

## Oral Administration of L-serine Modifies Amino Acid Metabolism in the Brain of Rats

Kazutaka Shigemi<sup>1</sup>,  
Shozo Tomonaga<sup>1</sup>,  
Nobuo Uotsu<sup>2</sup>,  
Michael Denbow, D<sup>3</sup>,  
Mitsuhiro Furuse<sup>1</sup>

### Abstract

**Objective:** Whether orally administered L-serine influences amino acid metabolism in the brain was investigated. **Methods:** L-Serine (6 mmol/10 ml/kg) was orally administered to rats after a 9 hr fast and plasma and brain samples were obtained at 30, 60 and 120 min in Experiment 1. In Experiment 2, the effect of L-serine and glycine on brain amino acid metabolism was compared. **Results:** In Experiment 1, plasma serine rapidly increased 30 min after administration and then decreased, but serine in both the cerebral cortex and hippocampus gradually increased with time. Plasma taurine and glutamine were positively and leucine, phenylalanine and lysine were negatively correlated with plasma serine concentration. Cerebral cortex glutamine, citrulline, tyrosine,  $\beta$ -alanine, and tryptophan were negatively and  $\alpha$ -amino adipic acid and glycine were positively correlated with serine concentration. In the hippocampus, glutamine and tyrosine were negatively correlated with L-serine. In Experiment 2, threonine concentrations were significantly decreased by L-serine treatment compared with the control (distilled water) and glycine in both brain regions. Serine concentrations in both brain regions were greatly increased by L-serine treatment, but were also moderately increased by glycine. Similarly, glycine in both regions was increased by glycine treatment and by L-serine treatment. Cysteine in both regions was increased by glycine treatment. Tyrosine in both regions was decreased by both L-serine and glycine treatments. **Conclusion:** Orally administered L-serine greatly influenced brain amino acid metabolism. L-Serine and glycine influenced each other, but the effect on brain amino acid metabolism was somewhat different between the two amino acids.

**Keywords:** L-Serine; Glycine; Cerebral cortex; Hippocampus; Plasma

1. Laboratory of Regulation in Metabolism and Behavior and Laboratory of Advanced Animal Bioresources, Graduate School of Bioresources and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan
2. FANCL Research Institute, 12-13 Kamishinano, Totsuka-ku, Yokohama, Kanagawa, 244-0806, Japan
3. Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA 24061-0306, USA

**Corresponding author:** Mitsuhiro Furuse

✉ furuse@brs.kyushu-u.ac.jp

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### Introduction

L-Serine, a nonessential amino acid, is synthesized mainly from metabolic intermediates of glycolysis, or partially from glycine. Serine-deficiency syndrome, a congenital disease associated with an inborn error generally in the L-serine biosynthetic enzyme 3-phosphoglycerate dehydrogenase, was reported [1]. Patients of this syndrome were identified because of very low concentrations of serine and glycine in the plasma and cerebrospinal fluid. Furthermore, they suffer from serious neurological symptoms such as congenital microcephaly, seizures and severe psychomotor retardation. However, these symptoms are treatable with oral supplementation of L-serine alone or in combination with glycine [1-3].

Other studies indicate that L-serine is a novel neurotrophic factor in cultured cells [4,5]. These actions are presumed to be related to metabolic products of L-serine (e.g., lipids and nucleotides) since they are components of cell membranes and nucleic acids required for neurogenesis during brain development. This idea is supported by the findings that neurons cannot maintain synthesis of serine-derived lipids without an external supply of L-serine [6,7]. These facts indicate the importance of L-serine in the central nervous system (CNS).

Asechi et al. [8] conducted a series of experiments to investigate the relationship between L-serine in the CNS and stress-related behavior of animals using the chick separation stress paradigm. Intracerebroventricular (i.c.v.) injection of L-serine and its derivatives, including glycine and L-cysteine, induced sedative and

hypnotic effects under isolation-induced stress in neonatal chicks. Furthermore, it was shown that the sedative and hypnotic effects of i.c.v. L-serine were due to L-serine itself, and not its metabolites [9]. In addition, Shigemi et al. [10] exposed rats to a social isolation stress for 4 weeks, and a home cage test and open field test were included to evaluate the effect of L-serine administered orally in the water on behavior. L-Serine in the drinking water increased L-serine levels in some brain areas, but changes in its metabolites were almost negligible. L-Serine decreased locomotor activity in rats exposed to a familiar environment. In addition, L-serine decreased exploratory behavior of isolated rats, even in a novel environment. These facts suggest that daily intake of L-serine can attenuate symptoms induced by chronic stress.

L-Serine and glycine are reversibly metabolized by serine hydroxymethyltransferase. Shigemi et al. [11] investigated the involvement of glycine receptors for sedative and hypnotic effects of L-serine, since L-serine is suggested to act as the  $\alpha$ -homomeric glycine receptor agonist [12]. Glycine similarly induced sedative and hypnotic effects in chicks, but its effect was attenuated by the glycine receptor antagonist strychnine [11]. Therefore, whether the effect of L-serine was mediated through the glycine receptor was investigated using L-serine and strychnine. The effect of L-serine was inhibited by picrotoxin, a  $\gamma$ -aminobutyric acid (GABA)<sub>A</sub> receptor antagonist, but not strychnine. It appears that L-serine induces sedative and hypnotic effects by enhancing inhibitory neurotransmission via GABA<sub>A</sub> receptors.

Currently, it is not known how quickly orally ingested L-serine is transported to the brain. Furthermore, the short term effect of L-serine on peripheral and central amino acid metabolism is also not known. Therefore, the present study was conducted to investigate the time course changes of peripheral and central amino acid concentrations after oral administration of L-serine. In addition, effects of L-serine on brain amino acid metabolism were compared with those of glycine.

## Materials and Methods

### Animals

Male Wistar rats (three weeks old) were purchased from Japan SLC, Inc. (Hamamatsu, Japan). They were kept in cages (three per cage) at a room temperature of  $25 \pm 1^\circ\text{C}$  on a 12L:12D light-dark cycle (lights on 08:00, lights off 20:00), and given free access to a commercial diet (MF, Oriental Yeast, Co., Ltd., Tokyo, Japan) and water. They were allowed to habituate for one week before beginning the experiment. This study was performed according to the guidance for Animal Experiments in the 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

Following a habituation period, rats were randomly selected and divided into four groups, each with six rats. The rats were fasted for 9 hr. Except for the control group, rats were orally administered L-serine (6 mmol/10 ml/kg) in Experiment 1. The control group received no treatment. Thirty, 60, or 120 min after treatment, rats were decapitated and the blood was collected into heparinized tubes. Blood samples were centrifuged for 15

min at  $4^\circ\text{C}$  at 6,000 g and the plasma was removed and stored at  $-80^\circ\text{C}$  until analysis. The cerebral cortex and hippocampus were collected, weighed, rapidly frozen with liquid nitrogen and stored at  $-80^\circ\text{C}$  until analysis. In Experiment 2, rats were divided into three groups, i.e., the control (distilled water), L-serine (6 mmol/10 ml/kg) and glycine (6 mmol/10 ml/kg) given following a 9 hr fast. The cerebral cortex and hippocampus were collected 120 min post administration. The hippocampus was dissected according to the method of Hagihara et al. [13]. L-Serine and glycine were provided by Kyowa Hakko Bio (Tokyo, Japan).

## Analysis of Free Amino acids

The method of sampling for the amino acid analysis has been described elsewhere [14] with some modifications. Brains 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  $0^\circ\text{C}$ . These supernatants were adjusted to pH 3 with 1 M sodium acetate and were filtrated through a  $0.2 \mu\text{m}$  filter (Millipore, Bedford, USA). The plasma was diluted with sulfosalicylic acid solution (20 mg/ml) and shaken for 15 min. Then the homogenate was centrifuged at  $2,000 \times g$  for 15 min at  $4^\circ\text{C}$ . Next, the sample was filtered through a  $0.2\text{-}\mu\text{m}$  filter (Millipore, Bedford, USA). Then, 40  $\mu\text{l}$  of each filtrate was applied to the Amino Acid Analyzer L-8800 (Hitachi High-Technologies Co. Ltd., Hitachi, Japan). Standard solutions were prepared by diluting a commercially available L-amino acid solution (type AN II and type B; Wako, Osaka, Japan) with distilled water.

## Statistical Analysis

Results were subjected to one-way analysis of variance. When significant ( $P < 0.05$ ) effects were detected, comparisons between means were made using the Fisher PLSD test. All analyses were performed with StatView (version 5, SAS institute, Cary, N.C., United States). Results are presented as means  $\pm$  S.E.M. In Experiment 1, correlations between serine and other amino acids were determined in the plasma, cerebral cortex and hippocampus.

## Results

Amino acid concentrations were measured as the sum of L and D types. Thus, except for administered L-serine, amino acids were described as the name without discrimination of L and D types.

### Experiment 1: Time course changes in plasma and brain amino acids after a single L-serine administration

Figure 1 shows the time course of serine concentration changes in the plasma, cerebral cortex and hippocampus of rats after oral administration of L-serine. Plasma serine rapidly increased at 30 min after administration, and then decreased at 60 min although the concentrations remained higher than the initial values. Serine gradually increased with time in both the cerebral cortex and hippocampus.

Table 1 shows significant changes in plasma free amino acids after oral administration of L-serine. Among 11 amino acids, plasma taurine and glutamine were positively and leucine,

phenylalanine and lysine were negatively correlated with plasma serine concentration.

**Table 2** shows significant changes in brain free amino acids after oral administration of L-serine. In the cerebral cortex, 9 amino acids were changed by L-serine treatment. Glutamine, citrulline, tyrosine,  $\beta$ -alanine and tryptophan were negatively correlated with serine concentration as shown in **Figure 1**. In contrast,  $\alpha$ -amino adipic acid and glycine were positively correlated with serine. In the hippocampus, only glutamine, leucine and tyrosine were changed by L-serine treatment. Glutamine and tyrosine were negatively correlated with serine (**Figure 1**).

#### Experiment 2: Changes in brain amino acids after a single L-serine or glycine administration

**Figures 2 and 3** show significant changes in free amino acids in the cerebral cortex and hippocampus after oral administration of L-serine or glycine. Threonine, serine, glycine, cysteine and tyrosine showed similar changes in both the cerebral cortex and hippocampus. In both brain regions, threonine concentrations

were significantly decreased by L-serine treatment compared with the control (distilled water) and glycine. Serine concentrations in both brain regions were greatly increased with L-serine treatment, but were also moderately increased by glycine. Similarly, glycine in both regions was increased more by glycine treatment than L-serine treatment. Cysteine in both regions was increased by glycine treatment. Tyrosine in both regions was decreased by both L-serine and glycine treatments. Valine in the cerebral cortex was highest in the glycine treatment group while lowest in the L-serine treatment. Histidine concentration was decreased in the hippocampus more by glycine than serine.

## Discussion

Sedative and hypnotic effects of L-serine are induced 10 min after i.c.v. injection [9]. Asechi et al. [9] determined brain amino acid concentrations following behavioral tests and confirmed that serine itself was higher than the control, but its metabolites were not changed during the 10 min post-administration period. Following the administration of serine for 4 weeks in the drinking

**Table 1** Significant changes in plasma free amino acids (nmol/ml) and correlation between plasma serine concentrations after oral administration of L-serine.

	Time after administration (min)				Correlation coefficient between plasma serine
	0	30	60	120	
Taurine	226 ± 19 <sup>B</sup>	290 ± 15 <sup>A</sup>	225 ± 15 <sup>B</sup>	237 ± 15 <sup>B</sup>	0.503
Threonine	280 ± 8 <sup>A</sup>	293 ± 14 <sup>A</sup>	241 ± 17 <sup>B</sup>	229 ± 9 <sup>B</sup>	
Glutamic acid	96 ± 3 <sup>B</sup>	123 ± 4 <sup>A</sup>	116 ± 4 <sup>A</sup>	116 ± 5 <sup>A</sup>	0.708
Glutamine	897 ± 23 <sup>A</sup>	862 ± 24 <sup>A</sup>	753 ± 36 <sup>B</sup>	770 ± 9 <sup>B</sup>	
Glycine	368 ± 8 <sup>C</sup>	415 ± 13 <sup>BC</sup>	410 ± 14 <sup>BC</sup>	479 ± 26 <sup>A</sup>	
Citrulline	134 ± 5 <sup>A</sup>	147 ± 4 <sup>A</sup>	115 ± 9 <sup>B</sup>	110 ± 5 <sup>B</sup>	
Leucine	149 ± 6 <sup>A</sup>	105 ± 5 <sup>B</sup>	128 ± 20 <sup>AB</sup>	124 ± 8 <sup>AB</sup>	-0.667
Phenylalanine	70 ± 2 <sup>A</sup>	56 ± 1 <sup>B</sup>	64 ± 3 <sup>AB</sup>	65 ± 3 <sup>AC</sup>	-0.735
Tyrosine	109 ± 4 <sup>A</sup>	100 ± 5 <sup>A</sup>	74 ± 2 <sup>B</sup>	84 ± 4 <sup>B</sup>	
Lysine	578 ± 12 <sup>A</sup>	461 ± 16 <sup>B</sup>	439 ± 48 <sup>B</sup>	455 ± 20 <sup>B</sup>	-0.588
Hydroxyproline	106 ± 4 <sup>A</sup>	105 ± 3 <sup>A</sup>	77 ± 8 <sup>B</sup>	97 ± 7 <sup>A</sup>	

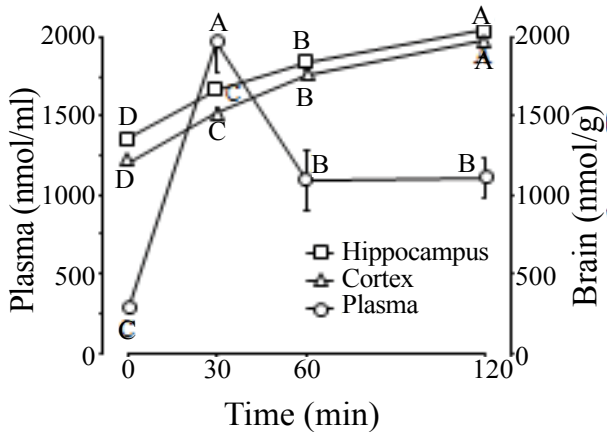
Values are means ± S.E.M. n=5-6. Values with different superscripts are significantly different at P<0.05.

**Table 2** Significant changes in brain free amino acids (nmol/g) and correlation between brain serine concentrations after oral administration of L-serine.

	Time after administration (min)				Correlation coefficient between
	0	30	60	120	
	Cerebral cortex				serine in the cerebral cortex
Glutamine	6233 ± 79 <sup>A</sup>	6012 ± 54 <sup>A</sup>	5669 ± 90 <sup>B</sup>	5689 ± 102 <sup>B</sup>	-0.737
$\alpha$ -AAA	84 ± 5 <sup>B</sup>	89 ± 5 <sup>B</sup>	91 ± 4 <sup>AB</sup>	104 ± 5 <sup>A</sup>	0.570
Glycine	708 ± 17 <sup>B</sup>	714 ± 14 <sup>B</sup>	731 ± 18 <sup>AB</sup>	777 ± 20 <sup>A</sup>	0.541
Citrulline	66 ± 3 <sup>A</sup>	63 ± 2 <sup>AB</sup>	57 ± 2 <sup>BC</sup>	59 ± 2 <sup>BC</sup>	-0.469
Isoleucine	81 ± 3 <sup>A</sup>	71 ± 2 <sup>B</sup>	73 ± 3 <sup>AB</sup>	79 ± 3 <sup>A</sup>	
Leucine	115 ± 3 <sup>A</sup>	93 ± 2 <sup>C</sup>	101 ± 1 <sup>B</sup>	114 ± 2 <sup>A</sup>	
Tyrosine	124 ± 4 <sup>A</sup>	124 ± 3 <sup>A</sup>	108 ± 5 <sup>B</sup>	103 ± 5 <sup>B</sup>	-0.553
$\beta$ -Alanine	47 ± 1 <sup>A</sup>	45 ± 2 <sup>AB</sup>	42 ± 2 <sup>BC</sup>	41 ± 1 <sup>C</sup>	-0.615
Tryptophan	12 ± 1 <sup>A</sup>	7 ± 2 <sup>B</sup>	6 ± 1 <sup>B</sup>	8 ± 1 <sup>B</sup>	-0.465
	Hippocampus				serine in the hippocampus
Glutamine	6709 ± 116 <sup>A</sup>	6458 ± 137 <sup>A</sup>	6027 ± 70 <sup>B</sup>	6001 ± 105 <sup>B</sup>	-0.676
Leucine	129 ± 2 <sup>A</sup>	104 ± 2 <sup>C</sup>	107 ± 2 <sup>C</sup>	117 ± 3 <sup>B</sup>	
Tyrosine	135 ± 2 <sup>A</sup>	138 ± 4 <sup>A</sup>	117 ± 7 <sup>B</sup>	112 ± 5 <sup>B</sup>	-0.556

Values are means ± S.E.M. n=5-6. Values with different superscripts are significantly different at P<0.05.

$\alpha$ -AAA:  $\alpha$ -Amino Adipic Acid.



**Figure 1** Time course changes in serine concentration in plasma, cerebral cortex and hippocampus in rats after oral administration of L-serine. Different letters on the same line were significantly different at  $P < 0.05$ . Values are presented as means  $\pm$  S.E.M.  $n = 5-6$ .

water, L-serine levels increased in some brain areas, but changes in its metabolites were almost negligible [10]. These results differ from the current results in which serine was acutely administered following a 9 hr fast.

In Experiment 1, we applied a single oral administration of serine following a 9 hr fast. Plasma serine concentration was sharply increased at 30 min and then decreased but maintained a still elevated level at 60 and 120 min post-administration (**Figure 1**). We expected that the pattern of changes in amino acid concentration would be similar between serine and glycine since they are reversibly produced by serine hydroxymethyltransferase. However, plasma glycine concentration was only elevated at 120 min after L-serine administration. As a result, no significant correlation was detected between serine and glycine. The synthesis of glycine from L-serine may not be under static equilibrium.

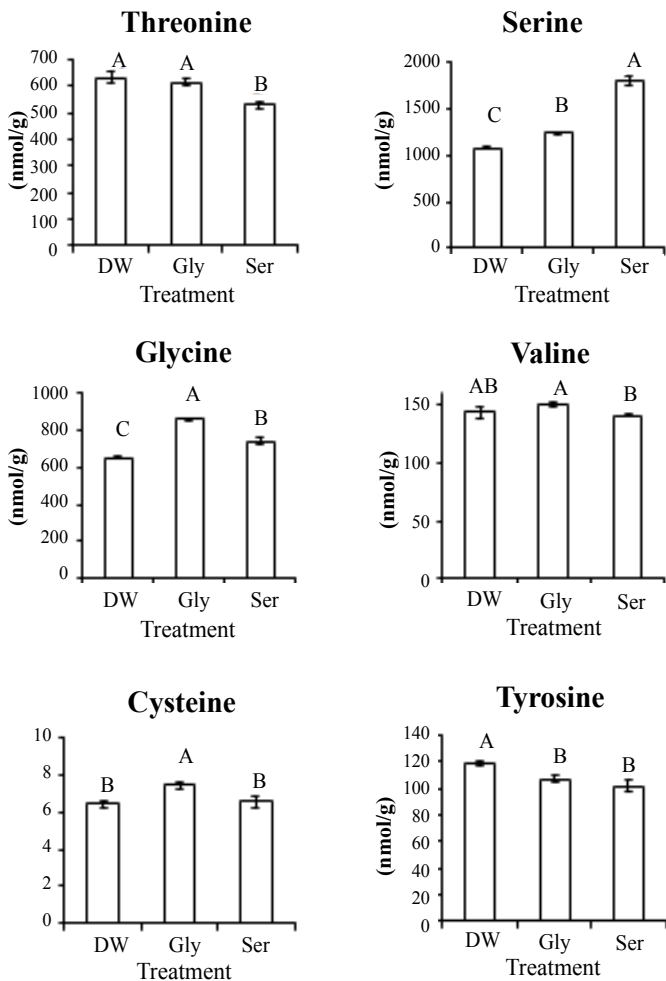
Taurine is a derivative of cysteine which is synthesized from serine. Plasma taurine concentration was correlated with plasma serine (**Table 1**). However, plasma cysteine was not significantly changed and not correlated with plasma serine (data not shown). Cysteine may be quickly utilized to synthesize taurine. The reasons that glutamic acid was positively correlated, and leucine, phenylalanine and lysine were negatively correlated with serine were unclear from the metabolic map.

In both Experiments 1 and 2, serine concentration was clearly increased in both the cerebral cortex and hippocampus by L-serine treatment. Our data support the report by Kasai et al. [15] who confirmed that L-serine crossed the blood brain barrier of rats.

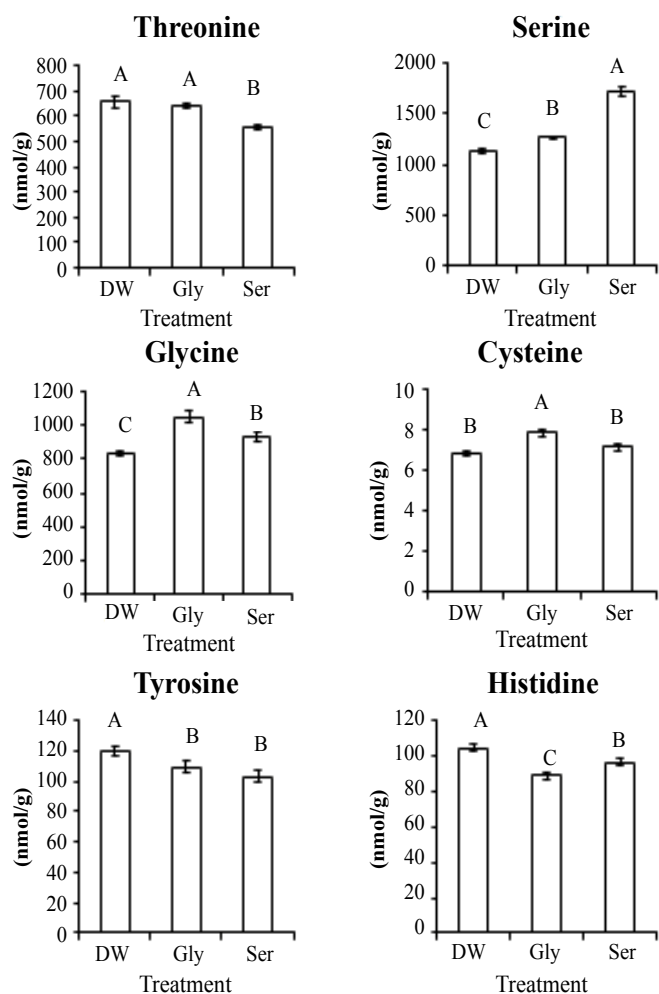
In Experiment 1, more amino acid changes were seen in the cerebral cortex than in the hippocampus following L-serine administration. In both brain regions, glutamine and tyrosine were decreased with the increase of serine (**Table 2**). Glycine administration also decreased tyrosine concentration in the cerebral cortex and hippocampus (**Figures 2 and 3**). Tryptophan in the cerebral cortex also decreased with increasing serine. These results suggest that serine incorporation in the brain is a regulator of synthesis and/or release of monoamine transmitters. This was the case for nitric oxide (NO) production in which citrulline in the cerebral cortex was decreased with an increase of serine. Brain citrulline, an NO co-product, could be an index of brain NO generation via NO synthase (NOS) [16]. L-Serine is converted to the enantiomer D-serine by serine racemase, which was purified from the mammalian brain [17,18]. D-Serine inhibits neuronal NOS but not endothelial NOS [19]. Orally ingested serine may contribute to lower NOS activity.

In Experiment 1, glycine concentration was increased in the plasma and cerebral cortex by L-serine administration, but not in the hippocampus. This was due to serine hydroxymethyltransferase which reversibly metabolizes L-serine and glycine. Similarly, serine concentration was increased by glycine treatment in Experiment 2 (**Figures 2 and 3**).

Cysteine is a metabolite of serine, but cysteine concentration in the cerebral cortex and hippocampus was not increased after L-serine treatment. Glycine rather than L-serine was effective in increasing cysteine concentration. In the



**Figure 2** Changes in free amino acids in the cerebral cortex after oral administration of L-serine or glycine. Different letters on the same graph were significantly different at  $P < 0.05$ . Values are presented as means  $\pm$  S.E.M.  $n = 6$ . DW, distilled water; Gly, glycine; and Ser, L-serine.



**Figure 3** Changes in free amino acids in the hippocampus after oral administration of L-serine or glycine. Different letters on the same graph were significantly different at  $P < 0.05$ . Values are presented as means  $\pm$  S.E.M  $n=5-6$ . DW, distilled water; Gly, glycine; and Ser, L-serine.

cysteine synthesis pathway, glycine is present up-stream of L-serine. Large amounts of L-serine may down regulate the enzymes of cysteine synthesis. In contrast, the amount of serine produced from glycine may match the enzyme activity.

Threonine and serine are metabolized by the same enzyme,

serine (threonine) dehydratase. The reduction in threonine of the cerebral cortex and hippocampus by L-serine treatment may be associated with serine dehydratase. However, the precise mechanism is unclear.

Shigemi et al. [10] reported the mechanism by which i.c.v. L-serine induced sedative and hypnotic effects in neonatal chicks exposed to acute stressful conditions. The involvement of GABA<sub>A</sub> receptors on the effect of L-serine was investigated using the GABA<sub>A</sub> receptor antagonist picrotoxin. Co-administration of picrotoxin attenuated the sedative and hypnotic effect of L-serine. Further, Shigemi et al. [10] also investigated the involvement of glycine receptors since L-serine is suggested to act as the  $\alpha$ -homomeric glycine receptor agonist [12]. Glycine similarly induced sedative and hypnotic effects in chicks, but its effect was attenuated by the glycine receptor antagonist strychnine. However, the effect of L-serine was not inhibited by strychnine. In the present study, L-serine did not change GABA concentrations in either Experiment 1 or 2. Accordingly, we believe that serine stimulates GABA release but not GABA synthesis. For instance, L-pipecolic acid (L-PA) is a major metabolic intermediate of L-lysine in the mammalian [20,21] and chick brain [22]. L-PA is incorporated into the crude synaptosomal fraction [23] and stimulates some brain sites in the rat [24]. It has also been reported that L-PA was able to increase the release of GABA stimulated by mild depolarization with potassium from brain slices and decrease the uptake of GABA by brain [25]. L-PA has similar effects such as hypnosis and sedation [26] as observed by L-serine [8,9,11].

In conclusion, orally administered L-serine greatly influenced both plasma and brain amino acid metabolism. These changes may be related to the central functions of L-serine. L-Serine and glycine influence the metabolism of each other, but the effect on brain amino acid metabolism was somewhat different between the two amino acids.

## Conflict of Interest

Declared none.

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