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Short communication

Effect of aminooxyacetic acid on extracellular level of D-serine in rat striatum: An in vivo microdialysis study

Atsushi Hashimoto *, Masanobu Yoshikawa

Department of Pharmacology, School of Medicine, Tokai University, Isehara, Kanagawa, 259-1143, Japan

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Abstract

To elucidate the effect of an inhibitor of pyridoxal phosphate-dependent enzymes, aminooxyacetic acid, on the activity of serine racemase in vivo, we have investigated the effect of aminooxyacetic acid on the extracellular concentration of D-serine in the rat striatum using an in vivo microdialysis technique. The intrastriatal perfusion of aminooxyacetic acid caused a significant decline in the extracellular concentration of D-serine. These data, together with the fact that serine racemase is a pyridoxal phosphate-dependent enzyme, suggest that the aminooxyacetic acid-induced reduction of the extracellular D-serine may be at least in part due to the drug's ability to inhibit serine racemase.

Keywords: Aminooxyacetic acid; *N*-Methyl-D-aspartate receptor; Microdialysis; D-Serine; Serine racemase

1. Introduction

High concentrations of D-serine, previously assumed to be unnatural in mammals, have recently been observed in the mammalian brain (Hashimoto and Oka, 1997; Hashimoto et al., 1993, 1995, 2000; Schell et al., 1995). Accumulating evidence indicates that D-serine plays a regulatory role as an indispensable co-agonist for the glutamate activation of *N*-methyl-D-aspartate (NMDA) receptors (Hashimoto and Oka, 1997; Hashimoto et al., 1993; Kleckner and Dingledine, 1988; Matsui et al., 1995). D-Serine potentiates the NMDA receptor-mediated transmission by selective stimulation of the glycine site of the NMDA receptors (Kleckner and Dingledine, 1988; Matsui et al., 1995). In vivo microdialysis studies have also indicated that the extracellular concentration of D-serine is comparable to that of glycine in the prefrontal cortex and striatum, and that depolarizing stimuli such as veratrine, kainate or NMDA produce a reduction in the extracellular level of D-serine in a tetrodotoxin-, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione)- or MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine)-reversible manner, respectively (Hashimoto et al., 1995, 2000).

Concerning the de novo synthesis of D-serine, an intraperitoneal administration of L-serine has been shown to cause a significant enhancement in the D-serine concentrations in several brain areas and periphery (Hashimoto, 2002; Takahashi et al., 1997). Furthermore, serine racemase, which directly converts D-serine to L-serine, has been purified and cloned from the mammalian brain (Konno, 2003; Wolosker et al., 1999a,b). Several lines of evidence have revealed that the regional distribution of serine racemase corresponds well with those of the endogenous D-serine and NMDA receptors with the higher levels in the forebrain and the lower levels in the hindbrain (Hashimoto et al., 1993; Wolosker et al., 1999b; Yoshikawa et al., 2004). Interestingly, we have recently shown that MK-801 upregulates the expression of serine racemase mRNA in the rat brain (Yoshikawa et al., 2004).

The serine racemase has a pyridoxal phosphate binding consensus region, and mutations of the lysine 56, which is predicted to bind pyridoxal phosphate, diminish the activity of serine racemase (Wolosker et al., 1999a,b). Aminooxyacetic acid (AOAA) is a potent inhibitor of pyridoxal phosphate-dependent enzymes. In vitro studies using purified and recombinant serine racemase have recently demonstrated that AOAA potently reduced the enzyme activity (Wolosker et al., 1999a,b). Microdialysis studies have also shown that the local application of AOAA (0.3–2.0 mM) inhibits pyridoxal

* Corresponding author. Tel.: +81 463 93 1121; fax: +81 463 93 2896.
E-mail address: hashimot@is.icc.u-tokai.ac.jp (A. Hashimoto).

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phosphate-dependent enzymes in the brain (Speciale et al., 1990; Swartz et al., 1990; Wu et al., 1992). To ascertain the inhibitory effect of AOAA on the activity of serine racemase in vivo, we have attempted to quantify the extracellular levels of D-serine and L-serine when perfused with AOAA using a microdialysis technique. Because depolarizing stimuli cause a decline in the extracellular D-serine in a tetrodotoxin-reversible manner (Hashimoto et al., 1995, 2000), we have also investigated the effect of the co-application of tetrodotoxin with AOAA.

2. Materials and methods

The present animal experiments were performed in strict accordance with the guidelines of Tokai University, and were approved by the Animal Investigation Committee of the university. The in vivo microdialysis was performed as previously reported (Hashimoto et al., 1995, 2000). Briefly, male Wistar rats weighing 220–270 g were anesthetized with pentobarbital (40 mg/kg, intraperitoneally) and mounted on a stereotaxic frame. The dialysis probe was then implanted into the striatum (A +0.5 mm, V +7.0 mm, L −3.0 mm). Two days after surgery, the dialysis probe was perfused with Ringer solution (NaCl, 147 mM; KCl, 4 mM; $CaCl_2$, 1.3 mM; pH 7.3) at a flow rate of 2 μl/min. Dialysate samples were collected every 20 min. After collection of three basal fractions, 0.5 mM AOAA (WAKO, Japan) was added to the perfusion solution throughout the experiment, either in the presence or absence of 2 μM tetrodotoxin (Sigma, USA). The simultaneous determination of the free amino acid enantiomers and non-chiral amino acids in the dialysate was accomplished using high-performance liquid chromatography (HPLC) and fluorometric detection as previously described (Hashimoto et al., 1993). Briefly, the dialysate sample was derivatized with *N*-tert-butyloxycarbonyl-L-cysteine and *o*-phthaldialdehyde for 2 min at room temperature. The amino acid derivative was then immediately applied to the HPLC system.

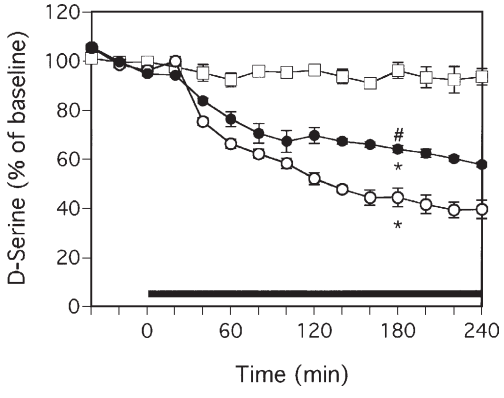

Fig. 1. Effect of AOAA perfusion on the extracellular concentration of D-serine in the striatum. The solid black bar indicates the length of application of Ringer solution containing AOAA (0.5 mM) in the presence (●) or absence (○) of tetrodotoxin (2 μM) through the dialysis probe. Results are means with S.E.M. of data obtained from four to five rats and expressed as percentages of the basal levels. Controls (□); AOAA-treated group (○); AOAA plus tetrodotoxin-treated group (●). $^{*}P<0.01$ as compared with controls, $^{\#}P<0.01$ as compared with the AOAA-treated group.

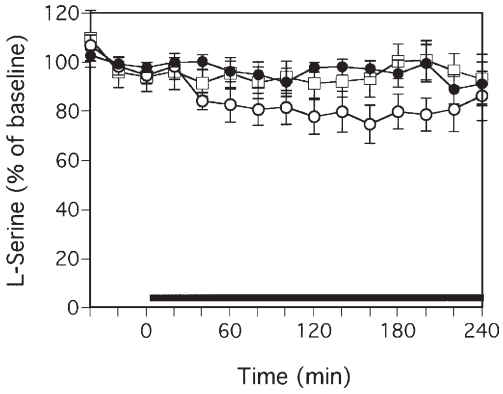

Fig. 2. Effect of AOAA perfusion on the extracellular concentration of L-serine in the striatum. The solid black bar indicates the length of application of Ringer solution containing AOAA (0.5 mM) in the presence (●) or absence (○) of tetrodotoxin (2 μM) through the dialysis probe. Results are means with S.E.M. of data obtained from four to five rats and expressed as percentages of the basal levels. Controls (□); AOAA-treated group (○); AOAA plus tetrodotoxin-treated group (●).

The average of the concentration of each amino acid during the period preceding the various treatments (three measurements performed every 20 min) was used as the control value (=100). Individual data are expressed as percentages of this baseline period. The means with S.E.M. of the results obtained from four to five animals were calculated using the corresponding periods. The areas under the curve (AUC) of the concentration vs. time plots for a dialysate at 0–240 min post-administration were calculated and used as overall measures of the treatment effects. Statistical comparisons were carried out between the groups on the AUC data using the student's or Cochran–Cox *t*-test.

3. Results

Fig. 1 shows the time course of changes in the extracellular concentration of D-serine in the striatum of rats treated with AOAA (0.5 mM), an inhibitor of serine racemase. The intrastriatal perfusion of AOAA caused a 60% decrease in the extracellular concentration of D-serine 200 min after the drug perfusion. Although co-perfusion of a sodium channel blocker, tetrodotoxin, with AOAA partially reversed the AOAA-induced reduction of the extracellular level of D-serine, there is a significant difference between the controls and AOAA plus tetrodotoxin-treated group. The L-serine concentration in the dialysate tended to decrease after the AOAA administration, but there is no significant difference between the controls and AOAA-treated group (Fig. 2). In addition, the perfusion of AOAA failed to enhance the extracellular concentration of L-glutamate compared with the controls (data not shown).

4. Discussion

In the present study, we demonstrated, using an in vivo microdialysis technique, that the intrastriatal perfusion of

AOAA produced a significant decline in the extracellular concentration of D-serine, but not L-serine. Because AOAA is an inhibitor of pyridoxal phosphate-dependent enzymes (Beal et al., 1991; Speciale et al., 1990; Wolosker et al., 1999a,b), the AOAA-evoked reduction in the extracellular D-serine may be at least in part due to the drug's ability to inhibit serine racemase activity. Several lines of evidence support this possibility: (a) in vitro studies using purified and recombinant serine racemase have indicated that AOAA potently inhibits the enzyme activity (Wolosker et al., 1999a,b); (b) although the striatal application of depolarizing agents, such as veratrine, kainate or NMDA, has been shown to cause a significant reduction in the extracellular level of D-serine in a tetrodotoxin-, CNQX- or MK-801-reversible manner, respectively (Hashimoto et al., 1995, 2000), the simultaneous perfusion of AOAA with tetrodotoxin also caused a significant decrease in the extracellular concentration of D-serine in the present study; and (c) the AOAA administration failed to reduce the extracellular level of L-serine, suggesting that the effect of AOAA is specific for D-serine. Further studies such as dose-dependency and measurement of D-serine content in tissue are needed to clarify the drug's ability to inhibit serine racemase.

The co-application of AOAA with tetrodotoxin partially reversed the AOAA-induced decline of the extracellular D-serine. Although the exact mechanism underlying the partial reversal is still unclear, this could be attributed to the indirect activation of the NMDA receptors by AOAA (Beal et al., 1991). The AOAA administration has been shown to produce excitotoxic lesions in a MK-801-reversible manner, although AOAA does not directly open the ion channels of the NMDA receptors (Beal et al., 1991). This possibility was further supported by the findings that the AOAA application leads to an NMDA-receptor dependent enhancement of the evoked potentials in cortical neurons (Scharfman, 1996). Alternatively, because the local perfusion of tetrodotoxin alone produced a slight but significant augmentation of the extracellular level of D-serine (Hashimoto et al., 1995), the tetrodotoxin administration could result in an elevation of the carrier-mediated release of D-serine due to the possible alterations in the equilibrium of the concentration gradient for sodium ions (Bernath and Zigmond, 1990; Hashimoto et al., 1995).

Because D-serine could play an important role in a variety of pathophysiological conditions for the hyper- and hypofunctions of the NMDA receptors as an endogenous indispensable co-agonist of NMDA receptors (Hashimoto and Oka, 1997), selective inhibitors or activators of serine racemase may be therapeutically beneficial for the treatment of diseases related to the hyper- and hypofunctions of the NMDA receptor-mediated neurotransmission.

Acknowledgments

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References

Beal, M.F., Swartz, K.J., Hyman, B.T., Storey, E., Finn, S.F., Koroshetz, W., 1991. Aminooxyacetic acid results in excitotoxin lesions by a novel indirect mechanism. J. Neurochem. 57, 1068–1073.

Bernath, S., Zigmond, M.J., 1990. Calcium-independent GABA release from striatal slices: the role of calcium channels. Neuroscience 36, 461–464.

Hashimoto, A., 2002. Effect of the intracerebroventricular and systemic administration of L-serine on the concentrations of D- and L-serine in several brain areas and periphery of rat. Brain Res. 955, 214–220.

Hashimoto, A., Oka, T., 1997. Free D-aspartate and D-serine in the mammalian brain and periphery. Prog. Neurobiol. 52, 325–353.

Hashimoto, A., Nishikawa, T., Oka, T., Takahashi, K., 1993. Endogenous D-serine in rat brain: *N*-methyl-D-aspartate receptor-related distribution and aging. J. Neurochem. 60, 783–786.

Hashimoto, A., Oka, T., Nishikawa, T., 1995. Extracellular concentration of endogenous free D-serine in the rat brain as revealed by in vivo microdialysis. Neuroscience 66, 635–643.

Hashimoto, A., Kanda, J., Oka, T., 2000. Effects of *N*-methyl-D-aspartate, kainate or veratridine on extracellular concentrations of free D-serine and L-glutamate in rat striatum: an in vivo microdialysis study. Brain Res. Bull. 53, 347–351.

Kleckner, N.W., Dingledine, R., 1988. Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. Science 241, 835–837.

Konno, R., 2003. Rat cerebral serine racemase: amino acid deletion and truncation at carboxy terminus. Neurosci. Lett. 349, 111–114.

Matsui, T., Sekiguchi, M., Hashimoto, A., Tomita, U., Nishikawa, T., Wada, K., 1995. Functional comparison of D-serine and glycine in rodents: the effect on cloned NMDA receptors and the extracellular concentration. J. Neurochem. 65, 454–458.

Scharfman, H.E., 1996. Hyperexcitability of entorhinal cortex and hippocampus after application of aminooxyacetic acid (AOAA) to layer III of the rat medial entorhinal cortex in vitro. J. Neurophysiol. 76, 2986–3001.

Schell, M.J., Molliver, M.E., Snyder, S.H., 1995. D-Serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc. Natl. Acad. Sci. U. S. A. 92, 3948–3952.

Speciale, C., Wu, H.Q., Gramsbergen, J.B.P., Turski, W.A., Ungerstedt, U., Schwarcz, R., 1990. Determination of extracellular kynurenic acid in the striatum of unanesthetized rats: effect of aminooxyacetic acid. Neurosci. Lett. 116, 198–203.

Swartz, K.J., During, M.J., Freese, A., Beal, M.F., 1990. Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. J. Neurosci. 10, 2965–2973.

Takahashi, K., Hayashi, F., Nishikawa, T., 1997. In vivo evidence for the link between L- and D-serine metabolism in rat cerebral cortex. J. Neurochem. 69, 1286–1290.

Wolosker, H., Sheth, K.N., Takahashi, M., Mothet, J.P., Brady, R.O., Ferris, C. D., Snyder, S.H., 1999a. Purification of serine racemase: biosynthesis of the neuromodulator D-serine. Proc. Natl. Acad. Sci. U. S. A. 96, 721–725.

Wolosker, H., Blackshaw, S., Snyder, S.H., 1999b. Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-*N*-methyl-D-aspartate neurotransmission. Proc. Natl. Acad. Sci. U. S. A. 96, 13409–13414.

Wu, H.Q., Ungerstedt, U., Schwarcz, R., 1992. Regulation of kynurenic acid synthesis studied by microdialysis in the dorsal hippocampus of unanesthetized rats. Eur. J. Pharmacol. 213, 375–380.

Yoshikawa, M., Kobayashi, T., Oka, T., Kawaguchi, M., Hashimoto, A., 2004. Distribution and MK-801-induced expression of serine racemase mRNA in rat brain by real-time quantitative PCR. Mol. Brain Res. 128, 90–94.