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NMDA-type Glutamatergic Modulation in Dopaminergic Activation Measured by Apomorphine-Induced Cage Climbing Behaviors

Choon-Gon Jang and Seok-Yong Lee

Department of Pharmacology, College of Pharmacy, Sungkyunkwan University, Suwon, 440-746, Korea

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The present study examined the hypothesis that NMDA, AMPA/Kainate, and metabotropic (mGlu) glutamate receptors contribute to a behavioral stimulation induced by activation of dopamine receptors by comparing responses in apomorphine-induced cage climbing behaviors in mice. MK-801, CNQX, and MCPG were served as the NMDA receptor, AMPA/Kainate receptor, and mGlu receptor antagonist, respectively, to elucidate the glutamatergic modulation in apomorphine-induced dopaminergic activation in mice. Drugs were administered intracerebroventricularly (i.c.v.) into the mouse brain 15 min before the apomorphine treatment (2 mg/kg, s.c.). I.c.v. injection of MK-801 inhibited the apomorphine-induced cage climbing behavior dose-dependently. However, treatments with CNQX and MCPG did not any significant change in apomorphine-induced cage climbing behavior in mice. These results suggest that stimulation of NMDA type of glutamate receptors could contribute to the dopaminergic stimulation, but not AMPA/Kainate and mGlu type glutamate receptors.

Key words: *Glutamate receptors, Dopamine receptors, Climbing behavior, NMDA receptors*

INTRODUCTION

Excitatory amino acids (EAA) such as glutamate and aspartate are the most abundant neurotransmitters in the CNS. Glutamate is the major rapid-acting excitatory neurotransmitter in the CNS and is in high concentration in glutamatergic synaptic vesicles (Taylor *et al.*, 1992). Glutamate receptor is involved in several forms of neuronal and behavioral plasticity, such as, neuronal development, long-term potentiation, kindling, learning and memory (McDonald and Johnston, 1990).

For over a decade it has been attracted much interest in the neuronal interactions between the EAA, particularly glutamate and dopamine receptor. Much current research is focused on describing the pharmacology of the different glutamate receptor subclasses and their interaction with dopamine receptor. The striatum has dopaminergic terminals arising from the midbrain and contains the high concentrations of glutamate. The corticostriatal pathway (Kemp and Powell, 1970; Webster, 1965) is thought to be the source of striatal glutamate (Fonnum, 1984; Hassler *et al.*, 1982; McGeer *et al.*, 1977; Walaas and Fonnum, 1979; Young and Bradford, 1986). In vivo, release of dopamine from nigrostriatal neurons is stimulated by low concentration of glutamate, suggesting the control of dopamine release by glutamatergic mechanisms (Cheramy *et al.*, 1986). Thus, the release of dopamine from striatal or nucleus accumbens slices can be stimulated by glutamate, NMDA, AMPA or kainate. Dopamine release induced by glutamate is blocked by L-glutamic acid gamma diethyl ester (GDEE) implying involvement of the quisqualate receptor (Marien et al., 1983), whereas that induced by NMDA is blocked by 2AVP or phencyclidine (Snell *et al.*, 1988; Jones *et al.*, 1987). Additionally, it has been demonstrated that MK-801 preferentially antagonizes the release of [^{3}H]dopamine from rat mesencephalic cells in culture induced by low concentration of L-glutamate (Mount *et al.*, 1990).

Recently it has been reported that DNQX injected into the nucleus accumbens inhibits the hyperactivity induced by amphetamine (Willins *et al.*, 1992). In addition to this, intra-accumbens microinjection of an NMDA antagonist

Correspondence to: Choon-Gon Jang, Ph.D., Assistant Professor, Department of Pharmacology, College of Pharmacy, Sungkyunkwan University, 300 Cheoncheon-dong, Jangan-ku, Suwon, 440-746, Korea
E-mail: jang@skku.ac.kr

prevents the locomotor activity of the heroin, cocaine and dopamine (Pulvirenti *et al.*, 1991). It has been also reported that the repeated treatment with MK-801, inhibits the development of reverse tolerance to the ambulation accelerating effects of cocaine and amphetamine, suggesting the modulation of dopaminergic system by MK-801 (Karler *et al.*, 1989; Pudiak and Bozarth, 1993). MK-801, also, prevents the development of opiate tolerance and dependence (Trujillo and Akill, 1991), and interferes with the development of behavioral sensitization to dopamine receptor stimulant drugs (Wolf and Khansa, 1991), suggesting that NMDA receptors might play crucial roles in the changes of these drugs-induced sensitivity to neurotransmitters.

Apomorphine-induced cage climbing behavior has been used as a convenient method of screening dopamine agonists or antagonists and to assess striatal dopamine activity. These behaviors are reduced after destruction of the striatum and are enhanced by 6-hydroxydopamine-induced lesions of dopamine input into the striatum (Protais *et al.*, 1976). Recently, it is reported that systemic injection of NMDA receptor antagonists (MK-801, ketamine, dextrorphan, and dextromethorphan) attenuate the apomorphine-induced cage climbing behavior, suggesting the glutamatergic modulation of dopaminergic function in mice (Kim *et al.*, 1996). However, it still remains unclear whether other glutamate receptors can modulate the dopaminergic system in mice which is administered supraspinally into the brain. Therefore, in the present study, we investigated the effects of glutamate receptor antagonists (MK-801, NMDA receptor antagonist; CNQX, AMPA/Kainate receptor antagonist; MCPG, mGlu receptor antagonist) on apomorphine-induced cage climbing behavior, to elucidate the glutamatergic modulation of dopaminergic stimulation in mice administered intracerebroventricularly (i.c.v.).

MATERIALS AND METHODS

Animals and materials

ICR male mice (MJ Ltd. Seoul, Korea) weighing 20-26 g in a group of 10-20, were used in all experiments. They were housed 10-14 mice in a cage with water and food available ad libitum under an artificial 12 h light dark cycle (light on: 7:00 a.m.) and constant temperature (22 ± 2°C).

The drugs used were (+)-MK-801 hydrogen maleate (dizocilpine, (+)-5-methyl-10,11-dihydroxy-5H-dibenzo-(a,d)cyclo-hepten-5,10-imine, Tocris Cookson Inc, USA), CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, Tocris Cookson Inc, USA), MCPG (Methyl-4-carboxyphenylglycine, Tocris Cookson Inc, USA), and apomorphine hydrochloride (Sigma Co. USA). MK-801 was dissolved in physiological saline. CNQX was dissolved in 7.5% DMSO and MCPG was dissolved in 0.1N NaOH. Apomorphine was dissolved in saline containing 0.1% ascorbic acid, just before the experiment.

Intracerebroventricular injection of drugs

Intracerobroventricular (i.c.v.) injection was given directly into the lateral ventricle according to the modification of Haley and McCormick (1957). Each mouse of each group received a single i.c.v. administration of MK-801 (0.01, 0.1 and 1 μg/mouse), CNQX (0.05, 0.1, and 0.5 μg/mouse), and MCPG (1, 5, and 15 μg/mouse), respectively. The injection volume was 5 μl/mouse. Each group consists of 10-14 mice. To assess the effects of the glutamate receptor antagonists, the drugs were administered i.c.v. to each mouse 15 min before the injection of apomorphine. Mice were observed immediately after apomorphine treatment (2 mg/kg, s.c.) and were scored as showing climbing response when the following mentioned behaviors were present.

Measurement of apomorphine-induced cage climbing behavior

The cage climbing behavior in mice was measured by modification of Protais et al's method (1976). Immediately after a subcutaneous injection of apomorphine 2 mg/kg, the mice were put into cylindrical individual cages: 12 cm in diameter and 14 cm in height, with walls of vertical metal bars (2 mm in diameter and 1 cm apart). After a 5-min period of exploratory activity, the climbing behavior was measured at 10, 20 and 30 min intervals for 1 min at each time. A summed score for the three evaluations was generated. The climbing behaviors were scored as all or none events as follows: four paws on the floor (0 point), forefeet on the bars (1 point), four paws on the bars (2 points).

Statistics

Data were expressed as a Mean ± S.E. The significant differences were first determined by one-way analysis of variance (ANOVA). In the case of significant variation, the individual values were compared by Newman-Keuls test.

RESULTS

Effects of MK-801, CNQX, and MCPG on apomorphine-induced cage climbing behavior

Our preliminary experiment showed that a single administration of apomorphine (0.5, 1, 2, and 4 mg/kg, s.c.) produced cage climbing behavior dose-dependently (data not shown). Apomorphine 2 mg/kg produced the greatest effect of climbing behavior in mice. Therefore, we used 2 mg/kg of apomorphine to induce climbing behavior in

these experiments.

I.c.v. injections of MK-801 0.1 and 1 mg/mouse attenuated the apomorphine-induced cage climbing behavior in mice by about 44 % (2.6 ± 0.6, $p<0.05$) and 72% (1.3 ± 0.5, $p<0.01$), when compared with the control group (4.5 ± 0.5), respectively (Fig. 1).

I.c.v. injections of CNQX 0.05, 0.1, and 0.5 μg/mouse did not change the apomorphine-induced cage climbing behavior in mice (Fig. 2). I.c.v. injections of MCPG 1, 5 and 15 μg/mouse did not also change the apomorphine-induced cage climbing behavior in mice (Fig. 3).

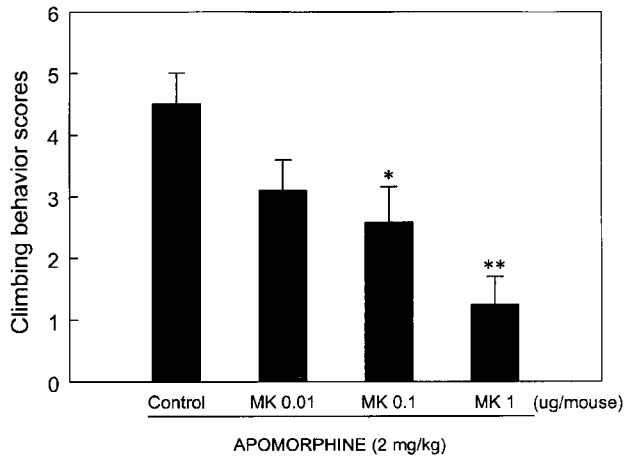

Fig. 1. Effects of MK-801 on apomorphine-induced cage climbing behavior in mice. Mice were injected with 0.01, 0.1, and 1 μg/5 μl/mouse of MK-801 (i.c.v.) 15 min before administration of 2 mg/kg of apomorphine (s.c.). After injection of apomorphine, the climbing behavior was measured at 10, 20, and 30 min for 1 min. The data are expressed as a mean composite score of the three time points with S.E.M. from 10-14 mice. *P< 0.05, **P<0.01, compared to control group.

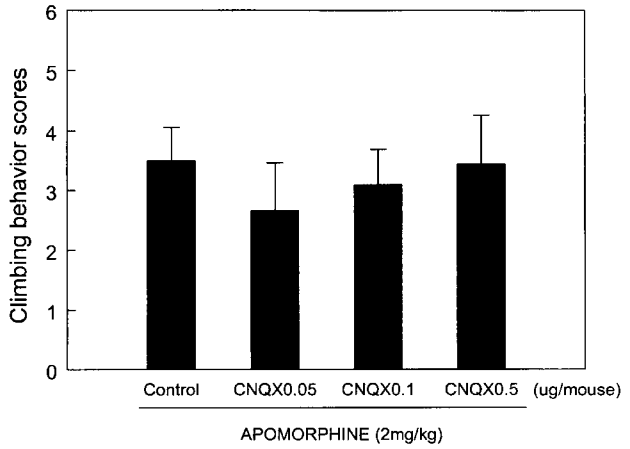

Fig. 2. Effects of CNQX on apomorphine-induced cage climbing behavior in mice. Mice were injected with 0.05, 0.1, and 0.5 μg/5 μl/mouse of CNQX (i.c.v.) 15 min before administration of 2 mg/kg of apomorphine (s.c.). After injection of apomorphine, the climbing behavior was measured at 10, 20, and 30 min for 1 min. The data are expressed as a mean composite score of the three time points with S.E.M. from 10-14 mice.

DISCUSSION

The present study investigated the effects of three different glutamate receptor antagonists, MK-801, CNQX, and MCPG, on apomorphine-induced climbing behavior in mice. I.c.v. injection of MK-801, an NMDA receptor antagonist, inhibited the apomorphine-induced cage climbing behavior dose-dependently. This result is consistent with the report (Kim *et al.*, 1996) that systemic administration of MK-801 blocked the apomorphine-induced climbing behavior in mice. However, i.c.v. injection of CNQX, an AMPA/Kainate receptor antagonist, and MCPG, mGlu receptor antagonist, did not change this behavior. In support of our result, Vanover (1998) has reported that intraperitoneal administration of AMPA receptor antagonists, GYKI 52466, LY300164, and NBQX, failed to attenuate apomorphine-induced climbing behavior, even though AMPA antagonists attenuate the behavioral effects of amphetamine and dizocilpine. They suggest that blockade by AMPA receptor antagonists of behavioral effect amphetamine and dizocilpine may be due to their presynaptic modulation of extracellular dopamine concentration. The previous study investigated the ability of mGlu receptor to modulate dopamine release in the striatum of freely moving rats assessed using the microdialysis technique (Bruton *et al.*, 1999). Administration of MCPG prevented the dopamine release facilitated by mGlu agonists, indicating that mGlu receptors modulate rat striatal dopamine release in vivo. In the present study, the fact that MCPG could not inhibit the

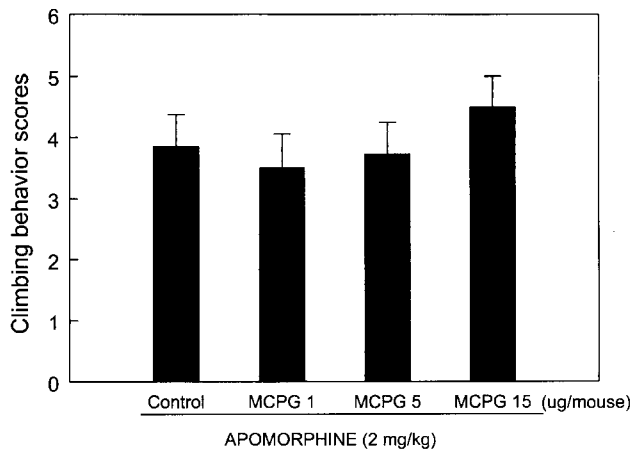

Fig. 3. Effects of MCPG on apomorphine-induced cage climbing behavior in mice. Mice were injected with 1, 5, and 15 μg/5 μl/mouse of MCPG (i.c.v.) 15 min before administration of 2 mg/kg of apomorphine (s.c.). After injection of apomorphine, the climbing behavior was measured at 10, 20, and 30 min for 1 min. The data are expressed as a mean composite score of the three time points with S.E.M. from 10-14 mice.

apomorphine-induced cage climbing behavior is somewhat surprising. However, considering previous suggestion that climbing behavior may be mediated in the nucleus accumbens, whereas stereotypy may be mediated in the striatum (Worms *et al.*, 1983), it is possible. Furthermore, the cage climbing behavior induced by apomorphine is due to the stimulation of dopamine receptors at the postsynaptic level (Protais *et al.*, 1976, Costentin *et al.*, 1975). Therefore, it is likely that blockade of non-NMDA receptor may result in a selective behavioral effect, such as modulation of locomotor behaviors induced by dopamine agonist, but not climbing behavior.

In the present study, the fact that NMDA receptor antagonist, but not AMPA/Kainate receptor antagonist and mGlu receptor antagonist, blocked climbing behavior induced by the direct dopamine receptor agonist, apomorphine, implies that NMDA type glutamatergic neurotransmission may modulate dopaminergic function at the postsynaptic level. Therefore, we propose that NMDA type glutamate receptors play an important role in dopaminergic stimulation at the postsynaptic level assessed by apomorphine-induced cage climbing behavior. Our present data suggest that NMDA type glutamate receptors are highly involved in producing cage climbing behavior induced by apomorphine.

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