

Effects of MK-801 on the expression of serine racemase and D-amino acid oxidase mRNAs and on the D-serine levels in rat brain

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Abstract

We have investigated the acute effects of the increasing doses of non-competitive *N*-methyl-D-aspartate receptor antagonist MK-801 (0.2–1.6 mg/kg) on the expression of serine racemase and D-amino acid oxidase (DAO) mRNAs in several brain areas of rats. We have also evaluated the effects of the chronic administration of MK-801 (0.4 mg/kg) on the gene expression of serine racemase and DAO and on the D-serine concentrations. A dose-dependent augmentation of the expression of serine racemase mRNA was seen in most brain areas at both 1 and 4 h after the administration. In contrast, a drastic decline in the expression of DAO mRNA was observed in most brain areas 1 h after the MK-801 administration, whereas a dose-dependent elevation in the expression of DAO mRNA was observed in most brain areas 4 h after the administration. The chronic MK-801 administration produced a significant increase in the expression of serine racemase mRNA in almost all brain areas, whereas no significant changes were found in the level of DAO mRNA in most brain areas. In addition, the chronic administration caused a slight but significant elevation in the concentrations of D-serine in the cortex and striatum. These present findings indicate that increasing the serine racemase mRNA and no changes in the DAO mRNA after the chronic administration could contribute to the elevation of the D-serine level in the forebrain, and that serine racemase and DAO could play an important role in the regulation of *N*-methyl-D-aspartate receptors via the D-serine metabolism.

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1. Introduction

The presence of high levels of D-serine, a D-amino acid thought to be unnatural in mammals, has been shown in the mammalian brain (Chouinard et al., 1993; Hashimoto and Oka, 1997; Hashimoto et al., 1993a,b; Nagata et al., 1994; Schell et al., 1995, 1997). D-Serine predominantly occurs in the forebrain where the *N*-methyl-D-aspartate (NMDA)-type glutamate receptors exist (Hashimoto and Oka, 1997; Hashimoto et al., 1993b; Schell et al., 1997). Because D-serine is an obligatory coagonist at the strychnine-insensitive glycine site of the NMDA receptor (Kleckner and Dingledine, 1988; Matsui et al., 1995), D-serine has been proposed as an endogenous

agonist for the NMDA-glycine site in the mammalian brain (Hashimoto et al., 1993b; Hashimoto and Oka, 1997; Schell et al., 1995).

D-Serine is synthesized by serine racemase, a pyridoxal phosphate-dependent enzyme enriched in the mammalian brain (Wolosker et al., 1999). Recently, serine racemase has been cloned from the mammalian brain (Konno, 2003; Wolosker et al., 1999). Several lines of studies have demonstrated that the distribution of serine racemase closely resembles those of the endogenous D-serine and NMDA receptors with the highest level in the forebrain and the lowest level in the hindbrain (Hashimoto et al., 1993b; Schell et al., 1997; Yoshikawa et al., 2004a). Interestingly, although it has long been assumed that D-serine and serine racemase are enriched exclusively in astrocytes (Schell et al., 1995, 1997; Wolosker et al., 1999), recent studies have revealed that significant amounts of D-serine and

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serine racemase are present in neurons (Kartvelishvili et al., 2006; Yasuda et al., 2001; Yoshikawa et al., 2006). In addition, D-amino acid oxidase (DAO), which catalyzes the oxidative deamination of neutral D-amino acids, is phylogenetically conserved in the mammalian kidneys and brain (D'Aniello et al., 1993; Hashimoto et al., 1993a; Horiike et al., 1994) and has been cloned from several mammalian species (Fukui and Miyake, 1992). DAO is confined to the hindbrain with the higher levels in the cerebellum and pons-medulla, decreasing levels in the midbrain and with low levels in the cortex and hippocampus (Horiike et al., 1994; Schell et al., 1995; Yoshikawa et al., 2004b). Recently, a new human gene, G72, on 13q34 that interacts with the gene for DAO on 12q24 was identified (Chumakov et al., 2002). Although both of these genes have been implicated with schizophrenia (Chumakov et al., 2002; Harrison and Weinberger, 2005), little information is available concerning the relationship between DAO and schizophrenia except for the genetic data. We have recently shown that mice lacking DAO activity exhibit a marked reduction in stereotypy and ataxia induced by the NMDA receptor antagonist, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a, d]cyclohepten-5,10-imine (MK-801), suggesting that the elevated D-serine in the brain of DAO-deficient mice could antagonize the MK-801-induced stereotypy and ataxia (Hashimoto et al., 2005).

The NMDA receptor hypofunction has been associated with the pathophysiology of schizophrenia (Coyle and Tsai, 2004; Deutsch et al., 1989; Javitt and Zukin, 1991). The NMDA receptor antagonists such as phencyclidine (PCP) and ketamine can induce positive, negative and cognitive schizophrenic-like symptoms in normal subjects. PCP and MK-801 also produces behaviors in rodents that include hyperlocomotion, stereotypy, ataxia and impaired social interactions (Coyle and Tsai, 2004; Deutsch et al., 1989; Javitt and Zukin, 1991). We have recently demonstrated that a transient augmentation in the expression of serine racemase and DAO mRNAs is observed in the brain after the acute administration of MK-801 and ketamine, respectively (Yoshikawa et al., 2004a,b; Takeyama et al., 2006). To obtain further insight into the relationship between the gene expression of the two enzymes and the blockade of the NMDA receptor, we have investigated the acute effects of increasing doses of MK-801 (0.2–1.6 mg/kg) on the expression of serine racemase and DAO mRNAs in several brain areas of rats. We have also evaluated the effects of the chronic administration of MK-801 on the expression of serine racemase and DAO mRNAs and on the D-serine concentrations.

2. Materials and methods

2.1. Animals and drugs

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. Male Wistar rats at postnatal week 7 were used in this study. In the experiment of the acute effects of MK-801, 0.2, 0.4, 0.8 or 1.6 mg/kg of MK-801 was dissolved in physiological saline and then intraperitoneally injected, whereas the control animals received only saline. One or 4 h after the administration, the rats were stunned and decapitated. In the experiment of the chronic effects of MK-

801, 0.4 mg/kg of MK-801 was dissolved in physiological saline and then intraperitoneally injected daily for 14 days. The control animals received only saline under the same conditions. The rats were stunned and decapitated 18 h after the final administration.

2.2. Real-time quantitative PCR analysis

The gene expression of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH; accession number NM017008), serine racemase (accession number NM0198757) and DAO (accession number NM053626) was determined by real-time quantitative polymerase chain reaction (PCR) using a method similar to the one described previously (Yoshikawa et al., 2004a,b). Briefly, the cDNA was amplified by real-time PCR using the DyNAmo SYBR green qPCR Kit (Finnzymes, Espoo, Finland) on the DNA Engine Opticon 2 System (Bio-Rad Laboratories; Hercules, CA, USA). The PCR products were separated by an Agilent 2100 Bioanalyzer (Agilent Technologies; Palo Alto, CA, USA), which utilizes chip-based nucleic acid separation technology. Furthermore, the identification of the amplified PCR products of the serine racemase, DAO and GAPDH cDNAs were determined by the dye terminator cycle sequencing.

2.3. High performance liquid chromatography (HPLC) analysis

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., 1993b). 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 $16,000 \times g$ 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*-phthalaldehyde for 2 min at room temperature. The amino acid derivative was immediately applied to the HPLC system.

2.4. Statistics

These results are given as means with S.E.M. of the data. For comparison between the two groups, statistical evaluations were carried out using the unpaired two-tailed Student's *t*-test or Mann–Whitney *U*-test. Statistical differences among more than three groups were estimated by one-way analysis of variance followed by a multiple comparison test. A *P*-value < 0.05 was considered as reaching statistical significance.

3. Results

3.1. Levels of serine racemase mRNA in several brain areas 1 h after the acute administration of MK-801 at various doses (0.2, 0.4, 0.8 and 1.6 mg/kg)

Because we have recently demonstrated that the levels of serine racemase mRNA in all brain areas significantly increased

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