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Short communication

Mice lacking D-amino acid oxidase activity display marked attenuation of stereotypy and ataxia induced by MK-801

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Abstract

The behavioral effects produced by MK-801 (0.4 mg/kg) were compared in mutant DAO−/− mice lacking D-amino acid oxidase activity and normal DAO+/+ mice. Mutant mice display marked diminution of stereotypy and ataxia induced by MK-801 compared to normal mice. Because the D-serine level in the brain of mutant mice is significantly higher than that of normal mice, the elevated D-serine in the brain of mutant mice could antagonize MK-801-induced stereotypy and ataxia.

Theme: Neurotransmitters, modulators, transporters, and receptors
Topic: Excitatory amino acids: physiology, pharmacology, and modulation

Keywords: D-Amino acid oxidase; *N*-Methyl-D-aspartate receptor; Schizophrenia; D-Serine; Serine racemase

The high heritability of schizophrenia has encouraged much research on identifying susceptibility genes [6]. Chumakov et al. [1] recently discovered a new human gene, G72, on chromosome 13q34 that interacts with the gene for D-amino acid oxidase (DAO) on 12q24. By positional genetics of the linkage and linkage disequilibrium, both of these genes have been shown to be associated with an increased susceptibility to schizophrenia. DAO, which metabolizes the oxidative deamination of neutral D-amino acids, is phylogenetically conserved in the mammalian brain and kidney and is able to catalyze D-serine [5,13,19,25,27]. D-serine is predominantly confined to the forebrain, where *N*-methyl-D-aspartate (NMDA) receptors are enriched [7,9,29]. In vivo microdialysis studies have indicated that the extracellular concentration of D-serine parallels that of glycine in the prefrontal cortex [11]. Because D-serine potentiates the NMDA receptor-mediated transmission by selective stimulation of the strychnine-insensitive glycine site of the NMDA receptor with an affinity similar to glycine, but no affinity for the inhibitory glycine receptor [7,16,22], D-serine has been proposed as an endogenous ligand for an NMDA receptor-related glycine site in the mammalian brain [7,9]. Furthermore, serine racemase that catalyzes the direct formation of D-serine from L-serine has been cloned from the mammalian brain [17,37]. Interestingly, a rapid induction of serine racemase mRNA after MK-801 ((+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5,10-imine) administration occurs in the rat brain [38].

The psychoses after administration of phencyclidine (PCP) are clinically indistinguishable from schizophrenia, including the positive, negative, and cognitive symptoms [4,15,35]. The blockade of NMDA receptors by uncompetitive antagonists such as PCP and MK-801 also induces behaviors in rats and mice that include hyperlocomotion, stereotypy, and ataxia. In fact, NMDA-glycine site agonists

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such as D-serine and D-alanine block the PCP-, MK-801-, and methamphetamine-induced hyperactivity, stereotyped behavior, and ataxia in rats [2,8,31,32]. These observations provided the basis for the hypothesis of the NMDA receptor hypofunction in schizophrenia. Therefore, PCP- or MK-801-treated animals have been utilized as models of schizophrenia.

Konno and Yasumura [18] established a mutant mouse strain (ddY/DAO$^-$) lacking DAO activity. The mutant mice were shown to have a missense mutation (Gly-181→Arg) in the DAO gene [28]. We have also demonstrated that the levels of D-serine, D-alanine, and L-glutamate in the cerebellum and rostral brain areas of the mutant DAO−/− mice are higher than those of normal DAO+/+ mice [10]. Although the DAO gene has been shown to be associated with schizophrenia [1,6], little information is available concerning the relationship between DAO and schizophrenia except for the genetic data. In this study, the behavioral effects produced by acute challenge with MK-801 were compared in normal DAO+/+ and mutant DAO−/− mice.

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 29–35 g at the time of the experiment were used. The mice were housed in groups under a 12-h light/dark cycle with food and water available ad libitum. MK-801 was purchased from Sigma (St. Louis, MO). 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. At first glance, the behavior of the non-treated mutant DAO−/− mice seemed to be normal. MK-801 was dissolved in saline. MK-801 was administered sc at 0.4 mg/kg and 0.1 ml/10 g of body weight. The test sessions were also videotaped. The locomotor activities (counts) of the mice were automatically measured every 10 min for 90 min with an animal activity meter, MK- ANIMEX (Muromachi Kikai, Tokyo, Japan). 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 MK-801 were evaluated based on the method of Costall and Naylor [3] for stereotyped behaviors and that of Tricklenbank et al. [33] for ataxia 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 0–4 where 0 = absent; 1 = equivocal; 2 = present (moderate rate of sniffing, head-weaving); 3 = intense (moderate rate of turning, sniffing, head-weaving); 4 = intense and continuous (continuous turning, sniffing, head-weaving). The intensity of the ataxia was scored on a scale of 0–3 where 0 = absent; 1 = equivocal; 2 = present (awkward-jerky movements, moderate rate of falling while moving about); 3 = intense (frequent falling on back and/or side while moving about). The number of turnings and fallings was also counted for 90 min. The results are given as means with SEM of the data. For comparison between the two groups, a statistical evaluation was performed using the unpaired two-tailed Student's t tests. A P value < 0.05 was considered as reaching statistical significance.

Fig. 1 shows the cumulative stereotypy score and the number of turnings for 90 min after MK-801 (0.4 mg/kg, sc) administration in normal DAO+/+ and mutant DAO−/− mice. In DAO+/+ mice, MK-801 produced severe stereotyped behaviors such as sniffing, head-weaving, and turn-

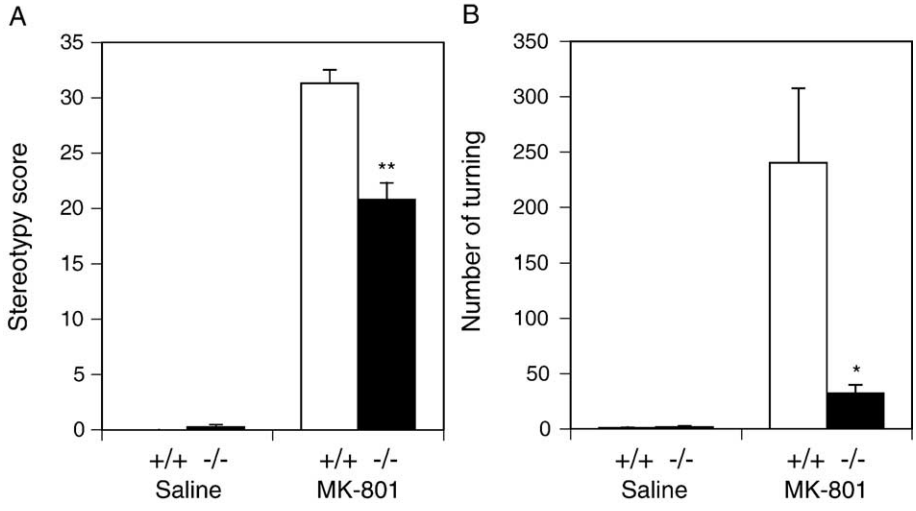

Fig. 1. Effect of MK-801 on the cumulative stereotypy score (A) and the number of turnings (B) of normal DAO+/+ and mutant DAO−/− mice. The behavioral ratings for the stereotypy score and the number of turnings were taken every 10 min for 90 min after MK-801 (0.4 mg/kg) administration. The results are means with SEM of data. Saline-treated DAO+/+ (n = 4), saline-treated DAO−/− (n = 4), MK-801-treated DAO+/+ (n = 6), MK-801-treated DAO−/− (n = 6). *P < 0.05; **P < 0.01 as compared with MK-801-treated DAO+/+ mice.