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ORIGINAL ARTICLE

Hypothesis with abnormal amino acid metabolism in depression and stress vulnerability in Wistar Kyoto rats

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Abstract While abnormalities in monoamine metabolism have been investigated heavily per potential roles in the mechanisms of depression, the contribution of amino acid metabolism in the brain remains not well understood. In additional, roles of the hypothalamus–pituitary–adrenal axis in stress-regulation mechanisms have been of much focus, while the contribution of central amino acid metabolism to these mechanisms has not been well appreciated. Therefore, whether depression-like states affect amino acid metabolism and their potential roles on stress-regulatory mechanisms were investigated by comparing Wistar Kyoto rats, which display depression-like behaviors and stress vulnerability, to control Wistar rats. Brain amino acid metabolism in Wistar Kyoto rats was greatly different from normal Wistar rats, with special reference to lower cystathionine and serine levels. In addition, Wistar Kyoto rats demonstrated abnormality in dopamine metabolism compared with Wistar rats. In the case of stress response, amino acid levels having a sedative and/or hypnotic effect were constant in the brain of Wistar Kyoto rats, though these amino acid levels were reduced in Wistar rats under a stressful condition. These results suggest that the abnormal amino acid metabolism may induce depression-like behaviors and stress vulnerability in Wistar Kyoto rats. Therefore, we hypothesized that abnormalities in amino acid and monoamine metabolism may induce depression, and amino acid metabolism in the brain may be related to stress vulnerability.

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Introduction

Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. These symptoms can become chronic or recurrent and lead to substantial impairments in an ability to take care of everyday responsibilities. The World Health Organization (WHO) reported that the prevalence of depression was 151 million in 2004 (Disease and Injury Regional estimates for 2004; WHO). Data from the American Psychiatric Association show that an estimated 10–25 % of women and 5–12 % of men will experience major depressive disorder in their lifetimes (Pincus and Pettit 2001). It is thought that major depression is caused by chronic stress. In fact, chronic mild stress induced depressive-like states in mice and rats (Elizalde et al. 2008; Gamaro et al. 2003; Lucca et al. 2009). In addition, acute stress altered amino acid metabolism in mice (Murakami et al. 2009) and in chicks (Hamasu et al. 2009). Therefore, relationships between stress and amino acid metabolism may partly explain the onset of depression.

It is well known that monoamines such as serotonin (5-HT) and norepinephrine (NE) are related to depression. Therefore, abnormality in monoamine metabolism has been much paid attention in the investigation of depression mechanisms. However, not only monoamine metabolism but also amino acid metabolism may contribute to these mechanisms. The precursors of these monoamines are amino acids, i.e., tryptophan and tyrosine, respectively. Furthermore, taurine-supplemented diet induced antidepressant-like effects in mice

(Murakami and Furuse 2010). Thus, amino acids are likely involved in major depression. In general, major depression is treated with antidepressants. It is thought that antidepressants exert their effects by increasing monoamine levels in synaptic clefts (Coppen 1967; Lanni et al. 2009). However, recent studies demonstrated that antidepressants affected not only monoamine system but also amino acid metabolism (Kokras et al. 2009; Murakami et al. 2009). Therefore, it can be interpreted that there are relationships between major depression and amino acid metabolism.

Wistar Kyoto (WKY) rats are a strain isolated from Wistar (WIS) rats and a control animal of spontaneously hypertensive rats. However, other studies reveal that WKY rats are hyper-responsive to stress from the view of stress ulcer (Pare' 1990, 1994; Tejani-Butt et al. 2003). On the other hand, O'Mahony et al. (2011) reported the difference in stress responsibility between Sprague–Dawley (SD) and WKY rats from the aspect of monoamine metabolism. In addition, WKY rats showed a high level of immobility in a forced swimming test compared to either outbred SD rats or other inbred strains (Lahmame and Armario 1996; López-Rubalcava and Lucki 2000). Therefore, WKY rats are thought as an animal model of depression. Previous study demonstrated the differences in monoamine levels in the various brain regions between WIS and WKY rats (De La Garza and Mahoney 2004). Moreover, Jiao et al. (2006) reported that WKY rats displayed the lower number of DA transporters compared with WIS rats. Therefore, depression-like behavior in WKY rats may be related to monoamine metabolism in the brain. However, no studies about the differences in amino acid levels in the peripheral and various brain regions between WIS and WKY rats have been performed yet.

In this way, though the relationships between major depression and amino acids in the brain are apparent, it is unclear whether major depression itself affects amino acids metabolism. Therefore, the purpose of the present study was to evaluate whether depression-like states affect amino acid metabolism by comparing WIS and WKY rats. In addition, we also aimed to elucidate stress-regulatory mechanisms by evaluating acute stress-induced differences in amino acid and monoamine metabolism between WKY and WIS rats; previous studies pertaining to stress-regulatory mechanisms have primarily focused on the hypothalamus–pituitary–adrenal axis.

Materials and methods

Animals

7-week-old male WIS and WKY rats (Charles River Japan, Yokohama, Japan) at the beginning of the experiment were used. Rats were housed four per cage with free access to food and water. They were maintained on a 12-h light/dark cycle (lights on at 07:00, lights off at 19:00) at the room temperature of 23 ± 1 °C and humidity 60 %. 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 Law (No. 105) and Notification (No. 6) of the Government.

Experimental procedure

7-week-old WIS and WKY rats were acclimated for 7 days on arrival. On a body weight basis, each strain was respectively assigned to two groups of 10 rats each, i.e., (1) control WIS rat group, (2) stress treatment WIS rat group, (3) control WKY rat group, and (4) stress treatment WKY rat group. We considered that behavioral tests are one of the stressors and all behavioral tests were performed in stress treatment WIS and WKY rat groups. Rats were tested during the light period and were kept in a closed room at constant temperature (23 ± 1 °C). Each test was recorded on a video recording system for analysis.

Open field test (OFT)

Open field test was performed on the 8th day after animals arrived. Motor activity in a novel environment was evaluated by an OFT. Each rat was transferred to the open field area from their home cage. The open field was a square arena (length 90 cm, width 90 cm and height 45 cm), and made of wood colored in black. The measurement of the motor activity was started as soon as rats were placed in the center of the arena. The motor activity of each rat was observed for 5 min. After each test, the open field area was cleaned with ethanol–water solution to unify the condition of each test. The travel distance was the parameter for motor activity, and was automatically analyzed with a computer-based video-tracking system (AXIS-90, Neuroscience, Inc., Tokyo, Japan).

Forced swimming test (FST)

Forced swimming test was performed on the 10th and 11th day to evaluate whether rats were in a depressive state. This test was conducted according to a previous report (Porsolt et al. 1978) with some modifications. In the present study, the FST was constituted of two exposure sessions: a pre-test and main-test session. First, rats were placed in an acrylic cylinder (30 cm in diameter, 45 cm height) containing water to the depth of 30 cm maintained at 24–26 °C for 15 min (pre-test session). 24 h later, they were re-placed in the cylinder and total duration of immobility was recorded by video camera for 5 min (main-test session). Immobility was defined as the index of

depressive state. A rat was evaluated as immobile when floating motionless or making only small movements to keep their head above water.

Analysis of monoamine in the brain

Immediately after FST, rats of stress treatment groups and the corresponding rats of control groups were decapitated under anesthesia with isoflurane (Escain®, Mylan, Osaka, Japan) which is an inhalation anesthetics, and trunk blood was collected. The brains were quickly removed and dissected in the prefrontal cortex, hippocampus, striatum, thalamus, hypothalamus, brain stem and cerebellum, and weighed. The samples were frozen in liquid nitrogen, and stored at −80 °C until analysis. To investigate the influences of strain difference and acute stress, brain monoamine levels were analyzed by high-performance liquid chromatography (HPLC). The tissue samples were homogenized in ice-cold 0.2-M perchloric acid solution containing 0.01 mM EDTA·2Na and left for deproteinization on ice for 30 min. Then, the tissue homogenates were centrifuged at 20,000 $\times g$ for 15 min at 0 °C. Supernatants were adjusted to pH 3 with 1 M sodium acetate and were filtrated through 0.20-μm filter. NE, 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and homovanillic acid (HVA) tissue concentrations in filtrates were analyzed using HPLC system (Eicom, Kyoto, Japan) with a 150 × 3.0-mm ODS column (EICOMPAK SC-5ODS, Eicom) and electrochemical detector (ECD-300, Eicom, Kyoto, Japan) at an applied potential of +750 mV versus an Ag/AgCl reference analytical electrode. The 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, pH 3.5, consisted of 0.1 M sodium acetate, 0.1 M citric acid, 1-octane sulfonate, EDTA·2Na (5 mg/ml). The retention time and height of the peaks in tissue homogenates were measured and compared to samples of the external calibrating standard solution containing 100 pg/μl of each NE, DOPAC, DA, 5-HIAA, HVA and 5-HT. Concentrations of these substances in the samples were calculated and expressed as pg/mg wet tissue. Monoamine turnover rates, DOPAC/DA, HVA/DA and 5-HIAA/5-HT were also calculated.

Analysis of free amino acid

To investigate the influences of strain difference and acute stress, free amino acid levels in brain were analyzed by HPLC. The supernatants which were collected in analysis of monoamine were adjusted to pH 7 with 1 M sodium hydroxide. Plasma was prepared by centrifuging at 3,000 $\times g$ for 15 min at 4 °C (KUBOTA 3740), and filtrated through ultrafiltration tube (Millipore, Bedford, USA). Each 20 μl sample of the brain and 10 μl sample of the plasma were dried under reduced pressure. The dried residues were dissolved in 10 μl of 1 M sodium acetate–methanol–triethylamine (2:2:1) and re-dried under reduced pressure, then dissolved in 20 μl of methanol–distilled water–triethylamine–phenylisothiocyanate (7:1:1:1) which was a derivatization solution. After phenylisocyanate finished reacting with amino groups at room temperature for 20 min, the samples were dried again and dissolved in 200 μl of Pico-Tag Diluent (Waters, Milford, USA). These diluted samples were filtrated through 0.20-mm filter (Millipore, Bedford, USA). The same methods were performed to standard solutions which were prepared by diluting a commercially available L-amino acid solution (type ANII, type B, L-asparagine, L-glutamine and L-tryptophan; Wako, Osaka, Japan) with distilled water. The derivatized samples were applied to a Waters HPLC system (Pico-Tag free amino acid analysis column (3.9 mm × 300 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 concentrations of free amino acids were determined by the absorbance at 254 nm wave length. The plasma amino acid concentrations were expressed as pmol/μl, and the amino acid concentrations in the brain were expressed as pmol/mg wet tissue.

Statistical analysis

All data were expressed as means ± SEM; the travel distance and duration of immobility were analyzed by t test in stress treatment WIS and WKY rat groups. Monamine and free amino acid levels were analyzed by two-way ANOVA and afterward by t test in all four groups. Significance was set at $p < 0.05$. All 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

Travel distance in the open field test and immobility in the forced swimming test

The effect of strain difference on travel distance (cm) in the open field test and immobility (s) in the forced swimming

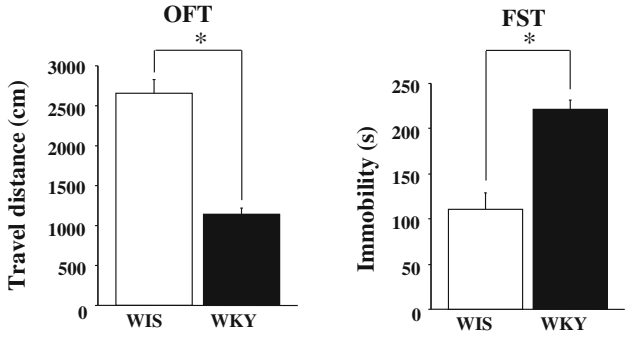

Fig. 1 The effect of strain difference on travel distance (cm) in OFT and immobility (s) in FST. The number of samples for analysis was 10. All results are expressed as mean ± SEM. *Significantly different at $p < 0.05$

test are shown in Fig. 1. Travel distance in WKY rats displayed significantly lower value than that in WIS rats. In addition, immobility in WKY rats was significantly higher than that in WIS rats.

Monoamine concentration in the brain

The concentration of monoamines and their metabolites differed by strain difference and were altered by stress treatment. All results for catecholamine metabolism are shown in Table 1.

In the prefrontal cortex, NE was significantly lower, but DOPAC/DA and HVA/DA were significantly higher in WKY than in WIS rats. Stress treatment significantly decreased DOPAC and HVA, and the reverse was true for 5-HT. No significant interactions between strain and stress treatment were observed in any parameters.

In the hippocampus, WKY rats showed higher values in HVA and HVA/DA compared with WIS rats. Stress treatment significantly increased DOPAC/DA, but significantly decreased HVA/DA. No significant interactions between strain and stress treatment were observed.

In the striatum, HVA/DA alone was significantly higher in WKY than in WIS rats. Stress treatment significantly decreased DOPAC, DOPAC/DA and HVA/DA. On the contrary, stress treatment significantly increased DA level. No significant interactions between strain and stress treatment were observed.

In the thalamus, DOPAC/DA and HVA/DA were of higher values in WKY than in WIS rats. Stress treatment significantly increased DOPAC, DA and HVA. No significant interactions between strain and stress treatment were observed.

In the hypothalamus, only significant strain differences were observed: DOPAC, HVA and HVA/DA were higher in WKY rats. Significant interaction between strain and stress treatment was observed in NE. NE level was different for stress between WIS and WKY rats: they remained constant in WIS and increased in WKY rats.

In the brain stem, DOPAC, HVA, DOPAC/DA and HVA/DA were significantly higher in WKY rats, but the reverse was true for NE, and DA. Stress treatment significantly increased NE, DOPAC, DA and HVA levels. No significant interactions between strain and stress treatment were observed.

In the cerebellum, DA was significantly lower, but HVA, DOPAC/DA and HVA/DA were higher in WKY rats. Stress treatment significantly increased in DA and HVA, but significantly decreased DOPAC/DA and HVA/DA. No significant interactions between strain and stress treatment were observed.

Amino acid concentration in the plasma and brain

There were significant differences in amino acid metabolism between WKY and WIS rats. Stress treatment significantly decreased many free amino acid levels in the plasma and brain.

In WKY rats, a significantly lower value in cystathionine level was observed in the prefrontal cortex, hippocampus, striatum, thalamus, hypothalamus, brain stem and cerebellum compared with WIS rats, and acute stress treatment decreased cystathionine level in the brain stem (Fig. 2). Moreover, WKY rats had a significantly lower level of serine compared with WIS rats in the prefrontal cortex, hippocampus, striatum, hypothalamus, brain stem, cerebellum and plasma (Fig. 3). In addition, acute stress treatment decreased serine levels in the hippocampus, thalamus, brain stem and plasma, and a significant interaction was observed in the plasma (Fig. 3). There were some alterations in not only cystathionine and serine but also other amino acids. Their alterations are described in the following paragraphs.

The concentrations of free amino acids in the striatum are shown in Table 2. Asparagine, GABA, threonine, alanine, arginine, leucine and ornithine were significantly lower, but carnosine alone was higher in WKY rats compared with WIS rats. Stress treatment was associated with decreases in aspartate, asparagine, glycine, β-alanine, GABA, arginine, proline, tyrosine, valine, isoleucine, leucine, ornithine and lysine. Significant interactions between strain and stress treatment were observed in ornithine and lysine. The values for ornithine and lysine were decreased by stress treatment in WIS rats, but remained intact under stressful conditions in WKY rats.

The concentrations of free amino acids in the thalamus are shown in Table 3. WKY rats showed significantly lower values in glycine and ornithine compared with WIS rats. Stress treatment decreased aspartate, glutamate, glycine, glutamine, taurine, threonine, alanine, arginine,

Table 1 Effects of strain difference and stress treatment on catecholamine concentration and metabolic turnover rate of several brain parts in WIS and WKY rats

Parts	Strain	WIS		WKY		p		
	Treatment	Control	Stress	Control	Stress	Strain	Stress	Strain × stress
Prefrontal cortex	NE	302 ± 3.3	331 ± 16	287 ± 8.1	298 ± 8.6	$p < 0.05$	NS	NS
	DOPAC	375 ± 29	239 ± 28	325 ± 51	221 ± 41	NS	$p < 0.01$	NS
	DA	486 ± 77	406 ± 77	387 ± 89	267 ± 71	NS	NS	NS
	HVA	112 ± 8.6	87 ± 3.9	114 ± 9.2	94 ± 10	NS	$p < 0.05$	NS
	DOPAC/DA	0.74 ± 0.06	0.63 ± 0.07	1.03 ± 0.16	0.89 ± 0.11	$p < 0.05$	NS	NS
	HVA/DA	0.25 ± 0.02	0.21 ± 0.03	0.43 ± 0.11	0.45 ± 0.1	$p < 0.01$	NS	NS
Hippocampus	NE	344 ± 14	346 ± 22	319 ± 8.8	358 ± 11	NS	NS	NS
	DOPAC	27.2 ± 4.4	20.1 ± 3.2	23.5 ± 1.9	19.8 ± 0.94	NS	NS	NS
	DA	17.9 ± 1.7	20.5 ± 3.5	14.3 ± 1.9	17.9 ± 1.2	NS	NS	NS
	HVA	9.13 ± 1.1	7.86 ± 0.74	12 ± 1.1	11.3 ± 0.24	$p < 0.01$	NS	NS
	DOPAC/DA	1.49 ± 0.14	0.92 ± 0.03	1.57 ± 0.19	1.02 ± 0.06	NS	$p < 0.01$	NS
	HVA/DA	0.49 ± 0.04	0.40 ± 0.05	0.92 ± 0.1	0.65 ± 0.04	$p < 0.01$	$p < 0.01$	NS
Striatum	NE	826 ± 48	792 ± 50	795 ± 75	866 ± 68	NS	NS	NS
	DOPAC	1,319 ± 51	1,077 ± 106	1,330 ± 52	1,131 ± 114	NS	$p < 0.05$	NS
	DA	1,806 ± 286	3,218 ± 441	1,973 ± 413	3,141 ± 358	NS	$p < 0.01$	NS
	HVA	423 ± 23	408 ± 51	489 ± 41	501 ± 45	NS	NS	NS
	DOPAC/DA	0.71 ± 0.11	0.36 ± 0.03	0.94 ± 0.2	0.44 ± 0.05	NS	$p < 0.01$	NS
	HVA/DA	0.22 ± 0.03	0.13 ± 0.01	0.31 ± 0.05	0.19 ± 0.01	$p < 0.05$	$p < 0.01$	NS
Thalamus	NE	356 ± 14	331 ± 20	322 ± 8.7	311 ± 12	NS	NS	NS
	DOPAC	71.7 ± 9.7	142 ± 25	83.6 ± 10	91.3 ± 7.1	NS	$p < 0.05$	NS
	DA	53.2 ± 4.0	127 ± 31	54.8 ± 9.7	61.4 ± 7.5	NS	$p < 0.05$	NS
	HVA	33.6 ± 3.5	50.9 ± 6.9	44.6 ± 5.0	49.2 ± 2.4	NS	$p < 0.05$	NS
	DOPAC/DA	1.38 ± 0.09	1.03 ± 0.12	1.57 ± 0.15	1.42 ± 0.11	$p < 0.05$	NS	NS
	HVA/DA	0.64 ± 0.03	0.49 ± 0.08	0.84 ± 0.07	0.79 ± 0.08	$p < 0.01$	NS	NS
Hypothalamus	NE	1,333 ± 81^{ab}	1,248 ± 40^{a}	1,212 ± 59^{a}	1,393 ± 52^{b}	NS	NS	$p < 0.05$
	DOPAC	167 ± 11	136 ± 11	200 ± 19	176 ± 12	$p < 0.05$	NS	NS
	DA	144 ± 16	157 ± 10	121 ± 12	166 ± 17	NS	NS	NS
	HVA	15.3 ± 1.5	17.9 ± 1.6	30.9 ± 2.1	32.9 ± 1.2	$p < 0.01$	NS	NS
	DOPAC/DA	1.28 ± 0.17	0.96 ± 0.11	1.60 ± 0.28	1.22 ± 0.12	NS	NS	NS
	HVA/DA	0.11 ± 0.01	0.11 ± 0.02	0.25 ± 0.03	0.20 ± 0.01	$p < 0.01$	NS	NS
Brain stem	NE	603 ± 16	673 ± 16	484 ± 6.6	555 ± 6.9	$p < 0.01$	$p < 0.01$	NS
	DOPAC	52.4 ± 1.1	55.9 ± 2.2	55.9 ± 3.0	61.6 ± 1.9	$p < 0.05$	$p < 0.05$	NS
	DA	114 ± 4.7	143 ± 3.3	111 ± 5.0	123 ± 2.6	$p < 0.01$	$p < 0.01$	NS
	HVA	28.7 ± 1.1	32.3 ± 1.3	34.5 ± 0.99	39.5 ± 0.56	$p < 0.01$	$p < 0.01$	NS
	DOPAC/DA	0.45 ± 0.02	0.38 ± 0.01	0.53 ± 0.04	0.51 ± 0.02	$p < 0.01$	NS	NS
	HVA/DA	0.25 ± 0.01	0.23 ± 0.01	0.33 ± 0.02	0.32 ± 0.01	$p < 0.01$	NS	NS
Cerebellum	NE	105 ± 4.5	119 ± 8.9	115 ± 2.3	120 ± 4.4	NS	NS	NS
	DOPAC	4.05 ± 0.27	4.21 ± 0.16	4.48 ± 0.26	4.54 ± 0.27	NS	NS	NS
	DA	1.74 ± 0.27	2.51 ± 0.29	1.34 ± 0.16	1.72 ± 0.10	$p < 0.05$	$p < 0.05$	NS
	HVA	4.05 ± 0.29	4.69 ± 0.08	5.69 ± 0.31	6.58 ± 0.25	$p < 0.01$	$p < 0.01$	NS
	DOPAC/DA	2.35 ± 0.31	1.80 ± 0.19	3.29 ± 0.39	2.67 ± 0.16	$p < 0.01$	$p < 0.01$	NS
	HVA/DA	2.31 ± 0.33	2.17 ± 0.26	4.41 ± 0.46	4.00 ± 0.32	$p < 0.01$	NS	NS

The number of samples used for analysis was 10. The values for NE, DOPAC, DA, 5-HIAA, HVA and 5-HT are expressed as mean ± SEM in pg/mg wet tissue, and the values for DOPAC/DA, HVA/DA and 5-HIAA/5-HT are expressed as mean ± SEM. Different letters indicate significant difference at $p < 0.05$

NS not significant

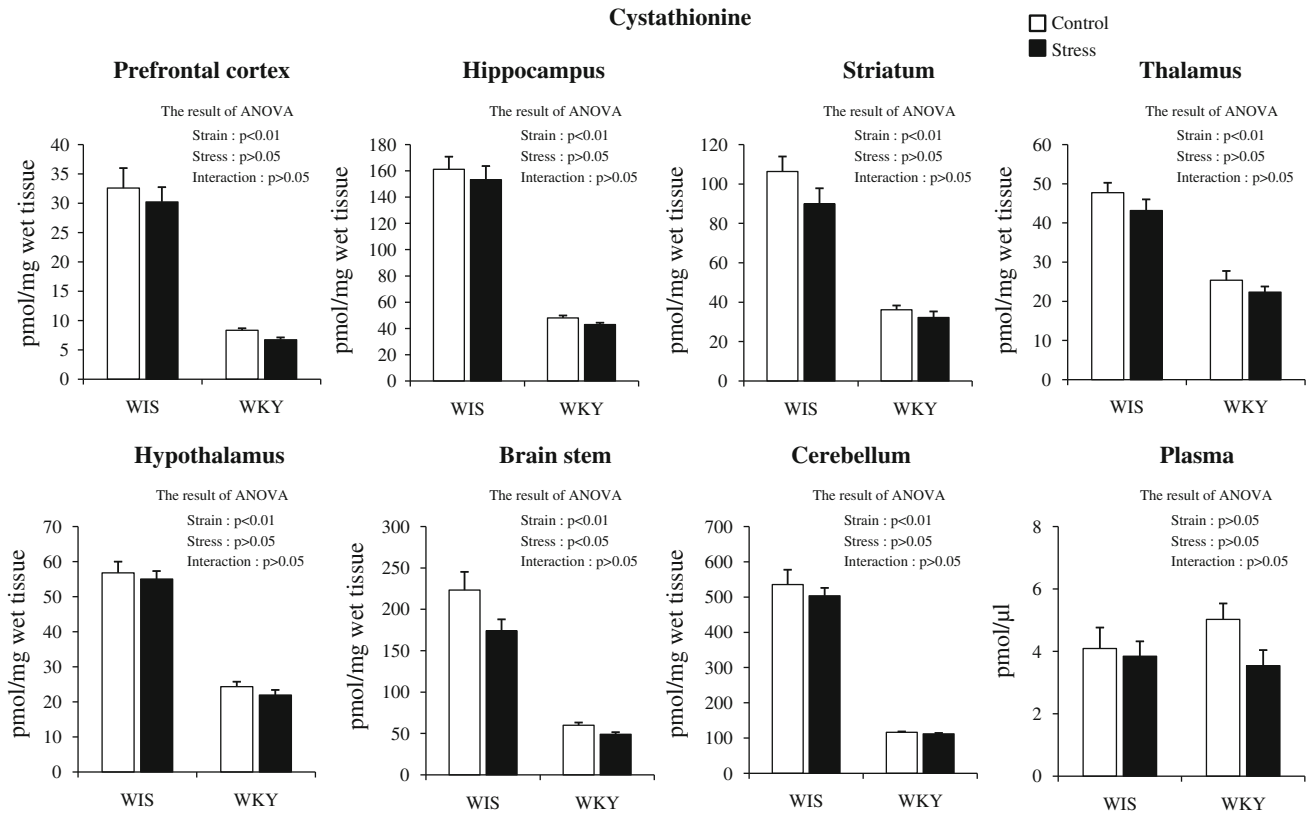

Fig. 2 The effect of strain difference on cystathionine level in the prefrontal cortex, hippocampus, striatum, thalamus, hypothalamus, brain stem cerebellum, and plasma. Values are presented as mean ± SEM. Brain concentrations were expressed in pmol/mg wet tissue, and plasma concentration was expressed in pmol/μl

proline, tyrosine, isoleucine, leucine and lysine. Significant interactions between strain and stress treatment were observed in aspartate, asparagine, threonine, alanine, arginine, proline, tyrosine, methionine, isoleucine, leucine and lysine. In WIS rats alone, stress treatment decreased aspartate, asparagine, threonine, alanine, arginine, proline, tyrosine, methionine, isoleucine, leucine and lysine.

Discussion

In the present study, WKY rats displayed significantly longer immobility in FST compared with WIS rats. This result was in accordance with the previous studies (Lahmame and Armario 1996; López-Rubalcava and Lucki 2000). In addition, we evaluated the motor activity in OFT, because the depressed patients displayed significantly lower motor activity compared with the healthy subjects (Hauge et al. 2011). In the present study, WKY rats demonstrated the hypoactivity in OFT, and this result corresponded with the previous reports (O'Mahony et al. 2011; Tejani-Butt et al. 2003). Therefore, we reconfirmed here the possibility that WKY rats are animal model of depression.

In WKY rats, significantly lower levels of cystathionine were observed in the prefrontal cortex, hippocampus, striatum, thalamus, hypothalamus, brain stem and cerebellum compared to WIS rats. Moreover, WKY rats had a significantly lower level of serine compared to WIS rats in the prefrontal cortex, hippocampus, striatum, hypothalamus, brain stem, cerebellum and plasma. Consequently, WKY rats displayed lower values of cystathionine within the brain and the systemic lower values of serine. Therefore, we hypothesized that abnormal behavior such as depression-like behavior and hypoactivity in WKY rats were caused by the abnormality in cystathionine and serine metabolism. In fact, the latest findings reveal that D-serine exerted an antidepressant-like effect (Malkesman et al. 2011). Data obtained here support their findings.

Metabolic pathway in cystathionine is shown in Fig. 4. Cystathionine is metabolized from serine and homocysteine by cystathionine β-synthase and to cysteine by cystathionine γ-lyase (Aitken et al. 2011; Yamada et al. 2012). Cystathionine and serine levels in WKY rat brain were displayed significantly lower values compared to WIS rat brain. These results may be induced by two phenomena. First, the synthesis of cystathionine was inhibited by the decrease in serine, one of the sources of cystathionine. As a

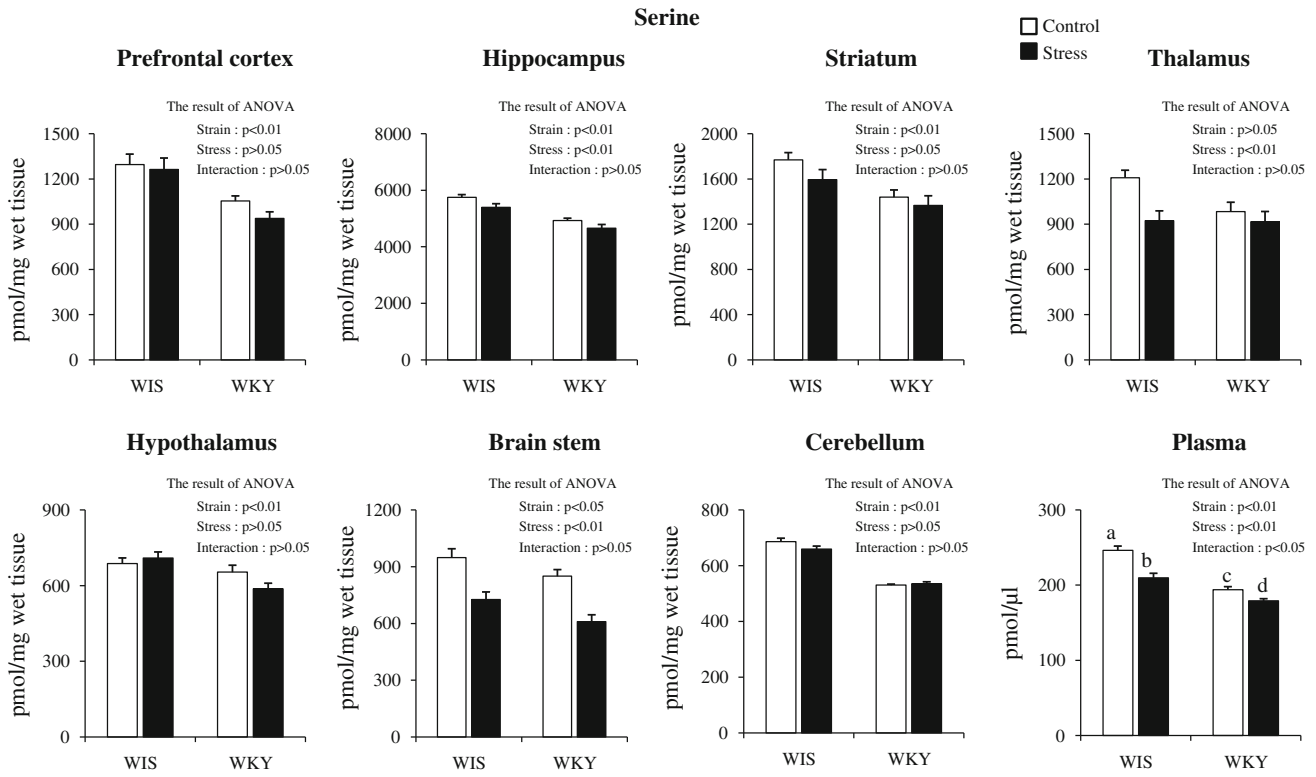

Fig. 3 The effect of strain difference on serine level in the prefrontal cortex, hippocampus, striatum, thalamus, hypothalamus, brain stem cerebellum, and plasma. Values are presented as mean ± SEM. Brain concentrations were expressed in pmol/mg wet tissue, and plasma concentration was expressed in pmol/μl

result, the increase in homocysteine, the other source of cystathionine may be induced by the inhibition of cystathionine synthesis in WKY rats. It was reported that homocysteine induced oxidative stress in the brain (Matte' et al. 2009), and there was a relationship between depression and oxidative stress (Ng et al. 2008; Tagliari et al. 2010). Therefore, depression-like behavior in WKY rats may be closely related to not only cystathionine and serine levels but also homocysteine levels in WKY rat brain. However, the relationship between depression and homocysteine levels in the brain is not fully understood, because homocysteine levels in the brain of depressed subjects or animal model of depression have not been investigated. Second, WKY rats may have an abnormality in cystathionine γ-lyase activity. Significant decreases in cystathionine and serine in the WKY rat brain may be caused by the acceleration of cystathionine metabolism with the elevation of cystathionine γ-lyase activity. However, the difference in cystathionine γ-lyase activity in the brain between WKY rats and other strains has not been demonstrated.

In the present study, WKY rats displayed the significantly higher values of HVA/DA turnover in the prefrontal cortex, hippocampus, striatum, thalamus hypothalamus, brain stem and cerebellum. In addition, catecholamine and its metabolites were altered in various brain regions of WKY rats. We cannot discuss whether the release levels of catecholamine and its metabolites were differed by strain difference because these results measured the monoamine concentration in the brain but not monoamine release levels into the synapse. However, the present results certainly demonstrated that WKY rats had abnormalities in DA and NE neuron systems in the dissected brain regions because catecholamine metabolism was different between WKY and WIS rats. In fact, a previous study revealed the abnormality in DA neuron systems in WKY rats of less DA transporters than in WIS rats in the hippocampus and hypothalamus (Jiao et al. 2006). Therefore, these results revealed that WKY rats had dysfunction in DA neuron systems with not only the hippocampus and hypothalamus but also the prefrontal cortex, striatum, thalamus, brain stem and cerebellum. In addition, chronic homocysteine treatment, which caused dysfunction in DA neuron system, induced hypoactivity (Lee et al. 2005). Adversely, nomifensine, a norepinephrine-dopamine reuptake inhibitor and increases DA level in the synaptic cleft, elevated the motor activity in WKY rats (Tejani-Butt et al. 2003). Taken together, it was indicated that hypoactivity in WKY rats may be induced by dysfunction in DA neuron systems.

In the present study, many free amino acids were significantly decreased by acute stress treatment in the brain

Table 2 Effects of strain difference and stress treatment on amino acid concentration of the striatum in WIS and WKY rats

Strain	WIS		WKY		*p*		
Treatment	Control	Stress	Control	Stress	Strain	Stress	Strain × stress
Aspartate	4,387 ± 214	3,366 ± 194	3,766 ± 249	3,376 ± 176	NS	$p < 0.01$	NS
Glutamate	13,348 ± 851	14,008 ± 770	13,158 ± 881	13,074 ± 1,001	NS	NS	NS
Asparagine	300 ± 12	259 ± 12	246 ± 11	220 ± 14	$p < 0.01$	$p < 0.05$	NS
Glycine	2,380 ± 106	1,736 ± 101	2,220 ± 116	1,839 ± 104	NS	$p < 0.01$	NS
Glutamine	10,614 ± 586	10,008 ± 608	10,695 ± 669	10,052 ± 678	NS	NS	NS
β-alanine	250 ± 17	166 ± 11	238 ± 17	199 ± 11	NS	$p < 0.01$	NS
Taurine	11,008 ± 488	11,254 ± 819	9,910 ± 720	10,598 ± 932	NS	NS	NS
Histidine	181 ± 16	171 ± 22	164 ± 20	176 ± 11	NS	NS	NS
GABA	5,600 ± 300	4,272 ± 231	4,617 ± 287	4,152 ± 177	$p < 0.05$	$p < 0.01$	NS
Threonine	1,083 ± 45	930 ± 49	910 ± 49	866 ± 52	$p < 0.05$	NS	NS
Alanine	1,300 ± 53	1,262 ± 92	1,073 ± 55	1,100 ± 80	$p < 0.05$	NS	NS
Carnosine	5.17 ± 0.59	4.20 ± 0.75	6.90 ± 0.86	6.25 ± 0.80	$p < 0.05$	NS	NS
Arginine	527 ± 25	389 ± 31	393 ± 22	319 ± 20	$p < 0.01$	$p < 0.01$	NS
Proline	168 ± 6.6	132 ± 8.2	158 ± 6.1	138 ± 9.0	NS	$p < 0.01$	NS
Tyrosine	240 ± 7.7	192 ± 15	217 ± 13	185 ± 12	NS	$p < 0.01$	NS
Valine	201 ± 4.9	163 ± 9.2	182 ± 7.8	162 ± 9.5	NS	$p < 0.01$	NS
Methionine	145 ± 12	104 ± 15	124 ± 12	126 ± 19	NS	NS	NS
Isoleucine	77.8 ± 2.8	59.4 ± 3.4	69.8 ± 3.2	60.7 ± 3.9	NS	$p < 0.01$	NS
Leucine	259 ± 11	216 ± 11	234 ± 8.2	192 ± 9.8	$p < 0.05$	$p < 0.01$	NS
Tryptophan	246 ± 38	231 ± 58	227 ± 61	255 ± 51	NS	NS	NS
Ornithine	44.3 ± 1.3[a]	33.5 ± 1.7[b]	31.3 ± 2.7[b]	30.0 ± 2.4[b]	$p < 0.01$	$p < 0.05$	$p < 0.05$
Lysine	713 ± 28[a]	466 ± 36[b]	578 ± 42[b]	517 ± 38[b]	NS	$p < 0.01$	$p < 0.05$

The number of samples used for analysis was 10. The values for amino acid are expressed as mean ± SEM in pmol/mg wet tissue. Different letters indicate significant difference at $p < 0.05$

NS not significant

and plasma. Chronic restraint stress caused the decrease in GABA level in the hippocampus (O'Mahony et al. 2011), and FST induced the decreases in free amino acid levels in the plasma (Murakami et al. 2009). In addition, restraint with isolation or fasting stress induced the decreases in GABA, proline, arginine, alanine, asparagine, aspartate, serine, tyrosine, valine, histidine and glutamine in the neonatal chicks brain (Hamasu et al. 2009). The present study revealed that stress treatment decreased a part of amino acids not only in the plasma (glutamate, asparagine, glycine, histidine, threonine, proline, methionine, ornithine and lysine (data not shown) as well as serine) but also in the various brain regions (data not shown) except for the striatum and thalamus. Especially interesting interactions were observed in the striatum and thalamus. The interactions were confirmed in ornithine and lysine in the striatum, and in aspartate, glycine, alanine, arginine, proline, tyrosine and lysine in the thalamus. In WIS rats, these amino acids decreased by acute stress treatment. In WKY rats however, these amino acids remained at constant levels compared to the control group. These amino acids without lysine have sedative or hypnotic-like effects under stressful conditions in neonatal chicks or mice (Asechi et al. 2006; Kabuki et al. 2009; Kurata et al. 2011; Kurauchi et al. 2006; Hamasu et al. 2010; Suenaga et al. 2008; Yamane et al. 2009). In the case of lysine, its metabolite, pipecolic acid, has sedative or hypnotic-like effects under stressful conditions in neonatal chicks (Takagi et al. 2001). Therefore, it is possible that these amino acids are involved in stress regulation. In turn, WIS rats may have utilized these amino acids in the brain to alleviate acute stress. On the other hand, WKY rats may have not utilized these amino acids, and they were not rescued from acute stress. Therefore, we hypothesized that WKY rats are vulnerable to acute stress. In fact, the previous studies revealed that WKY rats were highly sensitive to stress by counting the length of cumulative gastric ulcers after stress paradigm (Pare' 1990, 1994), and by comparing monoamine metabolism after chronic restraint stress (O'Mahony et al. 2011).

De La Garza and Mahoney (2004) reported that WKY rats displayed the lower values of DA and DOPAC in the prefrontal cortex, and the higher value of DOPAC/DA in the striatum compared with WIS rats. In addition, stress treatment caused the higher value of DOPAC, NE and

Table 3 Effects of strain difference and stress treatment on amino acid concentration of the thalamus in WIS and WKY rats

Strain	WIS		WKY		*p*		
Treatment	Control	Stress	Control	Stress	Strain	Stress	Strain × stress
Aspartate	3,330 ± 142[a]	2,401 ± 130[b]	2,949 ± 163[ac]	2,830 ± 148[c]	NS	$p < 0.01$	$p < 0.01$
Glutamate	8,542 ± 230	7,412 ± 253	8,442 ± 526	7,717 ± 368	NS	$p < 0.05$	NS
Asparagine	ND	ND	ND	ND			
Glycine	1,726 ± 78[a]	1,257 ± 53[b]	1,696 ± 91[a]	1,622 ± 94[a]	$p < 0.05$	$p < 0.01$	$p < 0.05$
Glutamine	6,907 ± 224	5,539 ± 297	7,196 ± 441	6,544 ± 315	NS	$p < 0.01$	NS
β-alanine	81.8 ± 6.8	63.9 ± 7.8	90.1 ± 9.6	79.8 ± 8.4	NS	NS	NS
Taurine	4,510 ± 157	4,009 ± 257	4,494 ± 181	4,123 ± 209	NS	$p < 0.05$	NS
Histidine	156 ± 22	108 ± 15	109 ± 13	123 ± 23	NS	NS	NS
GABA	3,237 ± 252	2,470 ± 249	2,646 ± 301	2,620 ± 259	NS	NS	NS
Threonine	794 ± 31[a]	564 ± 35[b]	719 ± 34[ac]	646 ± 49[bc]	NS	$p < 0.01$	$p < 0.05$
Alanine	978 ± 43[a]	650 ± 38[b]	775 ± 49[b]	696 ± 52[b]	NS	$p < 0.01$	$p < 0.05$
Carnosine	ND	ND	ND	ND			
Arginine	508 ± 19[a]	379 ± 22[b]	433 ± 27[b]	425 ± 29[b]	NS	$p < 0.01$	$p < 0.05$
Proline	150 ± 11[a]	97.5 ± 5.9[b]	132 ± 12[a]	124 ± 12[ab]	NS	$p < 0.01$	$p < 0.05$
Tyrosine	156 ± 7.6[a]	113 ± 7.0[b]	135 ± 11[ab]	125 ± 7.2[b]	NS	$p < 0.01$	$p < 0.05$
Valine	181 ± 14	138 ± 11	162 ± 16	173 ± 14	NS	NS	NS
Methionine	139 ± 7.8[b]	94.3 ± 5.7[a]	122 ± 9.1[b]	130 ± 12[b]	NS	NS	$p < 0.01$
Isoleucine	72.2 ± 4.4[a]	48.0 ± 1.5[b]	60.9 ± 3.2[a]	60.2 ± 4.0[a]	NS	$p < 0.01$	$p < 0.01$
Leucine	109 ± 8.3[b]	69.9 ± 4.5[a]	97.9 ± 4.0[b]	90.5 ± 4.9[b]	NS	$p < 0.01$	$p < 0.05$
Tryptophan	64.2 ± 12	72.3 ± 9.4	64.2 ± 17	74.9 ± 11	NS	NS	NS
Ornithine	27.0 ± 2.2	21.7 ± 2.4	20.1 ± 1.6	21.0 ± 1.3	$p < 0.05$	NS	NS
Lysine	467 ± 31[b]	289 ± 27[a]	416 ± 26[b]	437 ± 32[b]	NS	$p < 0.05$	$p < 0.01$

The number of samples used for analysis was 10. The values for amino acid are expressed as mean ± SEM in pmol/mg wet tissue. Different letters indicate significant difference at $p < 0.05$

NS not significant, *ND* not detectable

Fig. 4 Metabolic pathway of cystathionine, serine and homocysteine. Cystathionine is synthesized from serine and homocysteine by cystathionine β-synthase, and metabolized to cysteine by cystathionine γ-lyase. *CBS* cystathionine β-synthase, *CSE* cystathionine γ-lyase

DOPAC/DA in the prefrontal cortex. The results obtained in the present study were not in accordance with the previous report. This may be related to the difference in stress treatment. De La Garza and Mahoney performed FST for 15 min because they emphasized that this test duration activated the stress hormone responses in WKY rats. On the other hands, we selected normal FST for 5 min since we emphasized to evaluate the depression-like behavior. The differences in test duration for FST may have induced the different responses in the monoamine metabolism.

Previous study demonstrated the differences in amino acid levels, such as arginine, alanine and threonine in the cerebrospinal fluid between SD and WKY rats (Takasugi et al. 2003). In the present study, arginine, alanine and threonine levels in the striatum were lower in WKY rats compared with WIS rats, while those in the thalamus were similar in WKY and WIS rats. These data suggest that amino acid levels in the striatum depend on the cerebrospinal fluid, but those in the thalamus are independent.

In conclusion, WKY rats were abnormal in amino acid and monoamine metabolisms. In addition, WKY rats displayed the possibility of serving as animal models of depression and stress vulnerability from the aspect of abnormal behavior and amino acid metabolism compared to WIS rats, respectively. The present study may contribute to the elucidation of pathologic mechanisms for depression as well as stress-regulatory mechanisms. Furthermore, the elucidation about the relationships between abnormality in amino acid metabolism and depression or stress-regulatory mechanism may propose the new therapy.

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