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ORIGINAL ARTICLE

The effects of oral taurine administration on behavior and hippocampal signal transduction in rats

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Abstract Taurine, 2-aminoethylsulfonic acid, is one of the most abundant amino acids in the brain. It has various important physiological functions as a neuromodulator and antioxidant. Taurine is expected to be involved in depression; however, knowledge regarding its function in relation to depression is limited. In this study, we attempted to elucidate the effects of oral taurine administration on antidepressant-like behaviors in rats and depression-related signal transduction in the hippocampus. In behavioral tests, rats fed a high taurine (HT: 45.0 mmol/kg taurine) diet for 4 weeks (HT4w) showed decreased immobility in the forced swim test (FS) compared to controls. However, rats fed a low taurine (LT: 22.5 mmol/kg taurine) diet for 4 weeks or an HT diet for 2 weeks (HT2w) did not show a significant difference in FS compared to controls. In biochemical analyses, the expression of glutamic acid decarboxylase (GAD) 65 and GAD67 in the hippocampus was not affected by taurine administration. However, the phosphorylation levels of extracellular signal-regulated kinase1/2 (ERK1/2), protein kinase B (Akt), glycogen synthase kinase3 beta (GSK3β) and cAMP response element-binding protein (CREB) were increased in the hippocampus of HT4w and HT2w rats. Phospho-calcium/calmodulin-dependent protein kinase II (CaMKII) was increased in the hippocampus of HT4w rats only. Moreover, no significant changes in these molecules were observed in the hippocampus of rats fed an HT diet for 1 day. In conclusion, our findings suggest that taurine has an antidepressant-like effect and an ability to change depression-related signaling cascades in the hippocampus.

Keywords Taurine · Antidepressant · Behavior · CaMKII · Rat

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Introduction

Depression is a serious mental disorder that affects approximately 20 % of the population in the world (Berton and Nestler 2006). To treat depressive disorders, antidepressants such as selective serotonin reuptake inhibitors (SSRIs) have been widely used in developed countries. It is thought that SSRIs mediate their antidepressant effects by acting on serotonergic systems in the brain; however, the functions and effects of SSRIs on the central nervous system (CNS) are largely unknown. It has been reported that SSRIs have several harmful side effects including nausea, anorexia and headache (Vaswani et al. 2003). Recently, Kobayashi et al. reported that chronic administration of fluoxetine, one of the major SSRIs, induced dentate gyrus neurons in mouse hippocampus to become more immature. Dentate gyrus immaturity has been shown to be related to abnormal physiological properties in the hippocampus and to psychiatric disorder-like behaviors (Kobayashi et al. 2010; Kobayashi et al. 2011). Therefore, antidepressants without any severe side effects should be developed to improve quality of life for depressive patients. Diet may be one candidate for the prevention and treatment

of depressive disorders. Dietary nutrients should be widely screened for antidepressant-like activity. However, the antidepressant-like effects of nutrients have not yet been clarified. The identification of such nutrients may raise the possibility of treating or preventing depression through dietary regimens.

Taurine, 2-aminoethylsulfonic acid, is one of the most abundant amino acids in the CNS (Hussy et al. 2000). Taurine transporter (TAUT) is ubiquitously expressed in tissues, including those of the blood–brain barrier (Tamai et al. 1995). Taurine is synthesized from cysteine, and is known to have various important physiological functions such as membrane stabilization, osmoregulation and neuroprotection (Hussy et al. 2000; Timbrell et al. 1995; Tanabe et al. 2010). Furthermore, taurine acts as an agonist for glycine and gamma-aminobutyric acid (GABA) receptors (Albrecht and Schousboe 2005; del Olmo et al. 2000a). In addition, taurine modulates intracellular calcium and calcium signaling molecules in the CNS (Wu and Prentice 2010). Taurine also plays a key role in development. Mice whose mother was infected with human influenza virus during the gestational period suffer from brain atrophy and show a decreased concentration of taurine in the brain (Fatemi et al. 2008). Down syndrome patients have an abnormal concentration of amino acids including taurine and monoamines in the frontal cortex (Whittle et al. 2007).

Previous reports demonstrated that the concentration of taurine in the plasma of depressive patients is increased, while its concentration in the cerebrospinal fluid of schizophrenic patients is decreased (Altamura et al. 1995; Do et al. 1995). Perry et al. reported that families that suffer from a hereditary taurine deficiency have a tendency to develop depression (Perry et al. 1975). These phenomena indicate the possibility that taurine is related to depression and other mental disorders. Moreover, exposure to acute stress with forced swim increased the plasma concentration of taurine in mice (Murakami et al. 2009). Furthermore, taurine administration showed anxiolytic-like effects in mice and rats (Chen et al. 2004; Kong et al. 2006). Recently, Murakami et al. reported that ICR mice fed a taurine-supplemented diet for 4 weeks showed antidepressant-like behaviors (Murakami and Furuse 2010). However, Whirley et al. presented inconsistent results that daily intraperitoneal injections of taurine for 3 or 8 days in C57BL/6 mice had neither antidepressant-like nor anxiolytic-like effects (Whirley and Einat 2008). Therefore, precise studies about the relation between taurine and depression are needed.

Depression is linked to various molecular changes in the CNS. Amino acids are thought to be related to depression. Tryptophan and arginine were reported to have an antidepressant effect on the forced swim test (FS) (Inan et al. 2004; Wong and Ong 2001). In addition, neurotrophic factors have been linked to depression. Brain-derived neurotrophic factor (BDNF) has been implicated in the pathophysiology of stress-related mood disorders, and BDNF expression decreases after exposure to stress, including social-defeat stress (Krishnan and Nestler 2008). Conversely, infusion of BDNF into the hippocampus has shown antidepressant effects (Shirayama et al. 2002). Murakami et al. reported that hippocampal BDNF expression is not influenced by chronic taurine administration (Murakami and Furuse 2010); however, the cascades downstream of BDNF and other extracellular signals related to depression have not been characterized. Depression is related to various molecules in the CNS including extracellular signal-regulated kinase1/2 (ERK1/2), protein kinase B (Akt), glycogen synthase kinase3 beta (GSK3β), calcium/calmodulin-dependent protein kinase II (CaMKII) and cAMP response element-binding protein (CREB) (Barbiero et al. 2007; Iio et al. 2011; Karege et al. 2007; Krishnan and Nestler 2008). These molecules play pivotal roles in depression and other psychiatric disorders.

Mice with a mutation in the ubiquitin-specific peptidase 46 (Usp46) gene showed prolonged mobility in FS (Tomida et al. 2009). An association between the Usp46 gene and major depressive disorder was observed in a haplotype analysis of the Japanese population (Fukuo et al. 2011). Because the 67-kDa isoform of glutamic acid decarboxylase (GAD)67 was decreased in the hippocampus of the Usp46 mutant mice, hippocampal GAD67 is thought to be involved in behavioral despair and antidepressant-like behaviors in FS.

In this study, we observed the effects of oral taurine administration on body weight, food intake, behavioral tests, and the expression and phosphorylation of depression-related proteins in the hippocampus.

Materials and methods

Animals

Five-week-old male Wistar rats were purchased from Charles River (Yokohama, Japan) and housed individually at room temperature (22 ± 1 °C), with lights on from 6:00 to 18:00 with ad libitum access to food and water. After arrival, they were handled daily for 1 week to habituate them to the environment. All experimental procedures followed the Guidelines of the Animal Care and Use Committee of Ibaraki University.

Experimental design and drugs

After acclimation, animals were divided into six groups: control, HT4w, HT2w, HT1d, LT4w, and LT2w. The

control group was fed a normal powder diet (MF; Oriental Yeast, Tokyo, Japan). The HT4w group was fed a high taurine (HT) diet (45.0 mmol taurine/kg diet) for 4 weeks. The HT2w and HT1d groups were fed an HT diet for 2 weeks and 1 day, respectively. The LT4w group was fed a low taurine (LT) diet (22.5 mmol taurine/kg diet) for 4 weeks, and the LT2w group was fed an LT diet for 2 weeks.

Behavioral tests

All behavioral tests in this study were performed between 13:00 and 17:00 at a room temperature of 22 ± 1 °C and a light intensity of 70 lx.

Open field test (OF)

The method for OF was described previously (Iio et al. 2011). Each subject was placed in the same corner of the open field apparatus. The total distance travelled (in cm), time spent in the center area (in sec) and average speed (in cm/sec) during the 10-min session were recorded, and the results were analyzed on a Windows computer using Image J XX (O'Hara & Co., Ltd.), a modified software program based on the public domain Image J program.

Forced swim test (FS)

The method for FS was described previously (Iio et al. 2011). Each rat was placed into an acrylic cylinder filled with water (24 ± 1 °C) to a height of 18 cm. After 15 min, the animal was transferred to a 35 °C environment for another 15 min (pre-test). Twenty-four hours later, the subject was placed into the cylinder again for 5 min (test). Prior to each test, the cylinder was cleaned and filled with fresh water.

Body weight and food intake

Body weight and food intake were measured at the end of the acclimation phase (baseline) and at weekly intervals during taurine administration. Body weight gain was calculated by subtracting baseline body weight from weight at the end of each week.

Protein preparation and western blotting

After behavioral tests, all animals were subjected to biochemical analyzes. On killing, the rat's brains were rapidly removed and chilled on ice and the hippocampi were dissected out. The tissue was homogenized in ice-cold RIPA buffer [50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1 % NP-40, 0.75 % sodium deoxycholate, 1 mM EDTA, 100 mM NaF, 2 mM Na_3VO_4 and a protease inhibitor mix (GE Healthcare)] with a Polytron homogenizer. The homogenate was centrifuged at 800×*g* for 15 min at 4 °C and the supernatant was collected. Protein concentration was determined using the BCA method (Thermo). The method of western blotting followed the protocols of the ECL plus western blotting detection reagents (GE Healthcare), except for the incubation time of the primary antibody. We changed the incubation time of the primary antibody from 1 h to overnight. Detection was performed using the ECL plus western blotting detection reagents and LAS-3000 mini (FUJIFILM). Antibodies are shown in Table 1. The results of western blotting were quantitatively analyzed using Image J.

Table 1 The antibodies used for western blotting in this study

Antibody	Company	Cat. No.	Blocking reagent	Dilution
ERK1/2	Invitrogen	136200	5 % Skim milk/TBST	1:2,500
pERK1/2 (Thr202/Thr202)	Cell Signaling	#9101	5 % BSA/TBST	1:2,000
Akt	Milli Pore	#05-591	5 % Skim milk/TBST	1:2,500
pAkt (Thr308)	Cell Signaling	#2965	5 % BSA/TBST	1:2,500
GSK3β	BD Transduction Laboratories	610202	5 % Skim milk/TBST	1:2,000
pGSK3β (Ser9)	Cell Signaling	#9336	5 % BSA/TBST	1:1,000
CaMKll	Cell Signaling	MAB8699	5 % Skim milk/TBST	1:2,000
pCaMKII (Thr286)	Thermo	MA1-047	5 % BSA/TBST	1:1,000
CREB	Milli Pore	#05-757	5 % Skim milk/TBST	1:1,000
pCREB (Ser133)	Cell Signaling	#9191	5 % BSA/TBST	1:1,000
GAD65&67	Milli Pore	AB1511	5 % Skim milk/TBST	1:5,000
GAPDH	Santa Cruz	sc-32233	5 % Skim milk/TBST	1:5,000
Goat Anti-Mouse IgG (H + L)	Jackson ImmunoResearch	115-035-003		1:5,000
Goat Anti-Rabbit IgG (H + L)	Jackson ImmunoResearch	111-035-003		1:5,000

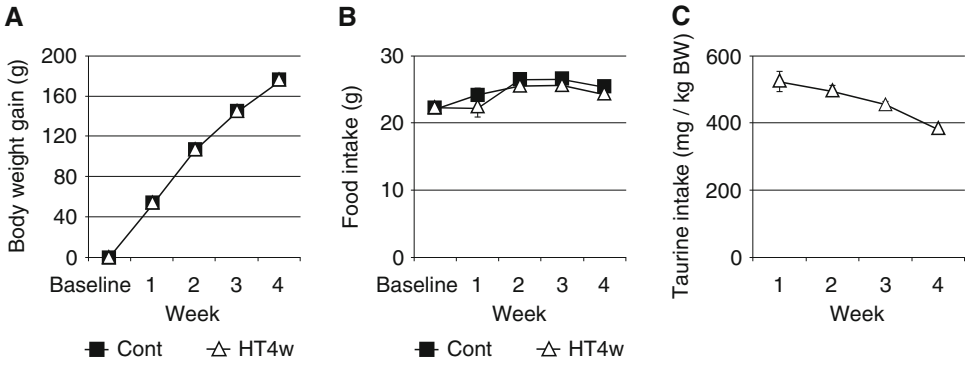

Fig. 1 The effects of 4 weeks of taurine administration on body weight gain and food intake. **a** Body weight gain, **b** food intake and **c** taurine intake. Data represent the mean ± SEM ($n = 10$/group). *Cont* control group, *HT4w* group fed a high taurine diet for 4 weeks

Statistical analysis

Data were analyzed using Excel Toukei 2006 for Windows (Social Survey Research Information Co., Ltd. Tokyo, Japan). The Western blotting data were analyzed using Student's *t* tests. The behavioral tests were analyzed using one-way ANOVAs and Bonferroni post hoc analysis. Body weight gain and food intake were analyzed using two-way repeated measures ANOVAs.

Results

The effects of chronic taurine administration on body weight gain and food intake

Our results showed that chronic taurine administration does not affect body weight gain (Fig. 1a). Before taurine administration, the body weights of both HT4w and control rats were similar (175.0 ± 1.9 g vs. 171.0 ± 2.1 g, $P = 0.1780$). A two-way repeated measures ANOVA revealed that taurine administration (F (1, 72) = 0.026, $P = 0.8748$) and taurine administration × time interaction (F (4, 72) = 0.042, $P = 0.9966$) had no significant effect on body weight gain. Furthermore, taurine supplementation did not affect food intake (Fig. 1b). A two-way repeated measures ANOVA revealed that taurine administration (F (1, 72) = 0.023, $P = 0.8805$) and taurine administration × time interaction (F (4, 72) = 1.283, $P = 0.2846$) had no significant effect on food intake. HT4w rats were fed taurine at 462.8 ± 12.0 mg/kg body weight/day during this study (Fig. 1c).

The effects of chronic taurine administration on behavior

We observed a concentration-dependent effect of taurine administration on rat behavior. HT4w rats showed decreased immobility in FS compared to controls (Fig. 2d, $P = 0.0430$). However, LT4w rats did not show any significant differences in FS compared to control or HT4w rats (Fig. 2d, control vs. LT4w; $P = 1.0000$, LT4w vs. HT4w; $P = 0.2441$). Furthermore, different taurine concentrations in the diet did not affect activity in any of these parameters of OF: total distance travelled (Fig. 2a, control vs. LT4w; $P = 1.0000$, control vs. HT4w; $P = 0.4966$, LT4w vs. HT4w; $P = 1.0000$), time spent in the center area (Fig. 2b, control vs. LT4w; $P = 1.0000$, control vs. HT4w; $P = 0.1728$, LT4w vs. HT4w; $P = 0.2994$), and average speed (Fig. 2c, control vs. LT4w; $P = 1.0000$, control vs. HT4w; $P = 0.5172$, LT4w vs. HT4w; $P = 0.9981$). Then, we observed the effect of 2 weeks of taurine administration on rat behavior. Rats in all groups did not show any significant difference in immobility in FS

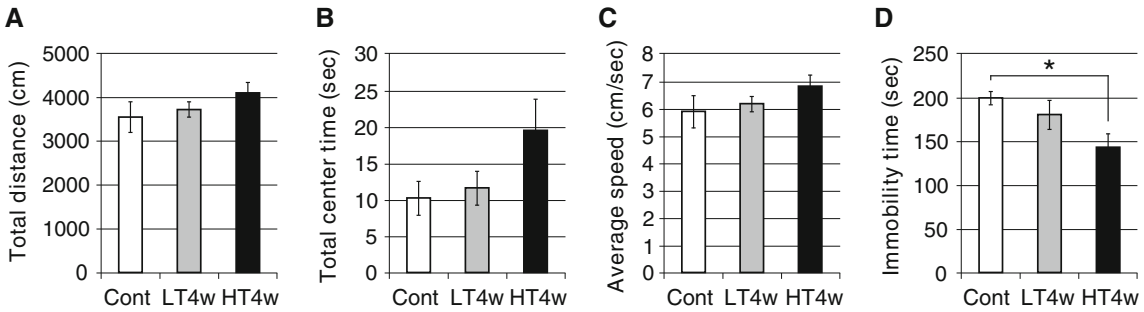

Fig. 2 The effects of 4 weeks of taurine administration on behavior. **a** Total distance, **b** total center time and **c** average speed during a 10-min test in OF. **d** Immobility time during 5-min test in FS. Data represent the mean ± SEM ($n = 5$/group); $^*P < 0.05$ (Bonferroni post hoc test). *Cont* control group, *LT4w* group fed a low taurine diet for 4 weeks, *HT4w* group fed a high taurine diet for 4 weeks

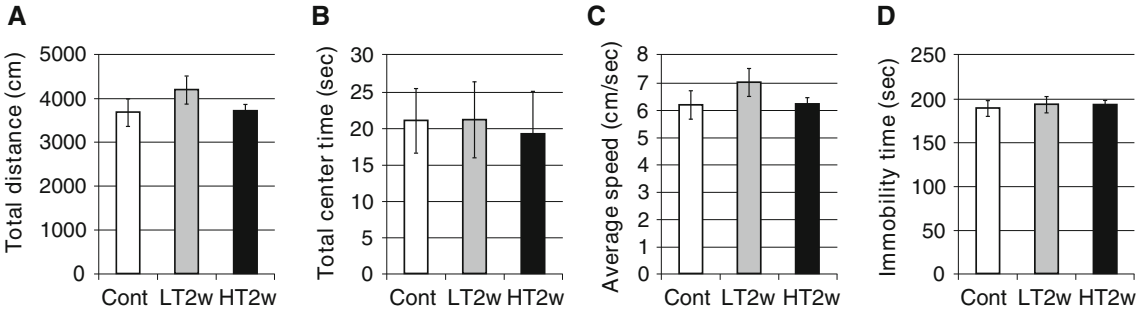

Fig. 3 The effects of 2 weeks of taurine administration on behavior. **a** Total distance, **b** total center time and **c** average speed during a 10-min test in OF. **d** Immobility time during 5-min test in FS. Data represent the mean ± SEM ($n = 5$/group). *Cont* control group, *LT2w* group fed a low taurine diet for 2 weeks, *HT2w* group fed a high taurine diet for 2 weeks

(Fig. 3d, control vs. LT2w; $P = 1.0000$, control vs. HT2w; $P = 1.0000$, LT2w vs. HT2w; $P = 1.0000$). Furthermore, 2 weeks of taurine administration had no significant effect on these parameters of OF: total distance travelled (Fig. 3a, control vs. LT2w; $P = 0.6185$, control vs. HT2w; $P = 1.0000$, LT2w vs. HT2w; $P = 0.6946$), time spent in the center area (Fig. 3b, control vs. LT2w; $P = 1.0000$, control vs. HT2w; $P = 1.0000$, LT2w vs. HT2w; $P = 1.0000$), and average speed (Fig. 3c, control vs. LT2w; $P = 0.6416$, control vs. HT2w; $P = 1.0000$, LT2w vs. HT2w; $P = 0.6736$).

The effects of chronic and acute taurine administration on hippocampal protein expression and phosphorylation

We assessed the effect of taurine administration on the expression of both GAD65 and GAD67 in the hippocampus. We defined HT1d, HT2w, and HT4w rats as acute-, subchronic-, and chronic-administrated rats, respectively. In our results, taurine administration did not affect the expression of either GAD65 or GAD67 in the hippocampus (Fig. 4, 4 weeks GAD65; $P = 0.3506$, GAD67; $P = 0.9759$, 2 weeks GAD65; $P = 0.9434$, GAD67; $P = 0.7155$, 1 day GAD65; $P = 0.8896$, GAD67; $P = 0.1817$). We then observed the effects of taurine administration on the expression and phosphorylation of important depression-related molecules in the hippocampus. First, we examined the expression and phosphorylation of ERK1/2 and CREB. The mitogen-activated protein kinase (MAPK)-CREB cascade plays a pivotal role in depression (Berton and Nestler 2006; Iio et al. 2011). In our results, the ratios of phospho-ERK1/ERK1, phospho-ERK2/ERK2, and phospho-CREB/CREB were increased in the hippocampus of HT4w rats compared to controls (Fig. 5c, phospho-ERK1/ERK1; $P = 0.0032$, phospho-ERK2/ERK2; $P = 0.0066$, phospho-CREB/CREB; $P = 0.0008$). Similar results were obtained in the hippocampus of HT2w rats (Fig. 5b phospho-ERK1/ERK1; $P = 0.0343$, phospho-ERK2/ERK2; $P = 0.0168$, phospho-CREB/CREB; $P = 0.0002$). However, the MAPK–CREB cascade was not changed in the hippocampus of HT1d rats (Fig. 5a, phospho-ERK1/ERK1; $P = 0.7448$, phospho-ERK2/ERK2; $P = 0.9737$, phospho-CREB/CREB; $P = 0.9938$). Next, we observed the expression and phosphorylation of Akt and GSK3β in the hippocampus. The phosphatidylinositol 3-kinase (PI3K)-Akt-CREB cascade in the hippocampus is also affected by mood disorders (Karege et al. 2007). In our results, the ratios of phospho-Akt/Akt and phospho-GSK3β/GSK3β were increased in the hippocampus of HT4w rats compared to controls (Fig. 5c, phospho-Akt/Akt; $P = 0.0010$, phospho-GSK3β/GSK3β; $P = 0.0093$). Similar results were obtained in the hippocampus of HT2w rats (Fig. 5b, phospho-Akt/Akt; $P = 0.0187$, phospho-GSK3β/GSK3β; $P = 0.0123$). However, the PI3K-Akt cascade was not changed in the hippocampus of HT1d rats compared to controls (Fig. 5a, phospho-Akt/Akt; $P = 0.5117$, phospho-GSK3β/GSK3β; $P = 0.6296$). In addition, we assessed the expression and phosphorylation of CaMKII, which is affected by antidepressant treatment (Barbiero et al. 2007). In our results, the ratio of

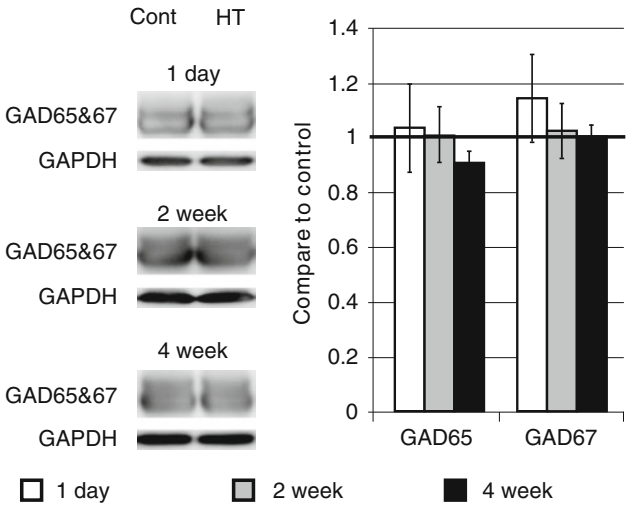

Fig. 4 The effects of taurine administration on the expression of GAD65 and GAD67 in the hippocampus. Data revealed the ratio of each molecule compared to the control group. Data represent the mean ± SEM. *Cont* control group, *HT* groups fed a high taurine diet

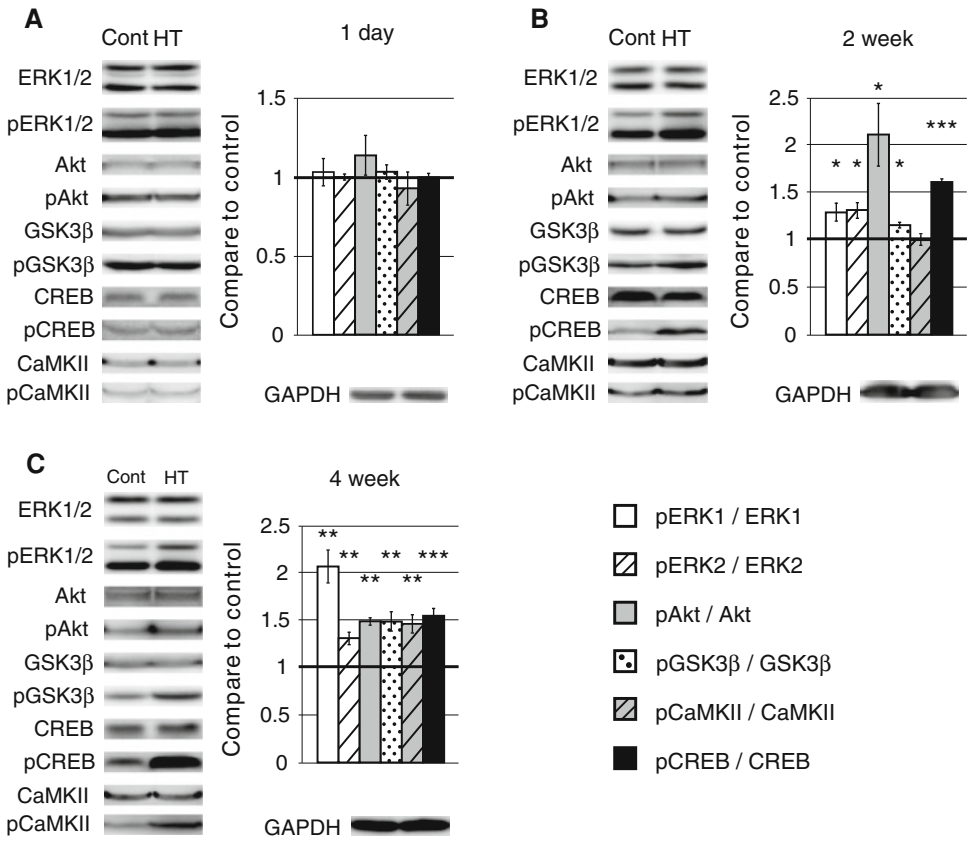

Fig. 5 The effects of taurine administration on several signaling cascades in the hippocampus. **a** Western blotting data for proteins from the hippocampus of rats administered taurine for 1 day, **b** for 2 weeks and **c** for 4 weeks, respectively. Data revealed the ratio of each molecule compared to that of its control group. Data represent the mean ± SEM. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ (Student's *t* test). *Cont* control group, *HT* groups fed a high taurine diet

phospho-CaMKII/CaMKII was increased in the hippocampus of HT4w rats compared to controls (Fig. 5c, phospho-CaMKII/CaMKII; $P = 0.0052$). However, the ratio of phospho-CaMKII/CaMKII was not changed in the hippocampus of HT2w or HT1d rats compared to controls (Fig. 5a, b, 2 weeks phospho-CaMKII/CaMKII; $P = 0.8850$, 1 day phospho-CaMKII/CaMKII; $P = 0.6176$). The protein expression levels of the aforementioned molecules were not changed between control and taurine-supplemented rats (Fig. 5a–c).

Discussion

We investigated the function of oral taurine administration on behaviors and signal transduction in the rat hippocampus. Specifically, we focused on characterizing the antidepressant-like effects of taurine using behavioral and biochemical approaches. A previous report showed that taurine has an antidepressant-like effect because mice fed a taurine-supplemented diet for 4 weeks displayed decreased immobility in FS (Murakami and Furuse 2010). However, another report indicated that mice given short-term taurine injections did not show antidepressant-like behaviors (Whirley and Einat 2008). These studies were carried out using different experimental methods; therefore, the inconsistency of the results was dependent on differences in experimental animal strains, methods and durations of taurine administration, among other factors. In this study, we confirmed an antidepressant-like effect of taurine using our original experimental methods with Wistar rats that were reared individually. Murakami et al. mentioned that mice were reared in pairs to observe an antidepressant-like effect of taurine because housing mice individually decreased antidepressant sensitivity in FS (Murakami and Furuse 2010). However, in our study, we observed an antidepressant-like effect of a taurine-containing diet (45 mmol taurine/kg diet for 4 weeks) in FS with Wistar rats (Fig. 2d). Taurine intake at 22 mmol/kg diet by ICR mice was nearly equaled to its intake at 45 mmol/kg by Wistar rats (Fig. 1c). Therefore, the duration of taurine administration and taurine concentration in the diet may be essential for revealing an antidepressant-like effect of taurine in FS. We observed the effects of chronic taurine administration on body weight gain and food intake in rats for the following reasons. If rats dislike eating taurine-containing diets and their food intake is decreased, the total calories that rats take from foodstuff are decreased. Previous reports described that calorie-restricted mice show antidepressant-like behaviors (Lutter et al. 2008), and that this antidepressant-like effect of caloric restriction was dependent on neuropeptides related to feeding behaviors, such as orexin and ghrelin (Berton and Nestler 2006; Lutter

et al. 2008). The taurine-containing diet (45.0 mmol taurine/kg diet) did not affect body weight gain or food intake in rats (Fig. 1a, b); thus, the results indicate that our behavioral and biochemical studies using taurine-fed rats are not influenced by the antidepressant-like effects of caloric restriction.

Next, we observed the effects of chronic taurine administration on rat behavior. The taurine-containing diet did not influence behaviors in OF (Fig. 2a, b, c). HT4w rats showed antidepressant-like behavior in FS (Fig. 2d), while HT2w rats did not (Fig. 3d). These results indicate that the antidepressant-like effects of taurine are not revealed after two successive weeks of administration, but that four successive weeks of administration are needed to reveal an antidepressant-like effect of taurine. Moreover, because LT4w rats did not show antidepressant-like behavior in FS (Fig. 2d), four successive weeks of taurine administration are insufficient to reveal antidepressant-like activity in rats. An HT diet may be necessary to obtain antidepressant-like effects of taurine in rats because the HT diet contained taurine at 45.0 mmol taurine/kg diet. Taurine supplementation is known to increase exercise performance. Rats fed with taurine at 100 mg taurine/kg body weight/day for 2 weeks increased exercise performance on a treadmill (Miyazaki et al. 2004; Yatabe et al. 2003). In our study, the rats fed with taurine at approximately 400–500 mg taurine/kg body weight/day for 4 weeks did not change their locomotor activity or average speed in OF (Figs. 1c, 2a, c). Therefore, OF was not influenced by the ability of taurine to improve physical endurance. Our FS data were also not influenced by this ability of taurine because taurine improves physical endurance with a dose less than that of a taurine-supplemented diet (Miyazaki et al. 2004; Yatabe et al. 2003). Therefore, we think that chronic taurine administration shows antidepressant-like effects in rats. The dose of taurine and duration of its administration are critical to reveal the antidepressant-like effects of taurine. Similarly, chronic exposure to antidepressants is required for clinical utility in depressive patients, while acute exposure is insufficient for recovery from depression. The action mechanisms of antidepressants are not yet completely understood. A previous report described that chronic imipramine (a tricyclic antidepressant) modifies the chromatin structure of the BDNF gene promoter, enhances BDNF expression in the hippocampus, and reverses depression-like behaviors in socially defeated mice (Tsankova et al. 2006).

Because TAUT is highly expressed in hippocampal CA3, taurine is implicated in significant functions in the hippocampus (Sergeeva et al. 2003). The antidepressant actions of chronic taurine may be based on long-term modifications in neurotransmitters and/or signal transduction in the hippocampus. Due to these estimations, we focused on signal transduction and enzymes related to depression in the hippocampus.

We observed the effects of chronic taurine administration on the GABAergic system in the hippocampus. Taurine acts as a neuromodulator and functions as an agonist for glycine and GABA receptors (Albrecht and Schousboe 2005; del Olmo et al. 2000a). Tomida et al. reported that Usp46 mutant mice show negligible immobility in FS and the tail suspension test. These anti-immobile behaviors in Usp46 mutant mice were dependent on the decreased expression of GAD67 in the hippocampus (Tomida et al. 2009). Furthermore, the Usp46 gene is implicated in major depression (Fukuo et al. 2011). Because immobility in FS has been linked to the regulation of GABA action in the hippocampus, we observed the expression of both GAD65 and GAD67 in the hippocampus of rats that were chronically administered taurine. Chronic taurine administration did not affect the expression of either GAD65 or GAD67 in the hippocampus (Fig. 4). Thus, the antidepressant-like effects of taurine are not based on the downregulation of GAD65 and GAD67 in the hippocampus.

Chronic taurine administration affected the phosphorylation of several key molecules related to depression in the hippocampus. In both HT4w and HT2w rats, increased phosphorylation of ERK1/2, Akt (Thr-308), GSK3β (Ser-9), and CREB (Ser-133) was observed in the hippocampus compared to control rats (Fig. 5b, c). Moreover, phosphorylation of CaMKII (Thr-286) was increased in HT4w rats (Fig. 5c). However, no significant change in the phosphorylation of these molecules was observed in HT1d rats (Fig. 5a). Acute oral administration of taurine could not induce profound effects on signal transduction in the hippocampus, but the long-term administration of taurine induced several profound changes in depression-related signal transduction. CREB, which is one of the molecules downstream of MAPK, serotonin (5-HT) and BDNF, plays a pivotal role in depression (Tsankova et al. 2006). Phospho-ERK1/2 and phospho-CREB were increased in the hippocampus after 2 weeks of taurine administration (Fig. 5b, c). A previous report showed that hippocampal ERK1/2 and CREB are activated by treatment with an antidepressant (Qi et al. 2006). Also, the expression of BDNF in the hippocampus is upregulated with antidepressant treatment, as described above (Tsankova et al. 2006). Furthermore, acute infusion of BDNF in the hippocampus induced antidepressant effects in a behavioral model of depression (Shirayama et al. 2002). BDNF also facilitates the PI3K-Akt cascade in the hippocampus (Zheng and Quirion 2004). Akt is activated by PI3K with its phosphorylation of Thr-308 and Ser-473, and then phospho-Akt phosphorylates the Ser-133 of CREB and the Ser-9 of GSK3β (Du and Montminy 1998). Phospho-CREB (Ser-133) is an active transcriptional form.

Phospho-GSK3β (Ser-9) is an inactive form of the kinase, and dephosphorylated-GSK3β phosphorylates the Ser-129 of CREB. Finally, phospho-CREB (Ser-129) has an attenuated DNA-binding activity, and its transcriptional activity is decreased (Grimes and Jope 2001). In our observations, both phospho-Akt (Thr-308) and phospho-GSK3β (Ser-9) were increased in the hippocampus of rats administered taurine for 2 weeks (Fig. 5b, c). Phospho-Akt (Ser-473) was also increased in the hippocampus with 2 weeks of taurine administration (data not shown). However, these molecules might not play an essential role in the antidepressant-like effect of taurine, because the phosphorylation of Akt, GSK3β, and CREB with taurine administration was observed in HT2w rats, which did not show any significant change in antidepressant-like behavior compared to controls (Fig. 5b). Moreover, Murakami et al. reported that the expression of the BDNF protein in the hippocampus of mice fed a taurine-containing diet for 4 weeks was not altered (Murakami and Furuse 2010). Taurine could possibly activate MAPK and PI3K cascades via a BDNF-independent pathway in the hippocampus, although these cascades may be not necessary to reveal an antidepressant-like effect of taurine.

CaMKII is an abundant serine/threonine protein kinase in the brain. The kinase is activated by the binding of the calcium/calmodulin complex that generates calcium-dependent enzymatic activity. CaMKII plays pivotal roles in synaptic plasticity, the process underlying learning and memory in the hippocampus (Silva et al. 1992a, b). In this study, we found that phospho-CaMKII was increased only in HT4w rats that revealed an antidepressant-like behavior in FS (Fig. 2d), while it was not observed in other groups of rats fed a taurine-supplemented diet. Therefore, the increase in phospho-CaMKII in the hippocampus may be critical to the antidepressant-like effect of taurine. CaMKII is also implicated in the pathophysiology and pharmacology of psychiatric disorders, because post-mortem brain studies of patients with bipolar or unipolar depression indicate significantly reduced CaMKII mRNA levels in certain brain regions (Xing et al. 2002). α-CaMKII-deficient mice exhibit abnormal behaviors resembling schizophrenia and other human psychiatric disorders (Yamasaki et al. 2008). Thus, CaMKII has been found to be one of the target molecules for antidepressants. Chronic treatment with antidepressants increased CaMKII activity in the hippocampus, but acute treatments did not induce any change in the kinase (Barbiero et al. 2007; Tiraboschi et al. 2004). Also, chronic treatment with antidepressants increased the phosphorylation of CaMKII (Thr286) in neuronal cell bodies in the hippocampus (Tiraboschi et al. 2004). However, chronic treatment with antidepressants downregulated the phosphorylation of CaMKII (Thr286) in synaptic terminals and synaptic membranes in the hippocampus. The decrease in CaMKII phosphorylation reduced its interaction with syntaxin-1, thereby changing protein–protein interactions at glutamatergic presynaptic terminals and reducing depolarization-evoked glutamate release (Barbiero et al. 2007; Bonanno et al. 2005). However, a previous report indicated that short-term treatment with taurine inhibited CaMKII activity in the hippocampus (Junyent et al. 2010). Therefore, there may be some different physiological mechanisms between the short- and long-term applications of taurine. Recently, Han et al. reported that chronic treatment with nefiracetam, a prototype cognitive enhancer, significantly improved depression-like behaviors in olfactory bulbectomized (OBX) mice, one of the popular animal models of depression. The improvement of depression-like behaviors was associated with activation of CaM kinases, including CaMKII, in the hippocampus, amygdala and prefrontal cortex. In addition to CaMKII autophosphorylation, CaMKI and CaMKIV may be required to counteract depressive behaviors through CREB phosphorylation in OBX mice (Han et al. 2009). Furthermore, CaMKIV knockout mice and calcineurin knockout mice showed symptoms like mood disorders (Miyakawa et al. 2003; Takao et al. 2010). Taurine plays a crucial role in cellular calcium homeostasis (Junyent et al. 2010), and chronic taurine administration may have antidepressant-like activities due to activation of CaMKII in the hippocampus.

We found that chronic taurine administration has a strong ability to induce modifications in various signaling cascades in the hippocampus, as described above, although the precise mechanisms remain unclear. Because taurine acts as an agonist for glycine and GABA receptors, neuronal activities in the hippocampus and in other brain regions may be changed by oral taurine administration (del Olmo et al. 2000a). Taurine also induces a long-lasting potentiation of excitatory synaptic potentials in hippocampal slices, which is related to the intracellular accumulation of taurine (del Olmo et al. 2000b, 2003; Galarreta et al. 1996). Taurine-induced synaptic potentiation requires calcium influx and shares some common mechanisms with tetanus-induced long-term potentiation (del Olmo et al. 2000b). Taurine potentiates presynaptic NMDA receptors in hippocampal Schaffer collateral axons (Suárez and Solís 2006). Chronic taurine administration may induce the intracellular accumulation of taurine in hippocampal neurons and modify intracellular calcium concentration and activate CaMKII. Because chronic, but not acute, taurine application was needed to facilitate the phosphorylation of ERK1/2, Akt, GSK3β, CREB, and CaMKII in the hippocampus, intracellular and/or extracellular accumulation of taurine in the brain may be essential to reveal antidepressant-like actions. In addition, some reports have indicated that taurine acts as a trophic factor in neuronal tissues and non-neuronal tissues (Hernández-Benítez et al. 2010; Jeon et al. 2007; Lima and Cubillos 1998). However, the precise function of taurine as a trophic factor in the brain remains to be

elucidated. In future studies, we need to investigate the relationship between taurine supplementation and the expression of neurotrophic factors in the brain, such as nerve growth factor, BDNF and neurotophin 3, and especially we should focus on the transcriptional regulation and epigenetics of these genes. Moreover, there is a possibility that intracellular taurine uptaken by the taurine transporter modifies signal transduction in the hippocampus. Oral taurine administration induces an increase in taurine concentration in various tissues including cerebral cortex and hypothalamus (Miyazaki et al. 2004; Murakami and Furuse 2010). However, hippocampal taurine concentration of taurine-fed rats has not been analyzed in this study. And oral taurine administration did not affect the concentration of serotonin in cerebral cortex and hypothalamus, but the serotonin synthesis and release in the hippocampus were not elucidated (Murakami and Furuse 2010). Thus, the effects of oral taurine administration on hippocampal taurine metabolism and serotonergic system should be investigated in the future studies. Intracellular taurine accumulation may be essential for inducing the modification of signal transduction in the hippocampus, although the precise mechanism should be investigated.

In conclusion, we found that chronic taurine administration at 45 mmol taurine/kg diet for 4 weeks induces antidepressant-like effects in rats. The beneficial effects of chronic taurine administration in rats might be mediated by phosphorylation of CaMKII in the hippocampus. In addition to CaMKII phosphorylation, the increase in hippocampal phospho-ERK1/2, phospho-Akt, phospho-GSK3β, and phospho-CREB may be required to reveal the antidepressant-like activities of taurine. Although taurine has various physiological effects on human health, its antidepressant action may be useful for combating depression. Further clinical study is required to evaluate the application of these findings for human health.

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References

Albrecht J, Schousboe A (2005) Taurine interaction with neurotransmitter receptors in the CNS: an update. Neurochem Res 30:1615–1621

Altamura C, Maes M, Dai J, Meltzer HY (1995) Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. Eur Neuropsychopharmacol 5(Suppl):71–75

Barbiero VS, Giambelli R, Musazzi L, Tiraboschi E, Tardito D, Perez J, Drago F, Racagni G, Popoli M (2007) Chronic antidepressants induce redistribution and differential activation of alphaCaM kinase II between presynaptic compartments. Neuropsychopharmacology 32:2511–2519

Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7:137–151

Bonanno G, Giambelli R, Raiteri L, Tiraboschi E, Zappettini S, Musazzi L, Raiteri M, Racagni G, Popoli M (2005) Chronic antidepressants reduce depolarization-evoked glutamate release and protein interactions favoring formation of SNARE complex in hippocampus. J Neurosci 25:3270–3279

Chen SW, Kong WX, Zhang YJ, Li YL, Mi XJ, Mu XS (2004) Possible anxiolytic effects of taurine in the mouse elevated plus-maze. Life Sci 75:1503–1511

del Olmo N, Bustamante J, del Río RM, Solís JM (2000a) Taurine activates GABA(A) but not GABA(B) receptors in rat hippocampal CA1 area. Brain Res 864:298–307

del Olmo N, Galarreta M, Bustamante J, Martín del Rio R, Solís JM (2000b) Taurine-induced synaptic potentiation: role of calcium and interaction with LTP. Neuropharmacology 39:40–54

del Olmo N, Handler A, Alvarez L, Bustamante J, Martín del Río R, Solís JM (2003) Taurine-induced synaptic potentiation and the late phase of long-term potentiation are related mechanistically. Neuropharmacology 44:26–39

Do KQ, Lauer CJ, Schreiber W, Zollinger M, Gutteck-Amsler U, Cuénod M, Holsboer F (1995) Gamma-Glutamylglutamine and taurine concentrations are decreased in the cerebrospinal fluid of drug-naive patients with schizophrenic disorders. J Neurochem 65:2652–2662

Du K, Montminy M (1998) CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem 273:32377–32379

Fatemi SH, Reutiman TJ, Folsom TD, Huang H, Oishi K, Mori S, Smee DF, Pearce DA, Winter C, Sohr R, Juckel G (2008) Maternal infection leads to abnormal gene regulation and brain atrophy in mouse offspring: implications for genesis of neurodevelopmental disorders. Schizophr Res 99:56–70

Fukuo Y, Kishi T, Kushima I, Yoshimura R, Okochi T, Kitajima T, Matsunaga S, Kawashima K, Umene-Nakano W, Naitoh H, Inada T, Nakamura J, Ozaki N, Iwata N (2011) Possible association between ubiquitin-specific peptidase 46 gene and major depressive disorders in the Japanese population. J Affect Disord 133:150–157

Galarreta M, Bustamante J, Martín del Río R, Solís JM (1996) Taurine induces a long-lasting increase of synaptic efficacy and axon excitability in the hippocampus. J Neurosci 16:92–102

Grimes CA, Jope RS (2001) CREB DNA binding activity is inhibited by glycogen synthase kinase-3 beta and facilitated by lithium. J Neurochem 78:1219–1232

Han F, Nakano T, Yamamoto Y, Shioda N, Lu YM, Fukunaga K (2009) Improvement of depressive behaviors by nefiracetam is associated with activation of CaM kinases in olfactory bulbectomized mice. Brain Res 1265:205–214

Hernández-Benítez R, Pasantes-Morales H, Saldaña IT, Ramos-Mandujano G (2010) Taurine stimulates proliferation of mice embryonic cultured neural progenitor cells. J Neurosci Res 88:1673–1681

Hussy N, Deleuze C, Desarménien MG, Moos FC (2000) Osmotic regulation of neuronal activity: a new role for taurine and glial cells in a hypothalamic neuroendocrine structure. Prog Neurobiol 62:113–134

Iio W, Matsukawa N, Tsukahara T, Kohari D, Toyoda A (2011) Effects of chronic social defeat stress on MAP kinase cascade. Neurosci Lett 504:281–284

Inan SY, Yalcin I, Aksu F (2004) Dual effects of nitric oxide in the mouse forced swimming test: possible contribution of nitric oxide-mediated serotonin release and potassium channel modulation. Pharmacol Biochem Behav 77:457–464

Jeon SH, Lee MY, Kim SJ, Joe SG, Kim GB, Kim IS, Kim NS, Hong CU, Kim SZ, Kim JS, Kang HS (2007) Taurine increases cell proliferation and generates an increase in [Mg2+]i accompanied by ERK 1/2 activation in human osteoblast cells. FEBS Lett 581:5929–5934

Junyent F, Romero R, de Lemos L, Utrera J, Camins A, Pallàs M, Auladell C (2010) Taurine treatment inhibits CaMKII activity and modulates the presence of calbindin D28k, calretinin, and parvalbumin in the brain. J Neurosci Res 88:136–142

Karege F, Perroud N, Burkhardt S, Schwald M, Ballmann E, La Harpe R, Malafosse A (2007) Alteration in kinase activity but not in protein levels of protein kinase B and glycogen synthase kinase-3beta in ventral prefrontal cortex of depressed suicide victims. Biol Psychiatry 61:240–245

Kobayashi K, Ikeda Y, Sakai A, Yamasaki N, Haneda E, Miyakawa T, Suzuki H (2010) Reversal of hippocampal neuronal maturation by serotonergic antidepressants. Proc Natl Acad Sci USA 107:8434–8439

Kobayashi K, Ikeda Y, Suzuki H (2011) Behavioral destabilization induced by the selective serotonin reuptake inhibitor fluoxetine. Mol Brain 4:12

Kong WX, Chen SW, Li YL, Zhang YJ, Wang R, Min L, Mi X (2006) Effects of taurine on rat behaviors in three anxiety models. Pharmacol Biochem Behav 83:271–276

Krishnan V, Nestler EJ (2008) The molecular neurobiology of depression. Nature 455:894–902

Lima L, Cubillos S (1998) Taurine might be acting as a trophic factor in the retina by modulating phosphorylation of cellular proteins. J Neurosci Res 53:377–384

Lutter M, Krishnan V, Russo SJ, Jung S, McClung CA, Nestler EJ (2008) Orexin signaling mediates the antidepressant-like effect of calorie restriction. J Neurosci 28:3071–3075

Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H, Caron MG, Tonegawa S (2003) Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. Proc Natl Acad Sci USA 100:8987–8992

Miyazaki T, Matsuzaki Y, Ikegami T, Miyakawa S, Doy M, Tanaka N, Bouscarel B (2004) Optimal and effective oral dose of taurine to prolong exercise performance in rat. Amino Acids 27:291–298

Murakami T, Furuse M (2010) The impact of taurine- and beta-alanine-supplemented diets on behavioral and neurochemical parameters in mice: antidepressant versus anxiolytic-like effects. Amino Acids 39:427–434

Murakami T, Yamane H, Tomonaga S, Furuse M (2009) Forced swimming and imipramine modify plasma and brain amino acid concentrations in mice. Eur J Pharmacol 602:73–77

Perry TL, Bratty PJ, Hansen S, Kennedy J, Urquhart N, Dolman CL (1975) Hereditary mental depression and Parkinsonism with taurine deficiency. Arch Neurol 32:108–113

Qi X, Lin W, Li J, Pan Y, Wang W (2006) The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress. Behav Brain Res 175:233–240

Sergeeva OA, Chepkova AN, Doreulee N, Eriksson KS, Poelchen W, Mönnighoff I, Heller-Stilb B, Warskulat U, Häussinger D, Haas HL (2003) Taurine-induced long-lasting enhancement of synaptic transmission in mice: role of transporters. J Physiol 550:911–919

Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS (2002) Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22:3251–3261

Silva AJ, Paylor R, Wehner JM, Tonegawa S (1992a) Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257:206–211

Silva AJ, Stevens CF, Tonegawa S, Wang Y (1992b) Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. Science 257:201–206

Suárez LM, Solís JM (2006) Taurine potentiates presynaptic NMDA receptors in hippocampal Schaffer collateral axons. Eur J Neurosci 24:405–418

Takao K, Tanda K, Nakamura K, Kasahara J, Nakao K, Katsuki M, Nakanishi K, Yamasaki N, Toyama K, Adachi M, Umeda M, Araki T, Fukunaga K, Kondo H, Sakagami H, Miyakawa T (2010) Comprehensive behavioral analysis of calcium/calmodulin-dependent protein kinase IV knockout mice. PLoS ONE 5:e9460

Tamai I, Senmaru M, Terasaki T, Tsuji A (1995) Na(+)- and Cl(-)-dependent transport of taurine at the blood–brain barrier. Biochem Pharmacol 50:1783–1793

Tanabe M, Nitta A, Ono H (2010) Neuroprotection via strychnine-sensitive glycine receptors during post-ischemic recovery of excitatory synaptic transmission in the hippocampus. J Pharmacol Sci 113:378–386

Timbrell JA, Seabra V, Waterfield CJ (1995) The in vivo and in vitro protective properties of taurine. Gen Pharmacol 26:453–462

Tiraboschi E, Giambelli R, D'Urso G, Galietta A, Barbon A, de Bartolomeis A, Gennarelli M, Barlati S, Racagni G, Popoli M (2004) Antidepressants activate CaMKII in neuron cell body by Thr286 phosphorylation. NeuroReport 15:2393–2396

Tomida S, Mamiya T, Sakamaki H, Miura M, Aosaki T, Masuda M, Niwa M, Kameyama T, Kobayashi J, Iwaki Y, Imai S, Ishikawa A, Abe K, Yoshimura T, Nabeshima T, Ebihara S (2009) Usp46 is a quantitative trait gene regulating mouse immobile behavior in the tail suspension and forced swimming tests. Nat Genet 41:688–695

Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ (2006) Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 9:519–525

Vaswani M, Linda FK, Ramesh S (2003) Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. Prog Neuropsychopharmacol Biol Psychiatry 27:85–102

Whirley BK, Einat H (2008) Taurine trials in animal models offer no support for anxiolytic, antidepressant or stimulant effects. Isr J Psychiatry Relat Sci 45:11–18

Whittle N, Sartori SB, Dierssen M, Lubec G, Singewald N (2007) Fetal Down syndrome brains exhibit aberrant levels of neurotransmitters critical for normal brain development. Pediatrics 120:e1465–e1471

Wong PT, Ong YP (2001) Acute antidepressant-like and antianxiety-like effects of tryptophan in mice. Pharmacology 62:151–156

Wu JY, Prentice H (2010) Role of taurine in the central nervous system. J Biomed Sci 17(Suppl 1):S1

Xing G, Russell S, Hough C, O'Grady J, Zhang L, Yang S, Zhang LX, Post R (2002) Decreased prefrontal CaMKII alpha mRNA in bipolar illness. NeuroReport 13:501–505

Yamasaki N, Maekawa M, Kobayashi K, Kajii Y, Maeda J, Soma M, Takao K, Tanda K, Ohira K, Toyama K, Kanzaki K, Fukunaga K, Sudo Y, Ichinose H, Ikeda M, Iwata N, Ozaki N, Suzuki H, Higuchi M, Suhara T, Yuasa S, Miyakawa T (2008) Alpha-CaMKII deficiency causes immature dentate gyrus, a novel candidate endophenotype of psychiatric disorders. Mol Brain 1:6

Yatabe Y, Miyakawa S, Miyazaki T, Matsuzaki Y, Ochiai N (2003) Effects of taurine administration in rat skeletal muscles on exercise. J Orthop Sci 8:415–419

Zheng WH, Quirion R (2004) Comparative signaling pathways of insulin-like growth factor-1 and brain-derived neurotrophic factor in hippocampal neurons and the role of the PI3 kinase pathway in cell survival. J Neurochem 89:844–852