Modulation of extracellular d-serine content by calcium permeable AMPA receptors in rat medial prefrontal cortex as revealed by in vivo microdialysis

Sayuri Ishiwata¹, Asami Umino¹, Masakazu Umino¹, Kazuko Yorita², Kiyoshi Fukui² and Toru Nishikawa¹

¹ Department of Psychiatry and Behavioral Sciences, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan
² Division of Enzyme Pathophysiology, The Institute for Enzyme Research, The University of Tokushima, Tokushima, Japan

Abstract

In mammalian brains, d-serine has been shown to be required for the regulation of glutamate neurotransmission as an endogenous co-agonist for the N-methyl-d-aspartate type glutamate receptor that is essential for the expression of higher-order brain functions. The exact control mechanisms for the extracellular d-serine dynamics, however, await further elucidation. To obtain an insight into this issue, we have characterized the effects of agents acting at the a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptor on the extracellular d-serine contents in the medial prefrontal cortex of freely moving rats by an in vivo microdialysis technique in combination with high-performance liquid chromatography with fluorometric detection. In vivo experiments are needed in terms of a crucial role of d-serine in the neuron-glia communications despite the previous in vitro studies on AMPA receptor-d-serine interactions using the separated preparations of neurons or glial cells. Here, we show that the intra-cortical infusion of (S)-AMPA, an active enantiomer at the AMPA receptor, causes a significant and concentration-dependent reduction in the prefrontal extracellular contents of d-serine, which is reversed by an AMPA/kainate receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide disodium salt, and a calcium permeable AMPA receptor antagonist, 1-naphthyl acetyl spermine. The d-serine reducing effects of (S)-AMPA are augmented by co-infusion of cyclothiazide that prevents AMPA receptor desensitization. Our data support the view that a calcium permeable AMPA receptor subtype may exert a phasic inhibitory control on the extracellular d-serine release in the mammalian prefrontal cortex in vivo.

Received 15 September 2012; Reviewed 28 October 2012; Revised 8 November 2012; Accepted 13 November 2012; First published online 9 January 2013

Key words: AMPA receptor, d-serine, in vivo dialysis, medial prefrontal cortex.

Introduction

It is widely accepted that, in mammalian brains, d-serine is an endogenous co-agonist for the N-methyl-d-aspartate (NMDA) type glutamate receptor that is essential for the control of a variety of higher-order brain functions and neuronal cell viability. This concept is based on essential observations that d-serine exhibits a brain-predominant and NMDA receptor-like distribution and selective binding to the glycine modulatory site of the glutamate receptor and that a specific elimination of d-serine from brain tissues results in reduced NMDA receptor activity (for a review, see Nishikawa, 2011). These features of d-serine suggest the presence of a fine regulatory system that maintains an appropriate extracellular d-serine signal in the brain. Indeed, substantial extracellular contents of d-serine have also been demonstrated to be highly correlated with the densities of the NMDA receptors in rat brains (Hashimoto et al., 1995) and to be altered by manipulation of the neural and glial cell activities in the prefrontal cortical region of freely moving rats (Hashimoto et al., 1995; Kanematsu et al., 2006), in brain slices or cell cultures (Rosenberg et al., 2010; Sullivan and Miller, 2010). However, the exact molecular and cellular mechanisms underlying the regulation of the extracellular d-serine contents still remain unclear.

Several lines of evidence have indicated that glutamate neurotransmission modifies the extracellular d-serine contents. Thus, in vitro studies using astrocytes, neurons in the primary culture, C6 glioma cells and intact retina
have shown that the extracellular release of D-serine is increased by glutamate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (for a review, see Nishikawa, 2011). In contrast, NMDA and kainic acid were reported to reduce the D-serine contents in the striatal extracellular fluid collected by in vivo dialysis experiments (Hashimoto et al., 2000). These discrepancies could at least partly be due to the differences in the interactions among the cells and molecules between the in vitro and in vivo preparations. In terms of the involvement of D-serine in the communications between neuronal and glial cells (Nishikawa, 2011), not only in vitro but also in vivo experiments are needed to analyse the control of brain extracellular D-serine concentrations. However, there is so far no in vivo evidence for the possible AMPA receptor-D-serine interactions in the brain.

In the present study, to gain a deeper insight into the glutamatergic control of extracellular D-serine signalling, we have characterized the effects of agents acting at the AMPA receptor on the extracellular D-serine contents in the rat medial prefrontal cortex (mPFC) using an in vivo dialysis technique. We also measured the extracellular levels of taurine that have been reported to be under an excitatory influence by the AMPA receptor in vivo and of some D-serine-related amino acids including L-glutamate, L-glutamine, glycine and L-serine.

**Materials and method**

**Animals and reagents**

The present animal experiments were performed in strict accordance with the guidance of the Tokyo Medical and Dental University and were approved by the Animal Investigation Committee of the Institution. Male Wistar rats (ST strain; Clea Japan, Inc., Japan) at post-natal day 56, weighing 200–250 g, were used. The animals were housed at 23.0 ± 0.5 °C in a humidity-controlled room under a 12-h light/dark cycle (lights on 08:00 hours) and had free access to food and water.

**Chemicals**

(S)-AMPA, cyclothiazide (CTZ), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[l]- quinoxaline-7-sulfonamide disodium salt (NBQX) and 1-naphthyl acetyl spermine trihydrochloride (NASPM) were purchased from Tocris Bioscience (USA). Recombinant human D-amino acid oxidase (hDAAO) was prepared according to the method of Kawazoe et al. (2006). All other chemicals were of ultrapure grade and commercially available. An ampoule solution of pentobarbital was diluted with physiological saline for the intraperitoneal injection of the animals. The doses always refer to the free bases.

**In vivo microdialysis**

The in vivo microdialysis was performed as previously reported with some modifications (Hashimoto et al., 1995; Nishijima et al., 1996; Kanematsu et al., 2006). The rats were anaesthetized with pentobarbital (40 mg/kg i.p.) and mounted on a stereotoxic frame. A straight-shaped cellulose dialysis tube (3.0 mm in length, 0.16 mm internal diameter, molecular weight cut-off 50,000; EICOM Co., Ltd., Japan) was then implanted into the mPFC (A +2.2 mm, V +5.0 mm, L −0.7 mm) according to the atlas of Paxinos and Watson (2005). Two days after surgery, the dialysis probe was perfused with a Ringer solution (147 mM NaCl; 4 mM KCl; 1.3 mM CaCl₂; pH 7.4) at the flow rate of 2 μl/min in a freely moving rat. After stabilizing for at least 80 min, the dialysate samples were collected every 20 min. The first three samples were used to determine the basal release of each amino acid and then the various experiments were started by the fourth sampling of the dialysate as time 0. A 20-min application of (S)-AMPA was performed by perfusing the Ringer solution, including different concentrations of (S)-AMPA from time 20 to 40 min. In some experiments, the Ringer solution with NBQX (100 μM), NASPM (300 μM) or CTZ (100 μM) was continuously infused from time 0. Our previous studies, in accordance with the data reported by other research groups (Korf and Venema, 1985; Westerink and Tuinve, 1986; Paulsen et al., 1987), confirmed that the above experimental conditions allowed us to observe: (1) a terodotoxin-reversible depolarization-induced increase in the extracellular release of classical amino acid transmitters, glutamate and glycine (Hashimoto et al., 1995); (2) a marked reduction or complete elimination of the extracellular release of a classical transmitter, dopamine, by cessation of the nerve impulse flow or calcium chelation (Nishijima et al., 1996; Kanematsu et al., 2006). (3) changes in the glial cell activity by monitoring the extracellular glutamine (Kanematsu et al., 2006).

**High-performance liquid chromatography (HPLC) with fluorometric detection**

The collected dialysates were stored at −80 °C until derivatization following the addition of D-homocysteic acid as the internal standard. For quantification of the amino acids by HPLC with fluorometric detection, an aliquot of each sample was derivatized with N-tert-butyloxycarbonyl-L-cysteine and o-phthalaldehyde for 2 min at room temperature. The derivatized sample was immediately applied to the HPLC system and then analysed on a 4-μM (particle size) Nova-Pak C18 column (300 mm × 3.9 mm) (Waters Co., Ltd., Japan). The column was operated at the constant flow rate of 0.8 ml/min at 35 °C. Mobile phase A was 0.1 M acetate buffer (pH 6.0) containing 12% acetonitrile and mobile phase B was the acetate buffer containing 20% acetonitrile. The separation of the amino acid derivatives was performed using a linear gradient from mobile phase A to B in 53 min. The fluorescent amino acid derivatives were detected using a Waters 2475 MultiL fluorescence detector spectrophotometer (Waters Co., Ltd.). The excitation and
emission wavelengths were 344 and 443 nm, respectively. The cortical dialysate levels of the L- and non-chiral amino acids determined in the present study are in good agreement with those previously reported (Hashimoto et al., 1995; Kanematsu et al., 2006).

We have proven the presence of endogenous D-serine by gas chromatography (GC) and GC with mass spectrometry (GC–MS) and demonstrated excellent agreement in the quantitative measurements among our GC, GC–MS and HPLC assay methods (for a review, see Nishikawa, 2011). Because the D-serine peak in the HPLC method was not inspected mass spectrometrically, we presently confirmed the precision of the D-serine identification in the dialysates by observing that the D-serine peak was specifically and completely removed after treating the supernatants with a D-serine degrading enzyme hDAAO (Fig. 1c, d), but not a boiled hDAAO (Fig. 1e, f) or vehicle (Fig. 1a, b).

Data analysis

The averaged concentration of each substance during the period preceding the drug treatment (three measurements were performed every 20 min) was used as the baseline control value (100%). The individual data are expressed as percentages of this baseline period. The means with s.e.m. of the results obtained from 4–7 animals were calculated using the corresponding periods. The areas under the curves (AUC) of the concentration vs. time plots for the dialysate amino acids at 40–180 min of infusion from time 0 (see above in 'In vivo microdialysis') were calculated and used as the overall measures of the treatment effects (Matthews et al., 1990).

For comparison between the two groups, the statistical evaluations were performed using the unpaired two-tailed Student’s t test (the homogeneous variance for each experimental group) or Aspin–Welch’s t test (the heterogeneous variance for each experimental group). Statistical differences among more than three groups were estimated by Bonferroni’s method. We evaluated using Barlett’s test the homogeneity of the variance of each experimental group). We estimated as the 100% means with S.E.M. of the results obtained from 4–7 animals expressed as percentages of this baseline period. The retention time of authentic D-Ser, but not L-Ser and L-Gln, was completely eliminated following hDAAO treatment (Fig. 1b, c, d). The peak eluted at the retention time of authentic D-Ser, but not L-Ser and L-Gln, was completely eliminated following hDAAO treatment (Fig. 1b, c, d). In these verification experiments, the standard mixture of amino acids or the prefrontal dialysates were incubated at 37 °C for 1 h in a total volume of 80 μl in the presence of DAAO at 50 μg/ml, boiled DAAO or its vehicle (a, b). The peak eluted at the retention time of authentic D-Ser, but not L-Ser and L-Gln, was completely eliminated following hDAAO treatment (Fig. 1b, c, d).

Results

Effects of AMPA receptor agents on the extracellular contents of d-serine in the rat mPFC

The concentration ranges from 10 to 500 μM of the intracortically infused (S)-AMPA via the dialysis probe were chosen based upon the following reasons: (1) the (S)-AMPA concentrations outside the dialysis probe is estimated as the 10⁻⁶–10⁻⁴ M ranges in case of application of the aforementioned original concentrations because we determined the efficiency of dialysis across the membrane as approximately 10% (mean with S.E.M.: 10.42 ± 0.17, n = 4) for (S)-AMPA (data not shown); (2) the 10⁻⁴ M concentrations of L-glutamate, which has been reported to be approximately one-fifth to 10 times potent...
Fig. 2. Effects of local perfusion of (S)-AMPA [an active enantiomer at the \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) receptor] alone or in combination with cyclothiazide (CTZ), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]-quinoxaline-7-sulfonamide disodium salt (NBQX) or 1-naphthyl acetyl spermine trichloride (NASPM) into the medial prefrontal cortex on the extracellular contents of D-serine in the cortical region of the rat. (a), as indicated by the open symbols, (S)-AMPA alone (10, 30, 100 and 500 \( \mu \)M) was infused into the medial prefrontal cortex of freely moving rats via dialysis tubing for 20 min from 20 to 40 min from the start of the present experiments (time 0). (c, e, g), the solid black symbols indicate the continuous local application of Ringer solution containing CTZ (100 \( \mu \)M; \( c \)), NBQX (100 \( \mu \)M; \( e \)) or NASPM (300 \( \mu \)M; \( g \)) and the open symbols display the period of (S)-AMPA (100 \( \mu \)M) infusion through the dialysis probe into the cortical portion. The concentrations of D-serine in the cortical dialysates collected every 20 min were quantitatively determined using high-performance liquid chromatography with fluorometric detection. Each point represents the mean with S.E. mean of data obtained from 4–7 animals and expressed as a percentage of the respective basal D-serine.
as AMPA for the AMPA receptor subunits including Glu_A, Glu_Q, and Glu_T (Traynelis et al., 2010), are considered to occur after depolarization, as depolarization by veratrine or veratrine causes a marked increase in the extracellular l-glutamate contents that is maximally about 10 times higher than the basal levels of around 35 μM in this study; (3) these concentrations do not seem to be cytotoxic because the 10^{-3}–10^{-4} M ranges of (S)-AMPA or AMPA have been used for bath application in the in vitro experiments using the cell culture or slice preparation.

As shown in Fig. 2a, the 20-min intra-mPFC perfusion of (S)-AMPA (10–500 μM) via the microdialysis tubing caused a concentration-related decrease in the extracellular contents of d-serine in the cortical portion as compared to the Ringer solution-perfused controls (Fig. 2a, b) in the rat. The local infusion of (S)-AMPA at 100 and 500 μM elicited a significant reduction in the extracellular levels of d-serine from 40 to 140 min post-infusion (80 to 180 min from time 0) while that at 30 μM diminished significantly the dialysate d-serine contents only at time 80 min (Fig. 2a). There was no significant change at any time following the intracortical application of 10 μM (S)-AMPA (Fig. 2a). The decreased responses as evaluated by AUC were statistically significant after perfusion of 100 and 500 μM, but not at 10 and 30 μM (S)-AMPA (Fig. 2b).

We also investigated the relationship of a rapid desensitization process following the stimulation of the AMPA receptor (Trussell et al., 1988; Sun et al., 2002) with the d-serine decreasing effects of (S)-AMPA using an allosteric potentiator of the AMPA receptor, CTZ, that slows its desensitization (Partin et al., 1993; Yamada and Tang, 1993; Fucile et al., 2006; Krintel et al., 2012). CTZ (100 μM) significantly enhanced the ability of 100 μM (S)-AMPA to reduce the extracellular d-serine contents without its own influence on the d-amino acid contents (Fig. 2c, d) when assessed both by the data at each time-point (from time 80 to 140 min and at time 180 min) and by AUC. These results suggest that the decreased responses of the extracellular d-serine concentrations to the prolonged (20 min) perfusion of (S)-AMPA are not due to the desensitization of the AMPA receptor, but produced by its stimulation. In the dialysates collected from 20 to 40 min following the start of the local perfusion of (S)-AMPA with CTZ, but not of (S)-AMPA at 10 to 500 μM alone, there was a small but significant increase in the d-serine contents (Fig. 2a, c).

The d-serine decreasing effects of (S)-AMPA at 100 μM were completely eliminated by its co-infusion with a competitive AMPA receptor-preventing antagonist, NBQX, 100 μM (Fig. 2e, f) or a selective calcium permeable AMPA receptor antagonist, NASPM, at 300 μM (Fig. 2g, h) when estimated both by the data at each time-point (from time 80 to 140 min for NBQX and at time 100 min for NASPM) and by AUC, while NBQX or NASPM alone failed to alter the cortical extracellular d-serine concentrations (Fig. 2e–h).

**Effects of AMPA receptor agents on the extracellular contents of taurine in the rat mPFC**

In contrast to d-serine, there was a concentration-dependent increase in the extracellular taurine contents in the rat mPFC by the local infusion of (S)-AMPA (10–500 μM) into the cortical area (Fig. 3a, b). The local infusion of (S)-AMPA at 100 and 500 μM elicited a significant elevation in the extracellular levels of taurine from 20 to 140 min post-infusion (60 to 180 min from time 0) whereas that at 30 μM augmented significantly the dialysate taurine contents only at time 60 min (Fig. 3a). There was no significant change in those at any time following the intracortical application of 10 μM (S)-AMPA (Fig. 3a). The augmented responses as evaluated by AUC were statistically significant after infusion of 100 and 500 μM, but not at 10 and 30 μM (S)-AMPA (Fig. 3b).

The taurine increasing effects of (S)-AMPA at 100 μM were significantly enhanced and completely abolished in the presence of CTZ (Fig. 3c, d) at 100 μM and of NBQX at 100 μM (Fig. 3c, f), respectively, when estimated both by the data at each time-point and by AUC, whereas neither CTZ (Fig. 3c, d) nor NBQX (Fig. 3e, f) changed the basal extracellular contents of taurine. In contrast, NASPM at 300 μM did not modulate the basal contents and the ability of (S)-AMPA at 100 μM to elevate the prefrontal extracellular taurine levels (Fig. 3g, h).

**Effects of AMPA receptor agents on the extracellular contents of l-serine, glycine, l-glutamate and l-glutamine in the rat mPFC**

The prefrontal extracellular contents of l-serine, which is converted to d-serine by serine racemase (Wolosker et al., 1999), and glycine, which is another endogenous co-agonist for the NMDA receptor in the brain region, were slightly and transiently, but significantly increased...
Fig. 3. Effects of local perfusion of (S)-AMPA [an active enantiomer at the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor] alone or in combination with cyclothiazide (CTZ), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]-quinoxaline-7-sulfonamide disodium salt (NBQX) or 1-naphthyl acetyl spermine trihydrochloride (NASPM) into the medial prefrontal cortex on the extracellular contents of taurine in the cortical region of the rat. (a), as indicated by the open symbols, (S)-AMPA alone (10, 30, 100 and 500 μM) was infused into the medial prefrontal cortex of freely moving rats via dialysis tubing for 20 min from 20 to 40 min from the start of the present experiments (time 0). (c, e, g), the solid black bars indicate the continuous local application of Ringer solution containing CTZ (100 μM; c), NBQX (100 μM; e) or NASPM (300 μM; g) and the open bars display the period of (S)-AMPA (100 μM) infusion through the dialysis probe into the cortical portion. The concentrations of taurine in the cortical dialysates collected every 20 min were quantitatively determined using high-performance liquid chromatography with fluorometric detection. Each point represents the mean with S.E. mean of data obtained from 4–7 animals and expressed as a percentage of the respective basal taurine contents (Table 1). ●, ■, and ▲, Indicate statistically significant differences in the time-point data at p < 0.05, 0.01 or 0.001 as compared to the Ringer solution alone-infused controls: open symbols, n.s. # p < 0.05 as compared to the animals treated with the intra-cortical infusion of (S)-AMPA in the presence of CTZ, NBQX or NASPM. The area under the curve (AUC) is also calculated by adding the areas under the graph of the concentration of the respective amino acid between each pair of every 20-min consecutive observation from 40 to
following the intra-mPFC infusion of 100 μM (S)-AMPA in a NBQX-sensitive fashion (Fig. 4a–d) when estimated both by the data at each time-point and by AUC. However, NASPM failed to affect the basal and increased levels of the extracellular l-serine and glycine (data not shown). The (S)-AMPA perfusion did not alter the l-glutamate (Fig. 4e, f) and l-glutamine (Fig. 4g, h) contents in the prefrontal dialysate.

Discussion

In the present study, by using an in vivo dialysis technique in freely moving rats, we first demonstrated that a locally applied AMPA type glutamate receptor selective agonist, (S)-AMPA, caused a concentration-related and an allosteric agonist-potentiating decrease in the extracellular contents of d-serine in the mPFC. This decrease is completely reversed by not only a competitive AMPA/kainate receptor antagonist, NBQX, but also by a calcium permeable AMPA receptor antagonist, NASPM. The differential effects of (S)-AMPA on the contents of l-serine, glycine, l-glutamate, l-glutamine and taurine in the extracellular fluid observed in this study appear to deny the possibility that the alterations in the d-serine contents are due to a non-specific phenomenon.

As AMPA receptor overstimulation has been reported to cause cell death or various types of tissue damages (Dugan et al., 1995; Larm et al., 1997; John et al., 1999), the (S)-AMPA-induced reduction in the extracellular d-serine contents could result from a complete or marked loss in the activities of the neural and/or glial cells around the site of the (S)-AMPA infusion. However, this is unlikely because: (1) an extreme attenuation of neural activity by tetrodotoxin failed to decrease the extracellular d-serine levels in the cortical area (Hashimoto et al., 1995); (2) in tetrodotoxin failed to decrease the extracellular d-serine contents in the prefrontal area of the rat by the following data: (1) (S)-AMPA is a highly selective and potent agonist for the AMPA receptors (Watkins et al., 1990); (2) CTZ enhances the effects of an AMPA receptor agonist acting at the glutamate binding site by attenuating the agonist-provoked desensitization of the receptor through selectively binding to its allosteric agonist site (Partin et al., 1993; Yamada and Tang, 1993; Fucile et al., 2006; Krintel et al., 2012); (3) the NBQX disodium salt is shown to be an AMPA receptor-prefering non-NMDA glutamate receptor antagonist based on in vitro experiments that a bath application of AMPA agonists facilitates the release of preloaded [3H]d-serine or endogenous d-serine from cultured astrocytes (Schell et al., 1995; Mothet et al., 2005; Rosenberg et al., 2010) or neurons (Kartvelishvily et al., 2006; Rosenberg et al., 2010) in a primary culture, C6 glioma cells (Mothet et al., 2005) or retinal cells (Sullivan and Miller, 2010). This discrepancy cannot be attributed to certain inappropriate conditions of this in vivo study because the (S)-AMPA-induced and NBQX-reversible increase in the prefrontal extracellular taurine contents in the present experiments is consistent with previous findings obtained by an in vivo dialysis method: (1) AMPA produces a rapid, concentration-related, prominent and selective antagonist-reversible increase in the extracellular taurine contents in the primary motor cortex (La Bella and Piccoli, 2000); (2) the AMPA/kainate receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione, significantly attenuated the elevations in the dialysate taurine levels produced by a selective glutamate uptake inhibitor, l-trans-pyrrolidine-2,4-dicarboxylic acid, in the prefrontal cortex (Del Arco et al., 1999). AMPA receptor-mediated property of the modification of taurine levels is also grounded by the augmentation of the (S)-AMPA-induced increase in taurine contents by an AMPA...
### Table 1. Baseline extracellular concentrations and control AUC values of d-serine and other amino acids measured in the medial prefrontal cortex of the rat for the control

<table>
<thead>
<tr>
<th>Experiments</th>
<th>d-serine</th>
<th>Taurine</th>
<th>l-serine</th>
<th>Glycine</th>
<th>l-Glutamate</th>
<th>l-Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Basal amino acid concentrations (μM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-AMPA</td>
<td>0.96 ± 0.04 (n = 23)</td>
<td>2.63 ± 0.20 (n = 23)</td>
<td>4.60 ± 0.23 (n = 23)</td>
<td>5.95 ± 0.38 (n = 23)</td>
<td>5.10 ± 0.54 (n = 23)</td>
<td>31.88 ± 1.99 (n = 23)</td>
</tr>
<tr>
<td>(S)-AMPA vs. CTZ</td>
<td>0.99 ± 0.04 (n = 24)</td>
<td>3.70 ± 0.23 (n = 22)</td>
<td>6.05 ± 0.31 (n = 22)</td>
<td>7.77 ± 0.92 (n = 22)</td>
<td>4.21 ± 0.37 (n = 22)</td>
<td>40.19 ± 3.04 (n = 22)</td>
</tr>
<tr>
<td>(S)-AMPA vs. NBQX</td>
<td>0.83 ± 0.04 (n = 21)</td>
<td>3.85 ± 0.41 (n = 21)</td>
<td>4.94 ± 0.28 (n = 21)</td>
<td>7.32 ± 0.48 (n = 21)</td>
<td>5.54 ± 0.82 (n = 21)</td>
<td>32.12 ± 3.35 (n = 21)</td>
</tr>
<tr>
<td>(S)-AMPA vs. NASPM</td>
<td>0.99 ± 0.02 (n = 21)</td>
<td>2.80 ± 0.14 (n = 21)</td>
<td>4.23 ± 0.18 (n = 21)</td>
<td>6.92 ± 0.64 (n = 21)</td>
<td>6.02 ± 0.43 (n = 21)</td>
<td>22.01 ± 1.29 (n = 21)</td>
</tr>
<tr>
<td>(b) Amino acid AUC control values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(S)-AMPA</td>
<td>13.489 ± 150.4 (n = 4)</td>
<td>14.275 ± 319.3 (n = 4)</td>
<td>13.301 ± 4.190 (n = 4)</td>
<td>14.678 ± 4.479 (n = 4)</td>
<td>13.346 ± 397.2 (n = 4)</td>
<td>12.685 ± 264.7 (n = 4)</td>
</tr>
<tr>
<td>(S)-AMPA vs. CTZ</td>
<td>13.180 ± 342.4 (n = 7)</td>
<td>12.819 ± 680.7 (n = 5)</td>
<td>14.856 ± 425.7 (n = 5)</td>
<td>14.617 ± 358.7 (n = 5)</td>
<td>14.805 ± 492.0 (n = 5)</td>
<td>12.685 ± 319.3 (n = 5)</td>
</tr>
<tr>
<td>(S)-AMPA vs. NBQX</td>
<td>13.026 ± 250.6 (n = 7)</td>
<td>13.305 ± 579.1 (n = 7)</td>
<td>14.805 ± 388.7 (n = 7)</td>
<td>14.653 ± 492.0 (n = 7)</td>
<td>13.949 ± 521.3 (n = 5)</td>
<td>13.990 ± 270.5 (n = 5)</td>
</tr>
<tr>
<td>(S)-AMPA vs. NASPM</td>
<td>13.744 ± 343.9 (n = 5)</td>
<td>12.612 ± 481.2 (n = 6)</td>
<td>14.394 ± 521.3 (n = 5)</td>
<td>13.590 ± 270.5 (n = 5)</td>
<td>13.590 ± 270.5 (n = 5)</td>
<td>13.590 ± 270.5 (n = 5)</td>
</tr>
</tbody>
</table>

(S)-AMPA, An active enantiomer at the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor; CTZ, cyclothiazide; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]-quinoxaline-7-sulfonamide disodium salt; NASPM, 1-naphthyl acetyl spermine trihydrochloride.

Basal amino acid concentrations show the average concentrations of each amino acid in the perfusates from the medial prefrontal cortex of all rats in each experimental group during the 60-min period prior to the drug treatment (three measurements were performed every 20 min). The concentrations are not corrected for recovery from microdialysis probe. Amino acid area under the curve (AUC) control values indicate the average overall measures for each amino acid in the dialysates from the medial prefrontal cortex of the Ringer solution-infused rats at 40–180 min of the infusion in each experimental group. The results represent the means with S.E.M. of the data obtained from 4–7 rats.

The number of animals is shown in parentheses.

As for the effects of extracellular d-serine on neuronal circuits, an increase in extracellular d-serine levels is likely to be mediated by a very low frequency burst of calcium-permeable AMPA receptor activation because NASPM, a selective antagonist for AMPA receptors with calcium-permeable AMPA receptor subunits, selectively increases extracellular d-serine contents [6]. As for the effects of extracellular d-serine on neuronal circuits, we have performed in vivo dialysis experiments in anesthetized animals and started to collect the brain perfusates in anesthetized animals at a selected stage of extracellular d-serine contents. As for the effects of extracellular d-serine on neuronal circuits, we have performed in vivo dialysis experiments in anesthetized animals and started to collect the brain perfusates in anesthetized animals at a selected stage of extracellular d-serine contents. As for the effects of extracellular d-serine on neuronal circuits, we have performed in vivo dialysis experiments in anesthetized animals and started to collect the brain perfusates in anesthetized animals at a selected stage of extracellular d-serine contents. As for the effects of extracellular d-serine on neuronal circuits, we have performed in vivo dialysis experiments in anesthetized animals and started to collect the brain perfusates in anesthetized animals at a selected stage of extracellular d-serine contents.
Fig. 4. Effects of local perfusion of (S)-AMPA [an active enantiomer at the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor] alone or in combination with 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]-quinoxaline-7-sulfonamide disodium salt (NBQX) into the medial prefrontal cortex on the extracellular contents of various amino acids in the cortical region of the rat. The solid black symbols indicate the continuous local application of Ringer solution containing NBQX (100 µM; a, c) and open symbols display the period of (S)-AMPA (100 µM) infusion (a, c, e, g) through the dialysis probe into the medial prefrontal cortex of the freely moving rats. The concentrations of L-serine, glycine, L-glutamate and L-glutamine in the cortical dialysates collected every 20 min were quantitatively determined using high-performance liquid chromatography with fluorometric detection. Each point represents the mean with S.E. mean of data obtained from 4–8 animals and expressed as a percentage of the basal L-serine, glycine, L-glutamate and L-glutamine contents (Table 1). * Indicates statistically significant differences in the time-point data at $p<0.05$, 0.01 or 0.001 as compared to the Ringer solution alone-infused controls: open symbols, n.s. # $p<0.05$, ## $p<0.01$, ### $p<0.001$ compared to the animals treated with the intra-cortical infusion of (S)-AMPA in the presence of NBQX. The area under the curve (AUC) is also calculated by adding the areas under the graph of the concentrations of L-serine and glycine between each pair of every 20-min consecutive observation from 40 to 180 min of treatment (b, d, f, h) and expressed as a percentage of the respective control L-serine, glycine, L-glutamate and L-glutamine contents (Table 1). ** $p<0.01$ as compared to the Ringer solution-infused controls; # $p<0.05$, ### $p<0.001$ between the two groups linked by the solid line; n.s. The statistical significance of the data was evaluated by two-tailed Student’s t test (homogeneous variance) or Aspin–Welch’s t test (heterogeneous variance) for comparison between the two groups and by that for comparison among more than three groups after adjustment of the $p$ values for multiple tests using Bonferroni’s method.
Alternatively, because interactions of the AMPA receptor with a D-serine synthesizing enzyme, serine racemase, via GRIP (Kim et al., 2005) and PICK1 (Fujii et al., 2006) have been reported, the reduced dialysate D-serine contents might be the result of a decrease in D-serine synthesis. From the fact that an inhibitory neurotransmitter taurine attenuates the augmented noradrenaline overflow by electrical stimulation of isolated perfused mesenteric arteries (Hano et al., 2009), we can also extrapolate that the elevated extracellular taurine levels could lead to a decrease in D-serine liberation in the cortical portion. However, the calcium-permeable AMPA receptor antagonist-resistant nature of the increase in this study opposes this assumption. The absence of significant influences of calcium-permeable or -impermeable AMPA receptor antagonist alone on the prefrontal extracellular D-serine contents indicates that the inhibitory control by the AMPA receptor is phasic in nature.

The (S)-AMPA-induced NASPM-sensitive drop in the D-serine contents was not observed immediately after the (S)-AMPA application while the taurine contents rapidly rose following the (S)-AMPA infusion in a NASPM-insensitive fashion. The delayed decline was preceded by a small and transient, but significant increase in extracellular D-serine when (S)-AMPA and CTZ were co-infused (Fig. 2). This biphasic phenomenon has also been observed following veratridine application in in vivo dialysis experiments by Rosenberg et al. (2010). These D-serine-selective complex changes could be associated with the plausible interactions between calcium-permeable and -impermeable AMPA receptors and/or complicated alterations in the D-serine metabolism in response to AMPA receptor activation or depolarization stimuli.

The exact physiological roles of the AMPA agonist-induced inhibition of D-serine release are still unclear. Because D-serine is required for the activation of the NMDA receptor by glutamate in the central nervous system (for a review, see Nishikawa, 2011) and because excessive D-serine signals have been presumed to enhance the neurotoxicity induced by the NMDA receptor stimulation (Inoue et al., 2008; Mustafa et al., 2010), the AMPA receptor-D-serine interaction could function as a feedback system in the glutamate synapse in that the surplus presynaptic glutamate release could impinge on the calcium-permeable AMPA receptor, which exerts an inhibitory control over the extracellular D-serine release, and thereby excitotoxic overactivation of the NMDA receptor might be avoided by the subsequent reduction in its co-agonist levels. While this series of in vivo studies definitely provide an improvement over limitations of in vitro studies, the question should be raised as to the above functional consequences of potentiating AMPA receptors in the mPFC when stimulating glutamatergic afferents with more discrete techniques, such as optogenetic recordings, etc.

In conclusion, the present findings indicate that prefrontal extracellular D-serine release may be under a phasic inhibitory control by the calcium-permeable AMPA receptor in mammalian brains in vivo. In terms of the fundamental role of D-serine in the NMDA receptor activation as its co-agonist, calcium-permeable AMPA receptor-mediated control could play an important role in the fine-tuning by D-serine of the glutamate transmission via the NMDA receptor. Therefore, further investigations on the types of cells that possess the D-serine-regulating calcium-permeable AMPA receptor and its downstream molecular cascades and the release machinery for D-serine should contribute to further understanding the biological mechanisms underlying neurological and mental functions and dysfunctions and to development of strategies for their novel treatment.

Acknowledgements
This work was supported by the CREST (Core Research for Evolutional Science & Technology) program funded by the Ministry of Education, Culture, Sports, Science and Technology of Japan. We thank the International Technology Exchange Society (an English editing agency) for their assistance in English editing for preparing our present manuscript. The authors are entirely responsible for the scientific content of this paper.

Statement of Interest
None.

References
Hano T, Kasano M, Tomari H, Iwane N (2009) Taurine suppresses pressor response through the inhibition of...


