F(2,25) = 5.223$ ,  $P = 0.0127$ ).

## Discussion

Plasma taurine concentration was significantly increased in taurine group compared to both controls and beta-alanine group. In contrast, plasma beta-alanine concentration was significantly increased in beta-alanine group compared to both controls and taurine group. These significant increases in taurine and beta-alanine corresponded to the reduction of the duration of immobility and the increase of the percentage of time spent and entries in the open arms, respectively.

The taurine group had reduced duration of immobility in the forced swimming test. This reduction was not attributed to increased locomotor activity because the distance of pass in the open field test was not altered by taurine-supplemented diet. The forced swimming test has been recognized as a useful experimental model for screening antidepressant activity, as it is sensitive to a wide range of antidepressants, including tricyclic antidepressants and monoamine oxidase inhibitors. The present study was the first case to demonstrate that chronic ingestion of taurine-supplemented diet had an antidepressant-like effect. However, taurine treatment had no effect on the behavior in the forced swimming test (Whirley and Einat 2008) in which mice were injected with taurine for only 3 days before the test and were housed singly from the start to the end of the experiment. It was revealed that housing mice

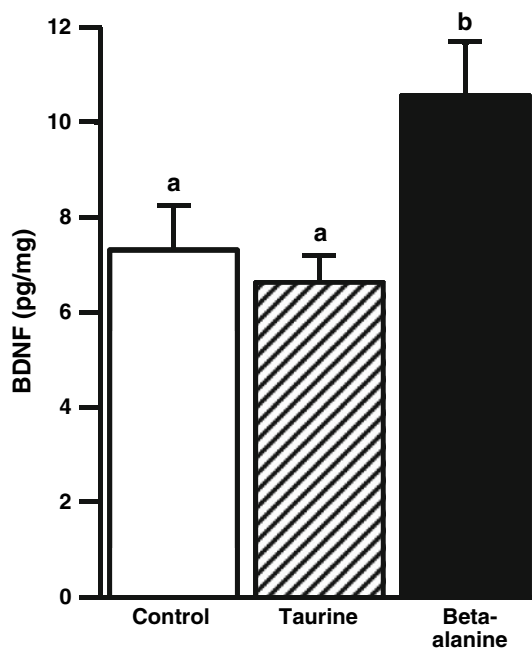
**Table 2** The effects of taurine- and beta-alanine-supplemented diets on the concentrations of free amino acids in the cerebral cortex and hypothalamus

	Taurine	Beta-alanine	L-Arg	Carnosine	GABA	Gly
Cerebral cortex						
Control	7,306 ± 275	41.1 ± 1.7	70.2 ± 1.4	16.5 ± 0.8 <sup>a</sup>	550 ± 12	520 ± 19
Taurine	7,880 ± 337	40.6 ± 2.1	76.8 ± 2.4	15.3 ± 1.0 <sup>a</sup>	571 ± 14	541 ± 17
Beta-alanine	7,205 ± 190	44.1 ± 1.5	71.5 ± 3.7	22.0 ± 1.2 <sup>b</sup>	573 ± 17	569 ± 23
Hypothalamus						
Control	5,224 ± 171 <sup>a</sup>	132 ± 4	161 ± 4 <sup>a</sup>	45.3 ± 0.9 <sup>a</sup>	5,375 ± 158	1,240 ± 28
Taurine	6,189 ± 253 <sup>b</sup>	132 ± 3	181 ± 5 <sup>b</sup>	37.3 ± 1.9 <sup>a</sup>	5,718 ± 193	1,191 ± 25
Beta-alanine	5,245 ± 168 <sup>a</sup>	134 ± 2	145 ± 4 <sup>a</sup>	67.6 ± 4.6 <sup>b</sup>	5,466 ± 117	1,263 ± 43

The numbers of samples used for analysis were 8–10. Values are means  $\mu\text{mol/kg}$  wet tissue  $\pm$  SEM

L-Arg L-arginine, GABA gamma-aminobutyric acid, Gly glycine

Groups with different letters are significantly different ( $P < 0.05$ )



**Fig. 3** The effects of taurine- and beta-alanine-supplemented diets on the concentration of BDNF in hippocampus homogenates. Data express mean + SEM of 9–10 mice per group. Groups with different letters are significantly different ( $P < 0.05$ )

individually decreased antidepressant sensitivity in the forced swimming test (Karolewicz and Paul 2001). In the present study, mice were reared in pairs and given the powder diet containing 22.5 mmol taurine per kg diet for 32 days. Therefore, it was clear that chronic ingestion of taurine-supplemented diet with two mice per cage housing had an antidepressant-like effect in the forced swimming test.

As a treatment for depression or depression-like behavior, NA and 5-HT have been given much attention. However, taurine-supplemented diet did not modify brain NA and 5-HT or their major metabolite MHPG and 5-HIAA concentrations. On the other hand, taurine-supplemented diet increased hypothalamic taurine concentration. In the central nervous system, taurine has a significant neuroprotective function and taurine deficiency results in neurodevelopmental pathology (Birdsall 1998). In addition, taurine has a role as an inhibitory neuromodulator acting GABA and Gly receptors (Oja and Saransaari 1996). Especially, GABA receptors were considered to be related to antidepressant-like effects (Aley and Kulkarni 1989). However, taurine function mentioned above appears when it is released into the synaptic cleft or intercellular space. Further study is needed to discriminate the extracellular taurine concentration from intracellular.

Moreover, L-Arg concentration in the hypothalamus was increased by taurine-supplemented diet. One of the most important functions of L-Arg is the precursor of nitric oxide

(NO), which has an antidepressant-like effect in animal models of depression (da Silva et al. 2000). Thus, L-Arg was also reported to have an antidepressant-like effect (Inan et al. 2004). In the present experiment, NO might be efficiently produced in the taurine group through L-Arg when the forced swimming test was conducted. Interestingly local NO-administration in the hypothalamic supra-optic nucleus increased local extracellular taurine concentrations that may affect the regulation of the endocrine stress response (Engelmann et al. 2002).

Beta-alanine-supplemented diet increased the percentage of time spent and entries in the open arms significantly in the elevated plus-maze test. This result suggested that chronic ingestion of beta-alanine-supplemented diet had an anxiolytic-like effect in mice.

In the hypothalamus, beta-alanine-supplemented diet decreased the concentration of 5-HIAA. 5-HIAA is considered to be metabolized from 5-HT, which is implicated to be related to the animal model of anxiety (Handley and McBlane 1993). Moreover, by beta-alanine-supplemented diet, increases in carnosine concentration in the cerebral cortex and hypothalamus were also observed. Carnosine, a dipeptide consisting of beta-alanine and L-histidine, has several functions including antioxidant activities (Kohen et al. 1988), buffering capacities (Abe 2000), and putative neurotransmitters action in the brain (Tomonaga et al. 2004, 2005).

Furthermore, the concentration of BDNF in the hippocampus was increased in the beta-alanine group. BDNF appears to be related not only to depression but also anxiety. For example, variant BDNF mice show increased anxiety-related behaviors (Chen et al. 2006). Furthermore, rats treated with BDNF in the hippocampus showed a clear tendency to spend a greater amount of time in the open arms in the elevated plus-maze test (Cirulli et al. 2004).

It is reported that chronic beta-alanine administration could decrease taurine concentration in the brain (Parildar-Karpuzoğlu et al. 2007). Taurine and beta-alanine are included in the beta-amino acid group, and they are antagonistic to each other at the blood–brain barrier (Takeuchi et al. 2000). Thus, it is considered that the increase of beta-alanine concentration in blood induces the decrease of taurine concentration in the brain. However, our data could not confirm these facts. Indeed, the beta-alanine concentration in the brain was not increased. Hence, taurine could pass through the blood–brain barrier without the antagonism.

In conclusion, taurine-supplemented diet had an antidepressant-like effect in the forced swimming test and beta-alanine-supplemented diet had an anxiolytic-like effect in the elevated plus-maze test. In addition, taurine-supplemented diet affected the concentrations of taurine and L-Arg in the hypothalamus, and beta-alanine-supplemented diet

affected the concentrations of 5-HIAA in the hypothalamus, carnosine in the cerebral cortex and hypothalamus, and BDNF in the hippocampus. The present study suggested that taurine- and beta-alanine-supplemented diet possibly have beneficial effects on brain functions related to depression- and anxiety-like behavior, respectively. Further studies are needed to clearly separate central from peripheral effects that resulted in the behavioral changes observed.

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