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European Journal of Pharmacology 586 (2008) 221–225
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Short communication

Mice lacking D-amino acid oxidase activity exhibit marked reduction of methamphetamine-induced stereotypy

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Received 29 November 2007; received in revised form 29 February 2008; accepted 14 March 2008
Available online 1 April 2008

Abstract

The behavioral effects induced by methamphetamine (5.0 mg/kg) were compared in the mutant mice lacking D-amino acid oxidase activity and normal mice. The mutant mice exhibited marked decline in the methamphetamine-induced stereotypy compared to the normal mice, whereas the mutant mice displayed a drastic augmentation in the locomotor activity evoked by methamphetamine compared to the normal mice. Because the D-serine levels in the brain of the mutant mice are significantly higher than those in the normal mice, the enhanced D-serine in the brain of the mutant mice could antagonize the methamphetamine-induced stereotypy via the *N*-methyl-D-aspartate receptors.

Keywords: D-amino acid oxidase; *N*-methyl-D-aspartate receptor; Schizophrenia; D-serine; Serine racemase

1. Introduction

Schizophrenia is a severe neuropsychiatric disorder that afflicts about 1% of the world's population. A variety of evidence has suggested that there are two major etiologies of schizophrenia. The dopamine hypothesis follows from the findings that amphetamine can induce a psychosis that resembles the positive symptom of schizophrenia, due to excessive dopamine release and that dopamine D_2 antagonists are efficacious in the treatment of schizophrenia (Carlsson et al., 2001). Furthermore, brain imaging studies have shown that after an acute amphetamine challenge, striatal dopamine release is increased more in schizophrenia patients compared to the controls, suggesting a dysregulated neuronal responsiveness to amphetamine in schizophrenia (Kegeles et al., 2000). In contrast, the *N*-methyl-D-aspartate (NMDA) hypothesis is based on the observations that noncompetitive NMDA antagonists, such as phencyclidine (PCP), ketamine and (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine (MK-801), induce positive, negative, and cognitive schizophrenia-like symptoms in healthy subjects and exacerbate these psychotic symptoms in schizophrenic subjects (Tsai and Coyle, 2002). NMDA-receptor knockdown mice also display behavioral abnormalities, including increased locomotion and stereotyped behaviors closely resembling those seen in the PCP- or MK-801-treated mice (Mohn et al., 1999).

The NMDA receptor has a variety of regulatory sites including a binding site for L-glutamate and a binding site for glycine or D-serine (Danysz and Parsons, 1998; Hashimoto and Oka, 1997). Especially, the occupation of the glycine site on the NMDA receptor by glycine or D-serine is absolutely required for the activation of the NMDA receptor (Danysz and Parsons, 1998). Because D-serine and serine racemase are predominantly concentrated in the forebrain, where the NMDA receptors are enriched, D-serine has been proposed as an endogenous coagonist for the NMDA-receptor-associated glycine site in the mammalian brain (Hashimoto and Oka, 1997; Hashimoto et al.,

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doi:10.1016/j.ejphar.2008.03.031

1993b; Yoshikawa et al., 2004a). In contrast, D-amino acid oxidase (DAO), which catalyzes the oxidative deamination of neutral D-amino acids, is confined to the hindbrain (Horiike et al., 1994; Yoshikawa et al., 2004b). Thus, the regional distribution of DAO in the brain inversely correlates with both those of D-serine and serine racemase (Hashimoto et al., 1993b; Horiike et al., 1994; Yoshikawa et al., 2004a,b). 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 associated with schizophrenia (Chumakov et al., 2002; Harrison and Weinberger, 2005), there is little available information regarding the relationship between DAO and schizophrenia except for genetic evidence. We have shown that a significant elevation in the gene expression of serine racemase and DAO is observed in the brain after the acute administration of MK-801 (Yoshikawa et al., 2004a,b).

Konno and Yasumura (1983) established a mutant mice strain (ddY/DAO$^-$) lacking DAO activity. The mutant mouse strain has a single point mutation in the DAO gene (Sasaki et al., 1992). We have revealed that these mutant DAO−/− mice show a drastic diminution of stereotypy and ataxia produced by MK-801, suggesting that the elevated D-serine and L-glutamate in the brain of the mutant DAO−/− mice could antagonize the MK-801-induced stereotypy and ataxia via the NMDA receptors (Hashimoto et al., 1993a, 2005). To gain further insight into the relationship between DAO and schizophrenia, the behavioral effects produced by acute challenge with another psychotomimetic drug methamphetamine were compared in the normal DAO+/+ and mutant DAO−/− mice.

2. Materials and methods

The 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 normal ddY/DAO$^+$ (DAO+/+) mice and mutant ddY/DAO$^-$ (DAO−/−) mice weighing 28–36 g at the time of the experiment were used. Methamphetamine hydrochloride was purchased from Dainippon Pharmaceutical Company and was dissolved in physiological saline. On the days of the behavioral experiments, the mice were individually placed into 37×24×30 cm high plastic cages divided into quadrants by lines on the floor and allowed to acclimate for at least 30 min before the testing began. Methamphetamine was administered s.c. at 1.5 or 5.0 mg/kg. The test sessions were also videotaped. The locomotor activities (counts) of the mice were automatically measured every 10 min for 90 min by an animal activity meter, ANIMEX-AUTO (Muromachi Kikai, Japan). The locomotor activity was also assessed by counting the number of lines crossed by all four feet (crossing). Rearing was measured every 10 min for 90 min using digital counters with infrared sensors. The behavioral effects of methamphetamine were evaluated on the basis of the method of Costall and Naylor (1975) for stereotyped behaviors with slight modifications. The cumulative behavioral rating for each animal was determined as the summation of each 10 min score for 90 min. The intensity of the stereotypy was scored on a scale of 0–6 where 0=no stereotyped behavior; 1=increased exploratory activity, discontinuous sniffing; 2=continuous sniffing and/or intermittent turning; 3=intermittent head bobbing, head weaving and/or turning; 4=continuous head bobbing and/or head weaving; remains in one location for as long as five minutes; 5=continuous head bobbing and/or head weaving; remains in one location for more than five minutes; 6=continuous licking or biting; remains in one location. The number of turnings (the animal circled laterally to left or right over 360° within a relatively small area) was counted for 90 min. Data for each drug dose were compared by using a between subjects two-way ANOVA (genotype and treatments as factors of variation). Data for time course effects were compared by using two-way repeated measures ANOVA (genotype, treatment and time as factors of variation). Individual treatment effects in each group

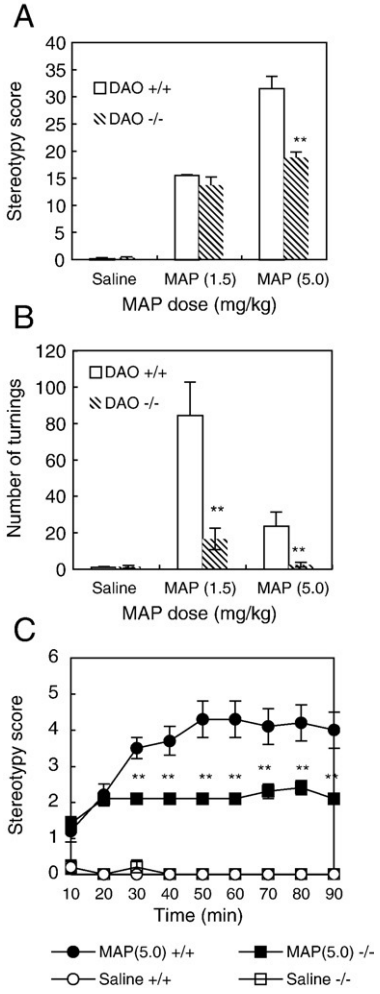

Fig. 1. Effect of methamphetamine (MAP) on the cumulative stereotypy score (A), the number of turnings (B) and time course changes in the stereotypy score after the MAP administration (C) in normal DAO+/+ and mutant DAO−/− mice. The behavioral ratings for the stereotypy score and the number of turnings were taken every 10 min for 90 min after the MAP (1.5 or 5.0 mg/kg) administration. The results are means with S.E.M. of data obtained from six to eight mice. Saline-treated DAO+/+ (open circles, $n=6$), saline-treated DAO−/− (open squares, $n=6$), MAP-treated (5.0 mg/kg) DAO+/+ (filled circles, $n=6$), MAP-treated (5.0 mg/kg) DAO−/− (filled squares, $n=8$). $^{**}P<0.01$ as compared with MAP-treated DAO+/+ mice.

(mutant and normal) were analyzed using one-way ANOVA between subjects. Post-hoc comparisons were made when required by using Bonferroni's test.

3. Results

Fig. 1A shows the cumulative stereotypy score for 90 min after methamphetamine (1.5 or 5.0 mg/kg) administration in the DAO+/+ and DAO−/− mice. In the DAO+/+ mice, methamphetamine (5.0 mg/kg) produced severe stereotyped behaviors such as sniffing, rearing, turning, head weaving, head bobbing and licking. Two-way ANOVA showed a significant effect of treatment ($F(2,33)=253.2$; $P<0.0001$), genotype ($F(1,33)=27.35$; $P<0.0001$), and interaction between treatment and genotype ($F(2,33)=19.59$; $P<0.0001$). Post-hoc analysis exhibited a marked diminution of methamphetamine-induced stereotypy score in the mutant DAO−/− mice when compared with the normal DAO+/+ mice at the dose of 5 mg/kg ($P<0.01$). In contrast, there was no significant difference in the stereotypy score elicited by a low dose of methamphetamine (1.5 mg/kg) between the two groups (Fig. 1A). Fig. 1B shows the number of turnings for 90 min after methamphetamine (1.5 or 5.0 mg/kg) administration. It was found that there was a significant effect of treatment ($F(2,30)=19.11$; $P<0.0001$), genotype ($F(1,30)=18.7$; $P=0.0002$), and interaction between treatment and genotype ($F(2,30)=8.74$; $P=0.001$). The number of turnings elicited by both 1.5 mg/kg ($P<0.01$) and 5.0 mg/kg ($P<0.01$) of methamphetamine was also significantly lower in the DAO−/− than in the DAO+/+ mice (Fig. 1B). However, there was no significant difference in the number of rearings induced by both 1.5 and 5.0 mg/kg of methamphetamine between the DAO+/+ (207±58 and 191±62) and DAO−/− mice (204±58 and 286±73), respectively. Fig. 1C shows the time course effects on the score of the methamphetamine-induced (5.0 mg/kg) stereotypy. The stereotypy score in the DAO+/+ mice after methamphetamine administration increased, and peaked at 50 min, and then remained relatively constant. In contrast, the stereotypy score in the DAO−/− mice remained relatively low throughout the experiment. Two-way repeated measures ANOVA revealed a significant effect of treatment ($F(1,176)=432.31$; $P<0.0001$), genotype ($F(1,176)=26.97$; $P<0.0001$), time ($F(8,176)=10.19$; $P<0.0001$), and interaction among treatment, genotype, and time ($F(8,176)=4.99$; $P<0.0001$). The stereotypy score was significantly lower in the DAO−/− than in the DAO+/+ mice around 30–90 min ($P<0.01$) (Fig. 1C). Fig. 2A shows the number of locomotor counts for 90 min after methamphetamine (1.5 or 5.0 mg/kg) administration in the DAO+/+ and DAO−/− mice. Two-way ANOVA showed a significant effect of treatment ($F(2,32)=54$; $P<0.0001$), genotype ($F(1,32)=11.24$; $P<0.01$), and interaction between treatment and genotype ($F(2,32)=22.29$; $P<0.0001$). Post-hoc analysis exhibited that the number of locomotor counts elicited by 5.0 mg/kg of methamphetamine was significantly ($P<0.01$) higher in the DAO−/− than in the DAO+/+ mice (Fig. 2A). Fig. 2B shows the number of crossings for 90 min after methamphetamine (1.5 or 5.0 mg/kg) administration. It was found that there was a significant effect of treatment ($F(2,32)=53.98$; $P<0.0001$), genotype ($F(1,32)=25.8$; $P<0.0001$), and interaction between treatment and genotype ($F(2,32)=34.88$; $P<0.0001$). The mutant DAO−/− mice displayed a drastic augmentation ($P<0.01$) in the number of crossings induced by a high dose of methamphetamine (5.0 mg/kg) compared to the DAO+/+ mice (Fig. 2B). The number of the locomotor counts and crossings induced by 1.5 mg of methamphetamine tended to be lower in the DAO−/− mice than in the DAO+/+ mice, whereas there was no significant difference between the two groups. Fig. 2C shows the time course effects on the number of methamphetamine-induced (5.0 mg/kg) crossings. The crossing in the DAO−/− mice drastically increased, and peaked at 40 min, and then gradually decreased, whereas the crossing in the DAO+/+ mice slightly increased, and peaked at 30 min, and then returned to the basal level around 60 min after

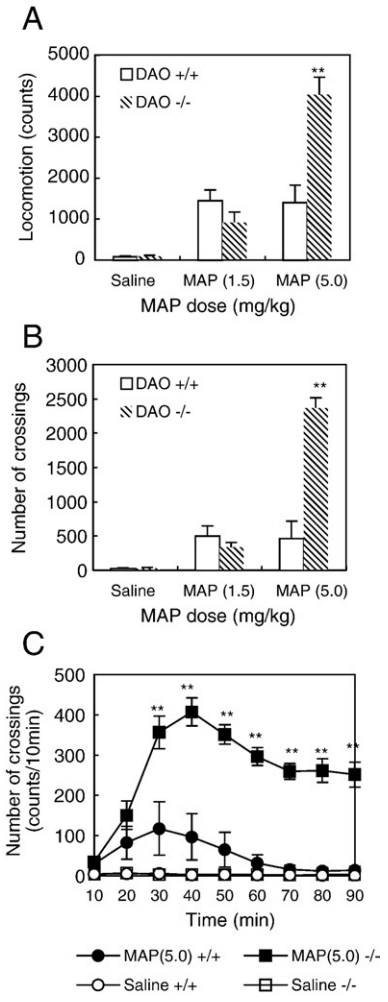

Fig. 2. Effect of methamphetamine (MAP) on the locomotion counts (A), the number of crossings (B) and time course changes in the crossings after the MAP administration (C) in normal DAO+/+ and mutant DAO−/− mice. The locomotion (counts) and crossings were quantified by ANIMEX-AUTO and by counting the number of lines crossed by all four feet (crossing), respectively, every 10 min for 90 min after the MAP (1.5 or 5.0 mg/kg) administration. The results are means with S.E.M. of data obtained from six to eight mice. Saline-treated DAO+/+ (open circles, $n=6$), saline-treated DAO−/− (open squares, $n=6$), MAP-treated (5.0 mg/kg) DAO+/+ (filled circles, $n=6$), MAP-treated (5.0 mg/kg) DAO−/− (filled squares, $n=8$). **$P<0.01$ as compared with MAP-treated DAO+/+ mice.

the administration. Two-way repeated measures ANOVA revealed a significant effect of treatment ($F(1,176)=86.07$; $P<0.0001$), genotype ($F(1,176)=40.72$; $P<0.0001$), time ($F(8,176)=11.85$; $P<0.0001$), and interaction among treatment, genotype, and time ($F(8,176)=6.95$; $P<0.0001$). The number of crossings was significantly higher in the DAO−/− than the DAO+/+ mice around 30–90 min ($P<0.01$) (Fig. 2C).

4. Discussion

The present study revealed that mice lacking DAO activity showed a significant decline in the stereotypy score elicited by the high dose of methamphetamine compared to the DAO+/+ mice, whereas there was no significant difference between the two groups in the locomotor activity induced by the low dose of methamphetamine. Although a genetic study has demonstrated that the DAO gene is associated with schizophrenia (Chumakov et al., 2002), little information is available on the in vivo association between DAO and schizophrenia. The present findings, together with the facts that treatment with DAO, which depletes the endogenous D-serine, has been demonstrated to diminish the NMDA-receptor-mediated neurotransmission using slices and cell culture preparations (Mothet et al., 2000), further support the possibility that there is an in vivo relationship between the DAO activity and psychotomimetic drug-induced abnormal behaviors.

The diminution of stereotypy in the DAO−/− mice, together with the fact that the concentrations of D-serine and L-glutamate in the rostral brain areas and cerebellum of the DAO−/− mice are higher than those in the DAO+/+ mice (Hashimoto et al., 1993a), suggested that the elevated D-serine and L-glutamate in the brain of the DAO−/− mice may antagonize the methamphetamine-induced stereotypy via the NMDA receptors. A variety of evidence supports this possibility: (a) D-serine and L-glutamate potentiate the NMDA-receptor-mediated transmission by stimulation of the glycine site and L-glutamate site of the NMDA receptor, respectively (Danysz and Parsons, 1998); (b) D-serine antagonizes the ability of PCP and MK-801 to produce stereotypy and ataxia in rats (Contreras, 1990; Tanii et al., 1994); (c) mice lacking DAO activity display marked attenuation of the stereotypy and ataxia induced by MK-801 (Hashimoto et al., 2005); (d) a brain imaging study has demonstrated that under the conditions of enhanced dopamine activity, such as amphetamine challenge, blockage of the NMDA transmission in healthy volunteers results in excess amphetamine-induced dopamine release (Kegeles et al., 2000); and (e) a pretreatment with the noncompetitive antagonist MK-801 results in a large potentiation of the amphetamine-induced dopamine release as measured with microdialysis (Millar and Abercrombie, 1996).

A high dose of methamphetamine (5.0 mg/kg) produced a drastic elevation in both the locomotion counts and the number of crossings in the DAO−/− mice compared to the DAO+/+ mice. The marked augmentation of the locomotion in the DAO−/− mice might be due to the decrease in severe stereotypy observed at around 30–90 min after the methamphetamine administration. In fact, the reduction of amphetamine-induced stereotypy by the cholecystokinin$_2$ receptor antagonist causes a long-lasting increase in the amphetamine-induced locomotion (Tieppo et al., 2000). Severe stereotypy and ataxia induced by a high dose of MK-801 or PCP also prevent forward locomotion (Hiramatsu et al., 1989; Sturgen et al., 1979). Both high and low doses of methamphetamine produced a reduction in the number of turnings, whereas there was no significant difference in the stereotypy score by a low dose of methamphetamine between the two groups. This difference might be derived from the stereotyped rating scale, because an intermittent display of turning without head bobbing and/or head weaving was assigned a rating of 2. In fact, the stereotypy score (90 min) induced by a low dose of methamphetamine was low (about 1.5/10 min). Further study is needed to clarify the mechanisms underlying the difference in the number of turnings between the two groups.

The association between DAO, which metabolizes D-serine, and schizophrenia has recently been demonstrated in French–Canadian populations (Chumakov et al., 2002). Because DAO−/− mice, which have the elevated levels of D-serine and L-glutamate in the brain, display the attenuation of stereotypy and ataxia evoked by MK-801 (Hashimoto et al., 2005) and because an administration of NMDA-receptor antagonist in healthy volunteers produces an increased striatal release of dopamine with an amphetamine challenge (Kegeles et al., 2000), the enhanced D-serine and L-glutamate in the brain of the DAO−/− mice could antagonize the methamphetamine-induced stereotypy via the NMDA receptors.

Acknowledgments

This study was supported in part by the grants from the 2006 Tokai University School of Medicine Research Aid and the Asahi Glass Foundation.

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