Characteristics of the Ambulation-Increasing Effect of the Noncompetitive NMDA Antagonist MK-801 in Mice: Assessment by the Coadministration with Central-Acting Drugs

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Abstract — Characteristics of the ambulation-increasing effect of MK-801, a non-competitive NMDA antagonist, were assessed through the coadministration of MK-801 with various central-acting drugs in mice. The MK-801 (0.3 mg/kg, i.p.)-induced ambulation-increment with a slight ataxia was maximum at around 50 min, and ambulation returned to the control level at about 3 hr after the administration. At 1 mg/kg, the mouse's activity transiently increased, followed by a decrease due to a marked ataxia, which was due to neither stereotypy nor convulsion, for 20–50 min, and then increased again; the ambulation-increment continued even at 4 hr after the administration. Coadministration of MK-801 (0.3 mg/kg, i.p.) with either methamphetamine (2 mg/kg, s.c.), cocaine (20 mg/kg, s.c.), GBR-12909 (10 mg/kg, i.p.), scopolamine (0.5 mg/kg, s.c.), caffeine (10 mg/kg, s.c.) or morphine (10 mg/kg, s.c.) produced a significant enhancement of the effect. However, 0.1 mg/kg of MK-801 had no effect on the interaction with these drugs. On the other hand, the ambulation-increasing effect of MK-801 (0.3 mg/kg) was significantly reduced by haloperidol (0.03 and 0.1 mg/kg, s.c.), ceruletide (0.01 and 0.1 mg/kg, i.p.), reserpine (0.05 and 2 mg/kg, s.c., pretreatment 4 hr before) and nimodipine (1 and 3 mg/kg, i.p.), but it was scarcely modified by α-methyl-p-tyrosine (100 and 200 mg/kg, i.p., pretreatment 24 hr and 4 hr before), imipramine (20 mg/kg, i.p.), 6R-L-erythro-5,6,7,8-tetrahydro-biopterin (100 mg/kg, i.p.), pilocarpine (1 and 4 mg/kg, s.c.), N°-(L-2-phenylisopropyl)-adenosine (0.03 and 0.1 mg/kg, s.c.) and naloxone (1 and 5 mg/kg, s.c.). These results indicate the MK-801 increases the mouse’s ambulatory activity through stimulation of the dopaminergic system which is strongly affected by a calcium-dependent mechanism.

The noncompetitive NMDA antagonist MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (1), has been receiving much attention in behavioral pharmacology as a tool for assessing the role of the central neurotransmission systems of the NMDA-component.

It has been reported that MK-801 shows anticonvulsant action (2), neuroprotective action (3), an antiparkinson property (4), and anticonflict action (5, 6). Furthermore, Clineschmidt et al. (7) demonstrated that MK-
801 increased the ambulatory activity of mice and produced an ipsilateral turning in the rat with unilateral nigrostriatal lesion, indicating that MK-801 possesses agonistic action on central dopaminergic systems through an acceleration of the dopaminergic transmission. Dimpfel and Spuler (8) also confirmed an MK-801-induced activation of dopaminergic transmission by recording the brain field potentials from the frontal cortex, hippocampus, striatum and reticular formation in rats that was similar to those observed after administration of amphetamine and L-dopa. However, Clineschmidt et al. (7) demonstrated that the MK-801 and amphetamine-induced increases in the ambulatory activity were differentially modified by \( \alpha \)-methyl-p-tyrosine and reserpine. Thus, further study was required to characterize the ambulation-increasing effect of MK-801.

In this study, we carried out experiments to assess the ambulation-increasing effect of MK-801 through the coadministration with various central-acting drugs having actions not only on dopaminergic systems but also on muscarinic cholinergic, opioid and adenosine systems as well as having a calcium-dependent mechanism.

MATERIALS AND METHODS

Animals
Seven-week-old male mice of the ddY strain (Japan Laboratory Animal) were used. Prior to the start of the experiment, they had been group housed in standard breeding cages with a free access to solid diet (MF, Oriental Ycast) and tap water. The breeding room was controlled so that the light-dark schedule (lighting time: 06:00–18:00) and temperature (23 ± 1°C) were almost constant.

Drugs
Drugs used were MK-801 hydromaleate (Merck/Banyu); methamphetamine HCl and haloperidol (Serenace Inj.) (Dainippon Pharm.); cocaine HCl and morphine HCl (Takeda Chem.); GBR-12909 (Gist-brocades); imipramine HCl (Tofranil Inj., Ciba-Geigy Japan); apomorphine HCl, scopolamine HBr, \( \alpha \)-methyl-p-tyrosine (AMPT), pilocarpine HCl, \( N^0 \)-[(l-2-phenylisopropyl)腺enosine (PIA) and naloxone HCl (Sigma Chem.); anhydrous caffeine (Kanto Chem.), \( 6R \)-l-erythro-5,6,7,8-tetrahydrobiopterin (R-THBP: Sunyory); ceruletide diethylamine (Shionogi Pharm.); reserpine (Apoplon Inj., Daiichi Pharm.) and nimodipine (Bayer AG). These drugs were dissolved or suspended in physiological saline or diluted by physiological saline immediately before administration. The concentration of each drug solution or suspension, expressed in terms of the salt forms, was adjusted so that each volume administered was constant at 0.1 ml/10 g body weight.

Experimental procedure
The mouse's ambulatory activity was measured using a tilting-type ambulometer (AMB-10, O'Hara & Co.). Mice were individually placed in plexiglas activity cages with a diameter of 20 cm, and their ambulatory activities were measured for 30 min and 3–4 hr before and after the drug administration, respectively.

Single administration: The ambulatory activities of mice were observed for 3 or 4 hr after administration of MK-801 (0: saline, 0.1, 0.3 and 1 mg/kg, i.p.).

Coadministration: In the first series of this experiment, the ambulatory activity of mice was measured for 3 hr after the coadministration of MK-801 (0, 0.1 and 0.3 mg/kg) with methamphetamine (2 mg/kg, s.c.), cocaine (20 mg/kg, s.c.), GBR-12909 (10 mg/kg, i.p.), imipramine (20 mg/kg, s.c.), apomorphine (0.5 mg/kg, s.c.), scopolamine (0.5 mg/kg, s.c.), caffeine (10 mg/kg, s.c.), morphine (10 mg/kg, s.c.), or R-THBP (100 mg/kg, s.c.). In the second series of this experiment, the mouse's activity was measured for 3 hr after the coadministration of MK-801 (0.3 mg/kg, i.p.) with haloperidol (0.03 and 0.1 mg/kg, s.c.), ceruletide (0.01 and 0.1 mg/kg, i.p.), reserpine (0.5 and 2 mg/kg, s.c., pretreatment 4 hr before), AMPT (100 and 200 mg/kg, i.p.) ×
2, pretreatment 24 and 4 hr before), pilocarpine (1 and 4 mg/kg, s.c.), PIA (0.03 and 0.1 mg/kg, s.c.), naloxone (1 and 5 mg/kg, s.c.) or nimodipine (1 and 3 mg/kg, i.p.).

In each drug test, 10–20 mice were used. All of these experiments were carried out between 9:00–16:30.

Statistical analyses

The mean overall ambulatory activity counts for 3 hr after the drug administration were calculated in individual groups of mice. These data were compared by the two-tailed paired test; when P values were equal to or less than 0.05, they were considered to be significantly different.

RESULTS

The single administration of MK-801

Figure 1 shows the time course changes in ambulatory activity counts after i.p.-adminis-
tration of MK-801 alone. The mouse's ambulatory activity increased only slightly at 0.1 mg/kg, but markedly increased at 0.3 mg/kg, attaining the maximum level at about 50 min, and disappeared by 3 hr after the administration. The mice exhibited a slight ataxia during the ambulation-increment. When 1 mg/kg of MK-801 was administered, the mice demonstrated a marked ataxia, but showed no stereotypy or convulsion for 20–50 min, and thereby their ambulation was obstructed. With the recovery from the ataxia, a prominent ambulation appeared again, and the activity continued even at 4 hr after the administration.

The overall ambulatory activity counts for 3 hr after the administration of 0.3 and 1 mg/kg of MK-801 were significantly higher than the saline-administered control value (data not shown, but refer to the left upper 3 columns of Fig. 2).

From these data, 0.1, 0.3 and 1 mg/kg of MK-801 could be considered as subeffective, optimum and over doses, respectively, for increasing the mouse's ambulatory activity under our experimental conditions. In these respects, 0.1 and 0.3 mg/kg of MK-801 were used in the experiment of coadministration with central-acting drugs.

The coadministration

As shown in Fig. 2, methamphetamine, cocaine, GBR-12909, scopolamine, caffeine or morphine in combination with saline, at each dose used in this experiment, increased the mouse's ambulatory activity. The coadministration of MK-801 at 0.3 mg/kg, but not 0.1 mg/kg, with these drugs produced a significant

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Fig. 2. The mean 3-hr overall ambulatory activity counts after the coadministration of MK-801 (0: saline, 0.1 and 0.3 mg/kg, i.p.) with methamphetamine (MAP: 2 mg/kg, s.c.), cocaine (COC: 20 mg/kg, s.c.), GBR-12909 (GBR: 10 mg/kg, i.p.), imipramine (IM: 20 mg/kg, i.p.), apomorphine (AP: 0.5 mg/kg, s.c.), scopolamine (SCP: 0.5 mg/kg, s.c.), caffeine (CAF: 10 mg/kg, s.c.), morphine (MOR: 10 mg/kg, s.c.) and 6R-l-erythro-5,6,7,8-tetrahydrobiopterin (R-THBP: 100 mg/kg, s.c.). After the coadministration of drugs, the mouse's ambulatory activity was observed for 3 hr. #: Significantly different as compared with both the values after the single administration of the test drug and MK-801 (P < 0.05). N = 10–20 in each experiment.
enhancement of the ambulation-increasing effect; i.e., the activity counts were significantly higher than the values after the single administration of MK-801 and the test drug. In contrast there was no marked change in the effect when MK-801 was coadministered with apomorphine. A gross observation revealed that the stereotypy induced by apomorphine was not modified by any dose of MK-801. Furthermore, in our preliminary experiments, low doses of apomorphine (0.1 mg/kg and less) and bromocriptine (1 mg/kg), which may selectively stimulate the presynaptic dopamine autoreceptors and reduce the motor activity of rat and mouse (9, 10), did not modify the effect of MK-801 (0.1 and 0.3 mg/kg) (H. Kuribara, unpublished data). Imipramine and R-THBP did not modify the effect of MK-801.

As shown in Fig. 3, the ambulation-increasing effect of MK-801 (0.3 mg/kg) was significantly reduced by haloperidol, ceruletide, reserpine and nimodipine, whereas AMPT and naloxone scarcely modified the effect of MK-801. Although pilocarpine and PIA tended to reduce the ambulation-increasing effect of MK-801 for 10–20 min, the difference in overall activity counts did not attain a level of statistical significance.

Table 1 shows the comparison among the semiquantitative changes in the ambulation-in-
creasing effects of MK-801, methamphetamine, cocaine and GBR-12909 by various central-acting drugs. The data for methamphetamine, cocaine and GBR-12909 were taken from our previous experiments (cf. ref. 11 and H. Kuribara, unpublished data).

**DISCUSSION**

MK-801 has been reported to facilitate dopaminergic transmission by means of brain field potential in 4 regions: frontal cortex, hippocampus, striatum, and reticular formation (8). In agreement with such a neurophysiological result, the present experiment demonstrated a prominent increase in the mouse’s ambulatory activity after the administration of MK-801 at 0.3 and 1 mg/kg. The effective doses of MK-801 that produced a significant increase in the mouse’s activity were almost comparable to those reported by Clineschmidt et al. (7). However, it is unlikely that MK-801-induced ataxia was non-specifically involved in the ambulation-increment, because the ambulometer used in this experiment detected ambulatory movements of longer than 15 cm as 1 count, and a small movement such swinging of the body was completely neglected (12).

The drugs tested in this experiment increased the mouse’s ambulatory activity through an activation of dopaminergic transmission by facilitating the release and/or inhibiting the uptake of catecholamines (methamphetamine, cocaine and GBR-12909) (11, 13, 14), by stimulating the opioid receptors (morphine) (15), by blocking the muscarinic cholinergic receptors (scopolamine) (16), and/or by blocking the adenosine receptors (caffeine) (17). MK-801 at 0.3 mg/kg interacted with all of these drugs to enhance the ambulation-increasing effect.

It is therefore curious that there was no marked interaction in the coadministration of MK-801 with apomorphine. Apomorphine stimulates presynaptic dopamine autoreceptors and reduces the mouse’s ambulatory activity, but at high doses, it stimulates postsynaptic dopamine receptors and increases the activity (10). The lack of a marked interaction be-

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**Table 1. Comparison of the interactions of MK-801, methamphetamine, cocaine and GBR-12909 with various central-acting drugs as evaluated by the ambulatory activity in mice**

|         | MK-801 | Methamphetamine | Cocaine | GBR-12909 |
|---------|--------|-----------------|---------|-----------|
| Imipramine    | NC     | +               | +       | ++        |
| Apomorphine   | NC     | NC              | NC      | NC        |
| Scopolamine   | ++     | ++              | ++      | ++        |
| Caffeine      | ++     | ++              | ++      | ++        |
| Morphine      | ++     | ++              | ++      | ++        |
| R-ThBP        | NC     | ++              | NC      | ++        |
| Haloperidol   | ---    | ---             | ---     | ---       |
| Ceruletide    | ---    | ---             | NC      | ---       |
| Reserpine     | ---    | NC              | +       | ---       |
| AMPT          | NC     | --              | NC      | --        |
| Filocarpine   | NC     | --              | --      | --        |
| PIA           | NC     | --              | --      | --        |
| Naloxone      | NC     | NC              | NC      | NC        |
| Nimodipine    | ---    | NC              | --      | NC        |

+ : Increased. ++ : Markedly increased. -- : Reduced. --- : Markedly reduced. NC: No significant change. The data for methamphetamine, cocaine and GBR-12909 are taken from our previous experiments (cf. ref. 11 and H. Kuribara, unpublished data).
tween apomorphine and MK-801 demonstrated in this experiment as well as in our preliminary experiment using low doses of apomorphine (H. Kuribara, unpublished data) may indicate that MK-801 scarcely possesses any direct action on both the post- and presynaptic dopamine receptors.

It is also interesting why imipramine and R-THBP, catecholamine uptake inhibitors and coenzyme of tyrosine hydroxylase (18), respectively, did not modify the ambulation-increasing effect of MK-801. Imipramine and R-THBP enhanced the ambulation-increasing effect of methamphetamine, cocaine and/or GBR-12909 even though the tested doses of these 3 drugs were subeffective or effective (11, 19). Furtheremore, 0.1 mg/kg of MK-801, which was a subeffective dose for increasing the mouse's ambulatory activity, did not show any interaction with the drugs mentioned above. Thus, it is probable that the ambulation-increasing effect of MK-801 appeared through a stimulation of dopaminergic transmission, although its mechanism was different from either that of amphetamines, cocaine or GBR-12909. It is also probable that the enhancements of the effect in the coadministration of MK-801 with these drugs were produced by an additive interaction, although the activity counts recorded were sometimes greater than the sums of the increased counts by the individual drugs.

On the other hand, the ambulation-increasing effect of MK-801 at 0.3 mg/kg was reduced by a dopamine receptor antagonist (haloperidol), monoamine depleter (reserpine), CCK-like decapetic (ceruletide) or calcium blocker (nimodipine) (20). Particularly, the action of ceruletide on MK-801 was about 10000 times as potent as that on methamphetamine and cocaine (21), but not that of GBR-12909 (11). These results indicate again that the characteristics of the ambulation-increasing effect of MK-801 are different from those of methamphetamine, cocaine and/or GBR-12909, and that a calcium-dependent mechanism plays an important role in it. This consideration may be supported by the facts that both tyrosine hydroxylase inhibitor (AMPT) and coenzyme of tyrosine hydroxylase (R-THBP) did not modify the ambulation-increasing effect of MK-801. Furthermore, a muscarinic cholinergic agonist (pilocarpine) and an adenosine receptor agonist (PIA) (24) only exhibited a very weak interaction with MK-801. These drugs can produce a significant reduction of the ambulation-increasing effect of methamphetamine, cocaine and/or GBR-12909. The opioid receptor antagonist naloxone also had no significant interaction with MK-801.

In these respects, it can be considered that the ambulation-increasing effect of MK-801 in mice is produced by modifying the dopaminergic process, probably through enhancement of dopamine release from the storage site at the presynaptic level, and that the dopamine release is strongly affected by a calcium-dependent mechanism. We are currently performing further studies to evaluate whether such a behavioral profile of MK-801 is produced through a direct action on the dopaminergic system or indirectly by affecting the NMDA system and whether MK-801 interacts with drugs that possess actions on the other neuronal systems which were not evaluated in the present experiments.

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