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Effect of systemic administration of D-serine on the levels of D- and L-serine in several brain areas and periphery of rat

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Abstract

To obtain further insight into the distribution and metabolism of exogenous D-serine, we have investigated the effect of the intraperitoneal administration of D-serine (10 mmol/kg) on the concentrations of D- and L-serine in several brain areas and periphery of infant and adult rats. The administration produced a significant augmentation of the D-serine levels not only in the cortex but also in the hippocampus, striatum, cerebellum and periphery. The rapid decline in the enhanced D-serine levels was observed in the periphery and cerebellum, whereas the injection caused a prolonged elevation of the D-serine levels in the cortex and hippocampus. The application caused a slight increase in the L-serine levels in several brain areas and periphery 3 or 6 h after the injection, whereas a significant decrease in the L-serine concentration was observed in the periphery, diencephalon and cerebellum 3 or 7 days after the injection. Because a structural abnormality and *N*-methyl-D-aspartate (NMDA) receptor hypofunction has been demonstrated in the cortex and hippocampus of schizophrenic subjects, D-serine treatment may offer a new therapeutic approach to diseases related to the hypofunction of NMDA receptors such as schizophrenia.
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Keywords: D-Amino acid oxidase; Glycine site; *N*-Methyl-D-aspartate receptor; D-Serine; L-Serine; Serine racemase

1. Introduction

Recently, growing evidence has been provided demonstrating that a high concentration of D-serine is present in the mammalian brain (Chouinard et al., 1993; Hashimoto et al., 1992, 1993a,b, 1995a,b, 2000; Hashimoto, 2002; Kumashiro et al., 1995; Schell et al., 1995, 1997; Wolosker et al., 1999a,b), although D-amino acids have long been assumed to be unnatural in mammals (Hashimoto and Oka, 1997). D-Serine is predominantly confined to the forebrain, where the *N*-methyl-D-aspartate (NMDA) type excitatory amino acid receptors are enriched (Hashimoto et al., 1993a; Schell et al., 1997). In vivo microdialysis studies have indicated that the extracellular concentration of D-serine parallels or is higher than that of glycine in the prefrontal cortex and in the striatum, respectively (Hashimoto et al., 1995a). Because D-serine potentiates the NMDA receptor-mediated transmission by selective stimulation of the strychnine-insensitive glycine site of the NMDA receptor with an affinity similar to glycine, but no affinity for the inhibitory glycine receptor (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Matsui et al., 1995), D-serine has been proposed as an endogenous ligand for an NMDA receptor-related glycine site in the mammalian brain (Hashimoto et al., 1993a; Hashimoto and Oka, 1997). Concerning the de novo synthesis of D-serine, an intraperitoneal administration of L-serine has recently been shown to produce a significant augmentation of the D-serine levels in several brain areas and periphery (Dunlop and Neidle, 1997; Hashimoto, 2002; Takahashi et al., 1997). Furthermore, serine racemase that catalyzes the direct formation of D-serine from L-serine has been purified and cloned from the mammalian brain (Wolosker et al., 1999a,b; Konno, 2003). Immunohistochemical studies using an antibody against serine racemase have demonstrated that the distribution of serine racemase closely resembles those of endogenous D-serine and NMDA (Wolosker et al., 1999b).

The NMDA receptor hypofunction has been implicated in the pathophysiology of schizophrenia (Deutsch et al., 1989; Javitt and Zukin, 1991; Tsai and Coyle, 2002). The psycho-

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ses after administration of NMDA receptor antagonists such as phencyclidine are clinically indistinguishable from schizophrenia, including the positive, negative and cognitive symptoms. Supporting the hypothesis of the NMDA receptor hypofunction in schizophrenia, the NMDA receptor-deficient mice display behavioral abnormalities, including increased motor activity and stereotypic behavior similar to that observed on treatment of normal mice with the NMDA receptor antagonists phencyclidine or MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine) (Mohn et al., 1999) and NMDA-glycine site agonists such as D-serine and D-alanine also blocks the phencyclidine- and methamphetamine-induced hyperactivity and stereotypic behavior in rats (Contreras, 1990; Hashimoto et al., 1991; Tanii et al., 1991, 1994). In fact, the administration of D-serine has been shown to improve the negative, positive and cognitive symptoms of schizophrenic subjects treated with conventional neuroleptics (Tsai et al., 1998).

Although researchers have administered D-serine to access their potential efficacy in patients with schizophrenia (Tsai et al., 1998, 1999), the distribution of D-serine and metabolism of D- and L-serine after D-serine administration are relatively poorly understood, in particular related to the distributional profile of exogenous D-serine in the brain except the cortex and the long-term effects for the metabolism of D- and L-serine (Imai et al., 1998; Takahashi et al., 1997). We have investigated the effect of the intraperitoneal administration of D-serine on the concentrations of D- and L-serine in several brain areas and the periphery of infant and adult rats.

2. Materials and methods

2.1. Animals and drugs

The present animal experiments were performed in strict accordance with the guidances of the Tokai University, and were approved by the Animal Investigation Committee of the university. Male Wistar rats at postnatal day 7 (14–17 g) or at postnatal week 7 (220–240 g) were used. D-Serine (10 mmol/kg) was dissolved in physiological saline and was intraperitoneally injected. The control animals received saline. Following this treatment, the infant rats were stunned and decapitated 6 h after the administration. The adult rats were stunned and decapitated 3 h, 6 h, 1 day, 3 days or 7 days after the administration. The brains and peripheries were dissected out on ice and stored at − 80 °C. D-Serine was purchased from Nacalai Tesque (Kyoto, Japan).

2.2. High performance liquid chromatography (HPLC) analysis of amino acids

The simultaneous determination of the free amino acid enantiomers and non-chiral amino acids in the tissue sample was accomplished using HPLC and fluorometric detection as previously described (Hashimoto et al., 1993a). Briefly, a tissue sample was homogenized in 10 volumes of 5% trichloroacetic acid after the addition of D-homocysteic acid, and the homogenate was centrifuged at 16,000 × *g* for 30 min at 4 °C. The supernatant was washed three times with water-saturated diethyl ether. The aqueous layer was then passed through a Millipore filter, HV (0.45 μm) and stored at − 80 °C until derivatization. The resultant sample was derivatized with *N*-*tert*-butyloxycarbonyl-L-cysteine and *o*-phthaldialdehyde for 2 min at room temperature. The amino acid derivative was immediately applied to the HPLC system.

2.3. Statistics

The results are given as means with S.E.M. of the data. For comparison between the two groups, a statistical evaluation was performed using either the unpaired two-tailed student's *t*-test or Mann–Whitney U-test. A *P*-value <0.05 was considered as reaching statistical significance.

3. Results

3.1. Time course of changes in the level of D- and L-serine in several brain areas of adult rats after the intraperitoneal administration of D-serine

Following the intraperitoneal administration of D-serine (10 mmol/kg), the D-serine concentration in the

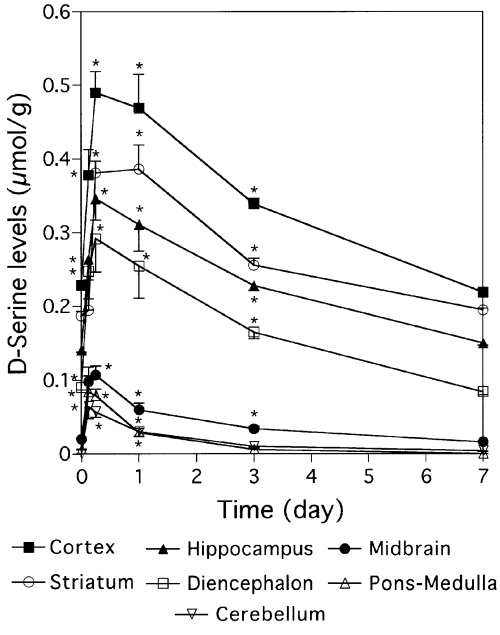

Fig. 1. Time course of changes in the concentrations of D-serine in several brain areas of the adult rats after the intraperitoneal administration of D-serine. Seven-week-old rats received an intraperitoneal injection of D-serine (10 mmol/kg) and were killed at each indicated point. The results are means with S.E.M. of data obtained from four to six animals. *$P<0.01$ as compared with controls.

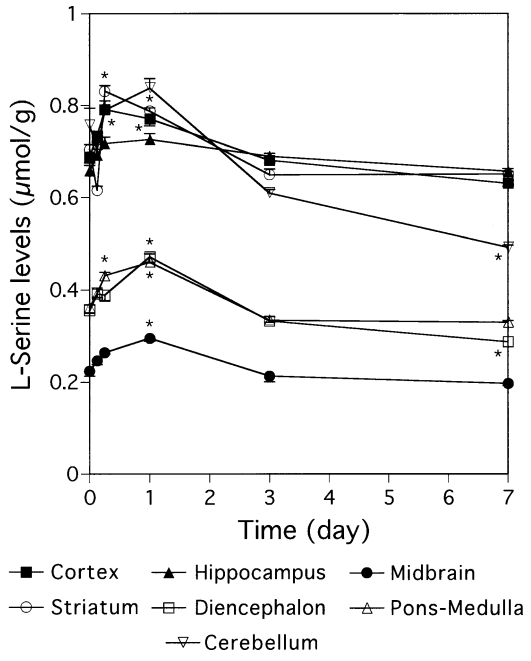

Fig. 2. Time course of changes in the concentrations of L-serine in several brain areas of the adult rats after the intraperitoneal administration of D-serine. Seven-week-old rats received an intraperitoneal injection of D-serine (10 mmol/kg) and were killed at each indicated point. The results are means with S.E.M. of data obtained from four to six animals. *$P<0.01$ as compared with controls.

cortex, striatum, hippocampus, diencephalon, midbrain, pons-medulla and cerebellum rapidly and moderately increased, and peaked at 6, 24, 6, 6, 6, 3 and 3 h, respectively (Fig. 1). The increased D-serine level in the cortex, striatum, hippocampus and diencephalon gradually decreased to the control level around 7 days after the injection, whereas a relatively rapid decrease in the enhanced D-serine level was observed in the cerebellum and pons-medulla (Fig. 1). The administration caused a slight but significant increase in the levels of L-serine in the cortex, striatum, diencephalon, midbrain and pons-medulla 6 or 24 h after the injection (Fig. 2). In addition, a slight but significant decrease in the L-serine concentration was observed in the diencephalon and cerebellum 7 days after the administration.

3.2. Time course of changes in the level of D- and L-serine in the periphery of adult rats after the intraperitoneal administration of D-serine

Following the systemic injection of D-serine, the D-serine concentrations in almost all the peripheral tissues except the testis rapidly and drastically increased, and peaked at 3 h, and then rapidly decreased to the control level around 3 days after the administration (Fig. 3). The increase in the D-serine level was highest in the thymus, followed by the spleen, lung, kidney, adrenal and liver, and was low in the testis. In addition, the rate of the reduction in the elevated D-serine levels is faster in all the peripheral tissues than in all the brain areas (Figs. 1 and 3). The administration caused a slight but significant increase in the levels of L-serine in the thymus, lung and liver 3 or 6 h after the injection, whereas a slight but significant decrease in the L-serine concentration was observed in almost all the peripheral tissues 3 days after the injection (Fig. 4). In addition, hypertrophy of the kidney was observed 7 days after the D-serine administration (data not shown).

3.3. Effect of the systemic administration of D-serine on D- and L-serine concentrations in the infant rat brain

In the infant rats, the levels of D-serine in all the brain areas dramatically and significantly increased by about 30–90 times 6 h after the D-serine administration (10 mmol/kg) (Table 1). The increase in the D-serine concentration was highest in the cerebellum, followed by the pons-medulla, cortex-striatum, diencephalon and midbrain. The magnitude of the increased D-serine concentration was much greater in all the brain areas of the infant than the adult rats (Table 1 and Fig. 1). A systemic injection of D-serine caused a slight but significant increase in the levels of L-serine in all the brain areas (Table 2).

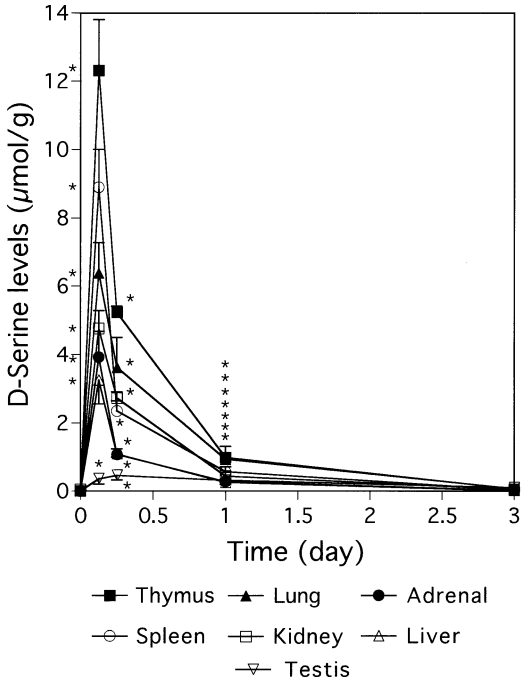

Fig. 3. Time course of changes in the concentrations of D-serine in peripheral tissues of the adult rats after the intraperitoneal administration of D-serine. Seven-week-old rats received an intraperitoneal injection of D-serine (10 mmol/kg) and were killed at each indicated point. The results are means with S.E.M. of data obtained from four to six animals. *$P<0.01$ as compared with controls.

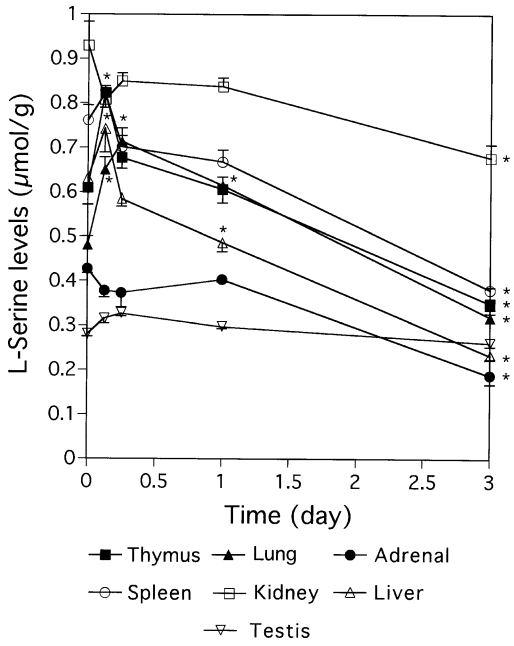

Fig. 4. Time course of changes in the concentrations of L-serine in peripheral tissues of the adult rats after the intraperitoneal administration of D-serine. Seven-week-old rats received an intraperitoneal injection of D-serine (10 mmol/kg) and were killed at each indicated point. The results are means with S.E.M. of data obtained from four to six animals. *$P<0.01$ as compared with controls.

3.4. Effect of the systemic administration of D-serine on D- and L-serine concentrations in the infant rat periphery

The levels of D-serine in all the peripheral tissues of the infant rats drastically increased by about 600–1700 times 6 h after the D-serine injection (Table 1). The increase in the D-serine concentration was highest in the kidney, followed by the thymus, spleen, testis and lung, and was low in the liver. The magnitude of the enhanced D-serine concentration was greater in almost all the peripheral tissues of the infant than the adult rats (Table 1 and Fig. 3). The systemic injection of D-serine caused a slight but significant increase in the levels of L-serine in almost all the peripheral tissues except the kidney (Table 2).

Table 1
Effect of intraperitoneal administration of D-serine on D-serine levels in several brain areas and periphery of infant rat

Tissues (μmol/g)	Saline	D-Serine	D-Serine/ Saline	*P*-value
Cortex-Striatum	0.16 ± 0.01	5.80 ± 0.41	36	<0.01
Diencephalon	0.15 ± 0.01	5.02 ± 0.36	33	<0.01
Midbrain	0.13 ± 0.01	4.62 ± 0.27	36	<0.01
Pons-Medulla	0.11 ± 0.01	7.34 ± 0.56	66	<0.01
Cerebellum	0.13 ± 0.01	12.06 ± 0.86	91	<0.01
Kidney	0.01 ± 0.01	17.01 ± 1.97	1701	<0.01
Liver	0.01 ± 0.01	6.68 ± 0.71	668	<0.01
Testis	0.01 ± 0.01	12.99 ± 0.98	1299	<0.01
Spleen	0.02 ± 0.01	13.12 ± 1.17	656	<0.01
Thymus	0.01 ± 0.01	13.86 ± 1.30	1386	<0.01
Lung	0.02 ± 0.01	12.26 ± 2.02	613	<0.01

Animals at postnatal day 7 received an intraperitoneal injection of D-serine (10 mmol/kg) and were killed at 6 h thereafter. Data are mean ± S.E.M. values obtained from five rats.

Table 2
Effect of intraperitoneal administration of D-serine on L-serine levels in several brain areas and periphery of infant rat

Tissues (μmol/g)	Saline	D-Serine	D-Serine/ Saline	*P*-value
Cortex-Striatum	0.79 ± 0.05	1.36 ± 0.12	1.7	<0.01
Diencephalon	0.70 ± 0.04	1.29 ± 0.12	1.8	<0.01
Midbrain	0.71 ± 0.04	1.22 ± 0.10	1.7	<0.01
Pons-Medulla	0.92 ± 0.04	1.58 ± 0.13	1.7	<0.01
Cerebellum	0.90 ± 0.04	1.55 ± 0.11	1.7	<0.01
Kidney	1.72 ± 0.08	2.14 ± 0.16	1.2	N.S.
Liver	0.85 ± 0.02	1.43 ± 0.13	1.7	<0.01
Testis	0.88 ± 0.05	1.40 ± 0.12	1.6	<0.01
Spleen	0.75 ± 0.03	1.27 ± 0.13	1.7	<0.01
Thymus	0.78 ± 0.06	1.27 ± 0.10	1.6	<0.01
Lung	1.02 ± 0.10	1.32 ± 0.11	1.3	<0.05

Animals at postnatal day 7 received an intraperitoneal injection of D-serine (10 mmol/kg) and were killed at 6 h thereafter. Data are mean ± S.E.M. values obtained from five rats. N.S.; not significant.

4. Discussion

The present study demonstrated that the intraperitoneal administration of D-serine produced a significant augmentation of the D-serine levels in all the brain areas and all the peripheral tissues of the infant and adult rats. The systemic injection caused a significant elevation of the L-serine levels in all the brain areas and almost all the peripheral tissues of the infant and adult rats. These observations, together with the fact that serine racemase can convert D- to L-serine (Wolosker et al., 1999a,b), provided the possibility that the enhanced L-serine after the D-serine administration may be formed from D-serine by serine racemase. Further support for this possibility comes from the fact that an intraperitoneal injection of L-serine (25 μmol/kg) at the dose of the calculated contamination in 10 mmol/kg of the D-serine reagent (10 mmol/kg × 0.25% = 25 μmol/kg) had no effect on the L-serine level in the cortex of the adult rats (data not shown).

The rapid decline in the enhanced D-serine levels was observed in almost all the peripheral tissues, pons-medulla and cerebellum of the adult rats after the D-serine administration, whereas a gradual decrease in the increased D-serine levels occurred in the forebrain such as the cortex, striatum, hippocampus and diencephalon. The rapid decline in the elevated D-serine levels may be due to the fact that the D-serine is excreted into the urine and is metabolized by D-amino acid oxidase (DAO), which catalyses the oxidative deamination of neutral D-amino acids (D'Aniello et al., 1993; Fleck, 1992; Horiike et al., 1994; Neims et al., 1966; Weimar and Neims, 1977). Several lines of evidence

support this possibility: (a) DAO activity is confined to the hindbrain such as the cerebellum and pons-medulla with low activity in the forebrain (Horiike et al., 1994; Neims et al., 1966), (b) the levels of D-serine in the cerebellum and rostral brain areas of mutant mice lacking DAO activity are higher than those of normal mice (Hashimoto et al., 1993b), and (c) comparable doses of D-serine cause acute tubular necrosis and amino aciduria (Ganote et al., 1974; Carone and Ganote, 1975). Because only 10–20% of ingested D-amino acids have been shown to excrete into the urine and feces within 2 days, percentage of D-serine metabolized by DAO might be relatively high (D'Aniello et al., 1993).

The magnitude of the elevated D-serine levels after the D-serine administration was highest in the cerebellum of the infant brain, but was lowest in the cerebellum of the adult brain. The differential changes in the enhanced D-serine levels between the infant and adult rats may be due to the fact that the blood–brain barrier and cerebellum of the infant rats are immature. In fact, the blood–brain barrier of the infant rats has been shown to have a greater permeability to neutral amino acids than that of the adult rats (Lefauconnier and Trouve, 1983). DAO activity of the infant cerebellum is also much lower than that of the adult cerebellum (Weimar and Neims, 1977). Together with the fact that the magnitude of the enhanced L-serine levels after the L-serine application is also highest in the cerebellum of the infant brain (Hashimoto, 2002), neutral amino acids might enter more readily into the cerebellum than the other brain areas in the infant period. The magnitude of the increase in the D-serine levels was also greater in the kidney of the infant than the adult rats. Because the urinary excretion capacity of the kidney and renal clearances of amino acids were lower in the young than adult rats (Braunlich, 1984; Fleck, 1992) and because the DAO activity in the kidney of the 7-day-old rats is much lower than that of the adult rats (Weimar and Neims, 1977), the infant kidney functions may also be immature.

The gradual and significant diminution in the L-serine levels was observed in almost all the peripheral tissues, cerebellum and diencephalon of the adult rats 3 or 7 days after the administration. Although the reason for the gradual decrease in the L-serine levels remains to be determined, the induction of L-serine-catabolic but not biosynthetic enzymes by D-serine could enhance the reduction of the L-serine concentration. Several other findings have supported this possibility: (a) several hormones and stimulants have been shown to induce L-serine-catabolic enzymes such as serine dehydratase, serine aminotransferase or serine hydroxymethyltransferase (Snell, 1984), (b) D-serine has been shown to induce D-serine-regulated transcript dsr-1 (Tsuchida et al., 2001), and (c) D-serine does not inhibit phosphoserine phosphatase, which catalyzes the final step in the major pathway of the L-serine biosynthesis (Hawkinson et al., 1997).

The hypofunction of the NMDA receptor subtype glutamate receptor has been implicated in the pathophysiology of schizophrenia (Deutsch et al., 1989; Javitt and Zukin, 1991; Tsai and Coyle, 2002). In fact, the administration of D-serine has been shown to improve the negative, positive and cognitive symptoms of schizophrenic subjects treated with conventional neuroleptics (Tsai et al., 1998). Because the intraperitoneal injection of D-serine caused a prolonged elevation of the D-serine level in the cortex, hippocampus and diencephalon (present study), where a structural abnormality and NMDA receptor hypofunction has been demonstrated in schizophrenic subjects (Deutsch et al., 1989; Javitt and Zukin, 1991; Tsai and Coyle, 2002) and because D-serine is an endogenous full agonist of the glycine site of the NMDA receptor (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Matsui et al., 1995), D-serine treatment may offer a new therapeutic approach to diseases related to the hypofunction of NMDA receptors such as schizophrenia and Alzheimer's disease. Further studies such as the dose dependency and the effect of chronic administration of D-serine are needed to clarify whether clinically effective doses would produce physiologically appropriate levels of D-serine in the brain.

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