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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.

Keywords: D-Amino acid oxidase; MK-801; NMDA receptor; Real-time quantitative PCR; Schizophrenia; Serine racemase

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 (Kartvelishvily 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 ×*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.

2.4. 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

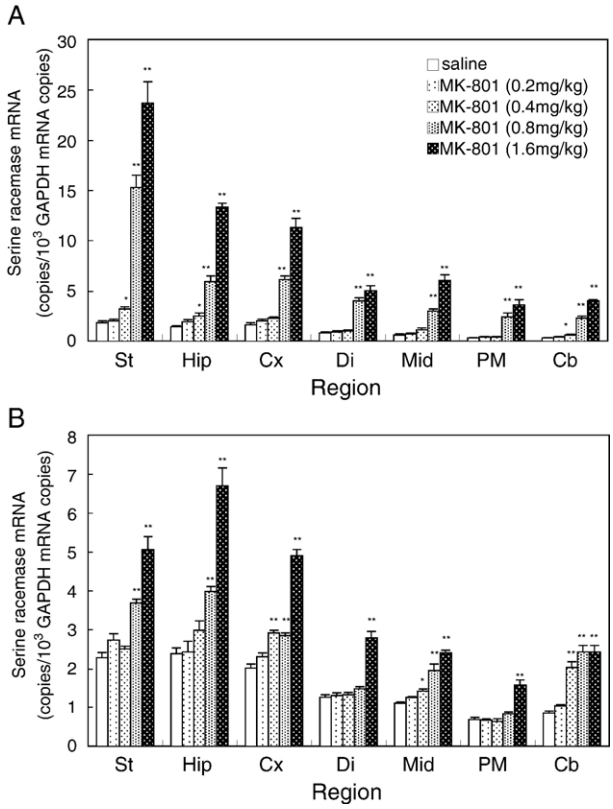

Fig. 1. Levels of serine racemase mRNA in several brain areas 1 h (A) and 4 h (B) after the acute administration of MK-801 at various doses (0.2, 0.4, 0.8 and 1.6 mg/kg). Rats received an intraperitoneal injection of MK-801 and then killed 1 h or 4 h thereafter. The results are means with S.E.M. of data obtained from five rats. ★$P<0.05$; ★★$P<0.01$ as compared with saline-treated group. St, striatum; Hip, hippocampus; Cx, cortex; Di, diencephalon; Mid, midbrain; PM, pons-medulla; Cb, cerebellum.

and peaked at 1 h after the acute administration of MK-801 (0.4 mg/kg) (Yoshikawa et al., 2004a), the rats were decapitated 1 h after the administration. As shown in Fig. 1A, the MK-801 (0.2–1.6 mg/kg) administration produced a drastic elevation in the serine racemase mRNA in all the brain areas in a concentration-dependent manner. Following the administration of MK-801 (1.6 mg/kg), the levels significantly increased by 525–1233% in the seven brain areas examined: striatum (1216% increase), hippocampus (850%), cortex (606%), diencephalon (525%), midbrain (900%), pons-medulla (1100%), and cerebellum (1233%).

3.2. Levels of serine racemase mRNA in several brain areas 4 h after the acute administration of MK-801 at various doses (0.2, 0.4, 0.8 and 1.6 mg/kg)

Fig. 1B shows data for the levels of serine racemase mRNA 4 h after the acute administration of MK-801 (0.2–1.6 mg/kg). The MK-801 administration caused a drastic augmentation of the serine racemase mRNA in all the brain regions in a concentration-dependent manner. Following the administration of MK-801 (1.6 mg/kg), the levels significantly increased by 218–284% in the seven brain areas examined: striatum (221% increase), hippocampus (281%), cortex (244%), diencephalon (221%), midbrain (218%), pons-medulla (227%), and cerebellum (284%). The magnitude of the elevated levels of serine racemase mRNA was much higher at 1 h than at 4 h after the administration in all the brain regions (Fig. 1A and B).

3.3. Levels of DAO 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)

As shown in Fig. 2A, the acute administration of MK-801 (0.8 and 1.6 mg/kg, 1 h) produced a drastic decline in the expression of the DAO mRNA in all the brain areas, whereas a slight but significant increase in the expression of DAO mRNA was observed in the pons-medulla 1 h after the administration of MK-801 (0.4 mg/kg). Following the administration of MK-801 (1.6 mg/kg), the levels were significantly reduced by 53–93% in the seven brain areas examined: striatum (88% decrease), hippocampus (53%), cortex (95%), diencephalon (97%), midbrain (82%), pons-medulla (60%), and cerebellum (76%).

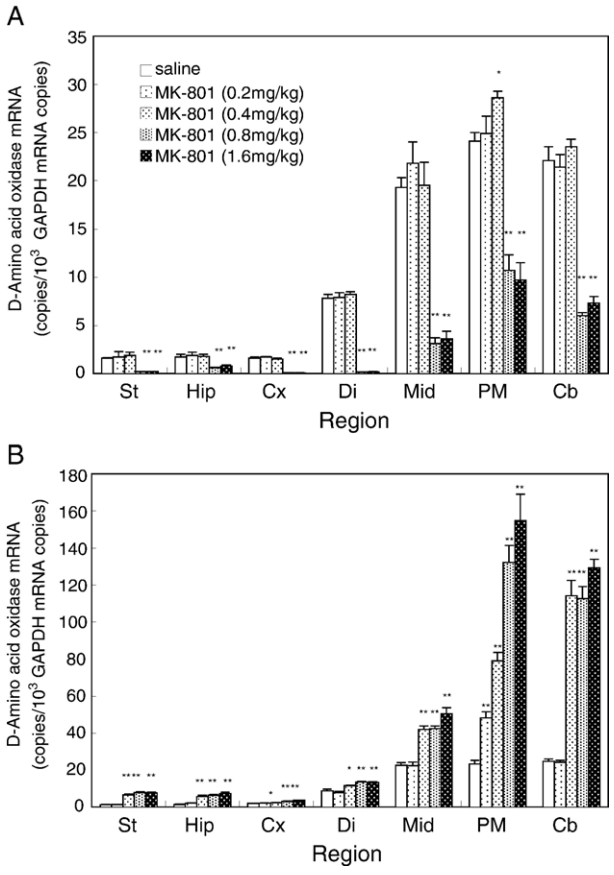

Fig. 2. Levels of D-amino acid oxidase mRNA in several brain areas 1 h (A) and 4 h (B) after the acute administration of MK-801 at various doses (0.2, 0.4, 0.8 and 1.6 mg/kg). Rats received an intraperitoneal injection of MK-801 and then killed 1 h or 4 h thereafter. The results are means with S.E.M. of data obtained from five rats. ★$P<0.05$; ★★$P<0.01$ as compared with saline-treated group. St, striatum; Hip, hippocampus; Cx, cortex; Di, diencephalon; Mid, midbrain; PM, pons-medulla; Cb, cerebellum.

3.4. Levels of DAO mRNA in several brain areas 4 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 shown that the levels of DAO mRNA in all brain areas significantly increased and peaked at 4 h after the acute administration of MK-801 (0.4 mg/kg) (Yoshikawa et al., 2004b), the rats were decapitated 4 h after the administration. As shown in Fig. 2B, the MK-801 administration produced a dramatic enhancement of the DAO mRNA in all the brain areas in a concentration-dependent manner. Following the administration of MK-801 (1.6 mg/kg), the levels significantly increased by 149–661% in the seven brain areas examined: striatum (589% increase), hippocampus (512%), cortex (174%), diencephalon (149%), midbrain (223%), pons-medulla (661%), and cerebellum (524%).

3.5. Effect of chronic administration of MK-801 (0.4 mg/kg) on the expression of serine racemase mRNA in several brain areas

The spontaneous behavior of the rats at 18 h after the final administration of MK-801 seemed to be normal. After the chronic MK-801 administration, the expression of the serine racemase mRNA was significantly increased in the striatum, hippocampus, cortex, diencephalon and cerebellum (Fig. 3A). The levels increased by 25–78% in several brain areas after the chronic administration of MK-801: striatum (78% increase), hippocampus (51%), cortex (25%), diencephalon (42%), and cerebellum (40%).

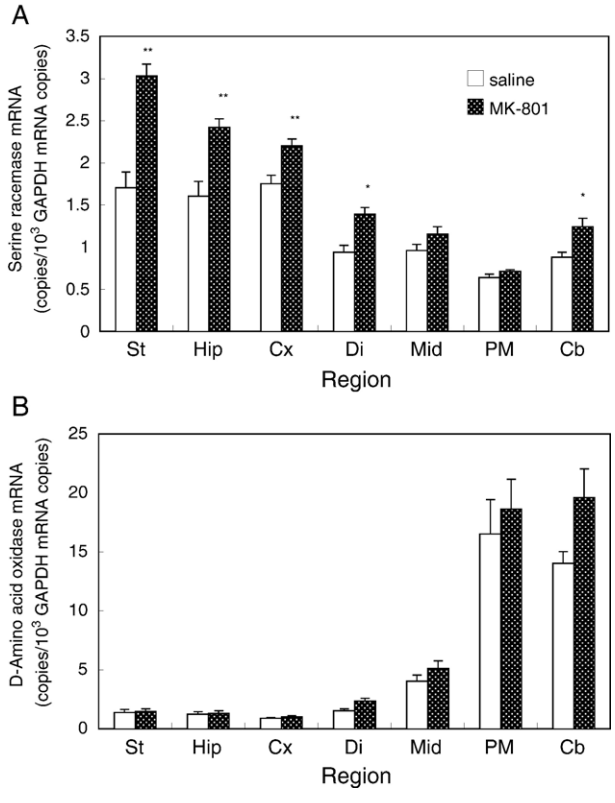

Fig. 3. Effect of chronic administration of MK-801 (0.4 mg/kg) on the mRNA expressions of serine racemase (A) and D-amino acid oxidase (B) in several brain areas. Rats received daily injections of either saline or MK-801 (0.4 mg/kg) for 14 days and then killed 18 h after the final administration. The results are means with S.E.M. of data obtained from five rats. ★$P<0.05$; ★★$P<0.01$ as compared with saline-treated group. St, striatum; Hip, hippocampus; Cx, cortex; Di, diencephalon; Mid, midbrain; PM, pons-medulla; Cb, cerebellum.

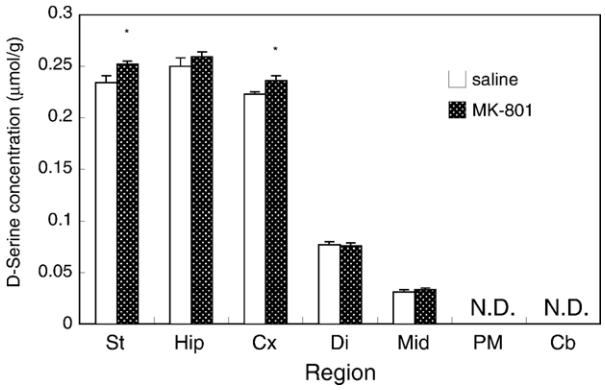

Fig. 4. Effect of chronic administration of MK-801 (0.4 mg/kg) on the levels of D-serine in several brain areas. Rats received daily injections of either saline or MK-801 (0.4 mg/kg) for 14 days and then killed 18 h after the final administration. The results are means with S.E.M. of data obtained from five rats. ★$P<0.05$ as compared with saline-treated group. St, striatum; Hip, hippocampus; Cx, cortex; Di, diencephalon; Mid, midbrain; PM, pons-medulla; Cb, cerebellum.

3.6. Effect of chronic administration of MK-801 (0.4 mg/kg) on the expression of DAO mRNA in several brain areas

As shown in Fig. 3B, no significant change was observed in the level of the DAO mRNA in all the brain areas after the chronic administration of MK-801 (0.4 mg/kg).

3.7. Effect of chronic administration of MK-801 (0.4 mg/kg) on the levels of D-serine in several brain areas

As shown in Fig. 4, a slight, but significant increase in the D-serine concentrations was observed in the striatum and cortex after the chronic administration of MK-801 (0.4 mg/kg).

4. Discussion

The present study demonstrated that increasing the serine racemase mRNA and no changes in the DAO mRNA after the chronic MK-801 administration could contribute to the elevation of the D-serine level in the forebrain. The distributional profile of both serine racemase and DAO mRNAs in the saline-treated rats coincides well with those of serine racemase immunoreactivity and DAO activity (Horiike et al., 1994; Schell et al., 1997; Wolosker et al., 1999). The regional distribution of D-serine levels in the saline-treated rats also corresponds well with those of D-serine and serine racemase immunoreactivity (Schell et al., 1997; Wolosker et al., 1999).

The augmentation in the levels of serine racemase mRNA was observed after the acute and chronic administration of MK-801. We have also demonstrated that the acute administration of

ketamine causes the transient elevation of serine racemase and DAO mRNAs (Takeyama et al., 2006). These findings, together with the fact that both MK-801 and ketamine are non-competitive NMDA receptor antagonists (Coyle and Tsai, 2004; Deutsch et al., 1989; Javitt and Zukin, 1991), provide further support for the view that there is a relationship between the gene expression of D-serine-related enzymes and the blockade of NMDA receptor function. In addition, because rat serine racemase has some activator protein-1 (AP-1) binding elements in the first intron of the gene (Wu and Barger, 2004) and because MK-801 has been shown to increase the DNA binding activity of the AP-1 and the expression of the Fos and Jun family members (Kontkanen et al., 2000), the AP-1 complex could play a regulatory role in the gene expression of serine racemase by MK-801. Further support for this possibility comes from the fact that the induction of serine racemase by inflammatory stimuli has recently been demonstrated to be dependent on AP-1 (Wu and Barger, 2004). Interestingly, Kartvelishvily et al. (2006) have revealed that significant amounts of serine racemase and D-serine occur in neurons. Because we have also shown that both serine racemase mRNA and protein are present in the cultured neurons and because both mRNA and protein levels in the neurons are higher than those in the astrocytes (Yoshikawa et al., 2006), the upregulation of serine racemase may occur in both neurons and astrocytes after the MK-801 administration.

A rapid and transient reduction in the expression of DAO mRNA 1 h after the MK-801 (0.8 and 1.6 mg/kg) administration and a dose-dependent elevation in the expression of DAO mRNA 4 h after the administration were seen in all the brain areas. The reason for the complicated changes in the levels of DAO mRNA after the acute administration remains to be determined. Because a sequence homologous to the cyclic AMP-responsive elements is found in the 5′-flanking region of the human DAO gene (Fukui and Miyake, 1992), and because a variety of early immediate genes and transcription factors is induced by MK-801 via the NMDA receptors (Castren et al., 1993; Hughes and Dragunow, 1995; Kontkanen et al., 2000; Storvik et al., 2000), the MK-801-induced transcription factors could play an important role in the regulation of the DAO mRNA. Interestingly, the expression of the cyclic AMP response element modulator and inducible cyclic AMP early repressor is increased in the rat brain after the MK-801 treatment (Storvik et al., 2000).

Both the moderate upregulation of serine racemase mRNA and no change in the expression of DAO mRNA after the chronic administration led to the slight but significant enhancement of the D-serine levels in the forebrain. These findings are the first to demonstrate that the drug enhancing gene expression of serine racemase could increase the D-serine concentrations in the forebrain in vivo. The slight augmentation of D-serine levels in the forebrain might be due to the α,β-elimination of endogenous D- and L-serine by elevated serine racemase (De Miranda et al., 2002; Foltyn et al., 2005; Strisovsky et al., 2003). In fact, serine racemase not only racemizes L-serine but it also converts D- and L-serine into pyruvate via its α,β-elimination activity (De Miranda et al., 2002; Foltyn et al., 2005; Strisovsky et al., 2003).

In contrast to serine racemase, no change was observed in the expression of the DAO mRNA after the chronic MK-801 administration, although the acute MK-801 treatment caused transient alterations in the level of DAO mRNA (Yoshikawa et al., 2004b; Fig. 2). Although the reason for no changes in the DAO mRNA after the chronic administration is unclear, it is likely that the MK-801-induced alterations in the levels of DAO mRNA could gradually be attenuated during the course of the repeated MK-801 administration. Alternatively, like the transient nature of the expression in the level of DAO mRNA after the acute MK-801 administration (Yoshikawa et al., 2004b), the elevated levels of DAO mRNA might immediately return to the control levels after the final MK-801 administration. Further studies are needed to clarify the signal transduction pathways and the regulatory mechanisms underlying the gene expression difference between the two enzymes after the acute and chronic MK-801 administration.

DAO, which metabolizes D-serine, has recently been shown to be associated with the susceptibility to schizophrenia (Chumakov et al., 2002; Harrison and Weinberger, 2005). We have also demonstrated that DAO-deficient mice, which have an enhanced level of D-serine in the brain, show diminution of stereotypy and ataxia induced by MK-801 (Hashimoto et al., 2005). Because the chronic MK-801 administration produces an upregulation of serine racemase mRNA and an increase in the D-serine levels in the present study, and because D-serine improves the negative, positive and cognitive symptoms of schizophrenic subjects treated with conventional neuroleptics (Tsai et al., 1998), drugs affecting serine racemase and DAO may offer a novel therapeutic target for the treatment of schizophrenia, especially for the negative symptoms and the cognitive impairments associated with schizophrenia.

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