Behavioral Study on Mergocriptine (CBM36-733) by Ambulatory Activity in Mice: Repeated Administration and Interaction with Methamphetamine

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Abstract—Effects of repeated administration of mergocriptine (CBM36-733: CBM), a long-acting ergot derivative with an agonistic action on both dopamine D1 and D2 receptors, as well as interaction between CBM and methamphetamine (MAP: 2 mg/kg, s.c.), were investigated by ambulatory activity in mice. CBM at 4 mg/kg significantly suppressed the ambulatory activity, but significantly increased it at 16 mg/kg in the drug-naive mice. However, 4 and 8 mg/kg of CBM were effective for increasing the ambulatory activity when these doses were repeatedly administered for 9 times at intervals of 7 days. The same treatment with 16 mg/kg of CBM produced a reverse tolerance to the ambulation-increasing effect. The mice that had received CBM at 1 and 2 mg/kg, but not 4-16 mg/kg, demonstrated a significantly lower sensitivity to MAP than the saline-experienced mice. On the other hand, the repeated MAP administration induced not only a reverse tolerance to itself, but also a cross reverse tolerance to 8 and 16 mg/kg of CBM. Furthermore, the established reverse tolerance to MAP was scarcely attenuated by the repeated treatment with any doses of CBM, but rather enhanced by 8 and 16 mg/kg of CBM. The present results indicate that, although the dose-effect relations are partially different, the behavioral characteristics of CBM were almost identical with those of bromocriptine, another long-acting ergot derivative having antagonistic and agonistic actions on dopamine D1 and D2 receptors, respectively.
been studied in a behavioral despair paradigm (5).

Hence, the purposes of this experiment were to investigate the behavioral effects of repeated administration of CBM and the interaction between CBM and methamphetamine by ambulatory activity in mice.

**Materials and Methods**

**Animals:** Experimental animals used were ddY mice (Japan Laboratory Animal Co.), which were purchased at 5 weeks of the age. Groups of 10 mice each were housed in aluminum cages of 30(W)×20(D)×10(H) cm with a wooden-flake floor mat (White Flake: Charles River Japan) and freely given solid diet (MF: Oriental Yeast Co.) and tap water until the start of the experiment. The breeding room had controlled illumination (light period: 6 a.m.–6 p.m.) and temperature (22±2°C). The experiment was started when mice were 7 weeks of the age and weighed 28–35 g.

**Apparatus:** The apparatus for measurement of the mouse’s ambulatory activity was a tilting-type ambulometer (AMB-10: O’Hara & Co.). The apparatus consisted of 10 bucket-like activity cages, each of which was made of plexiglass with a diameter of 20 cm. Thereby, the ambulatory activities of 10 mice could be measured individually at the same time.

**Drugs and administration schedules:** The drug used were CBM (Sandoz) and methamphetamine HCl (MAP: Dainippon Pharm. Co.). CBM was suspended in physiological saline vehicle with a very small amount (one drop/3 ml) of Tween-80. MAP was dissolved in the saline vehicle. The concentration of each drug solution was adjusted so that each volume injected was always constant at 0.1 ml/10 g.

**Repeated administration of CBM and cross-administration of MAP:** Six groups of mice were individually put into the activity cages; and after adaptation period of 30 min, CBM (0: physiological saline with Tween-80, 1, 2, 4, 8 or 18 mg/kg, i.p.) was administered to these mice, and then the ambulatory activity was additionally observed for 7 hr. The same treatment was repeated 9 times at intervals of 7 days. Seven days after the final (9th) administration, MAP (2 mg/kg, s.c.) was cross-administered to all of these mice, and their ambulatory activities were observed for 3 hr thereafter.

**Administration of CBM to the mice that showed reverse tolerance to MAP:** Five groups of mice were treated with repeated 5 times administration of MAP (2 mg/kg, s.c.) at intervals of 3–4 days, and their ambulatory activities were observed for 3 hr after each administration. Seven days after the final MAP administration, the mice were given one of the doses of CBM (0: physiological saline with Tween-80, 2, 4, 8 and 16 mg/kg, i.p.). The ambulatory activity of each mouse was observed for 7 hr thereafter. The same doses of CBM were additionally administered once a day for 4 days to the mice in their home cages. Seven days after the final (5th) CBM administration, all of these mice were again given MAP (2 mg/kg) in the activity cage to observe whether CBM was effective for modifying reverse tolerance of the mouse to MAP.

**Statistical analysis:** The mean overall ambulatory activity counts for 7 hr and 3 hr after administration of CBM and MAP, respectively, were compared using the paired t-test. When P values were equal to or less than 0.05, they were defined to be significantly different.

**Results**

Figures 2 and 3 show time-course changes and mean overall ambulatory activity counts,
respectively, for 7 hr after administration of CBM (0: saline with Tween-80, 1, 2, 4, 8 and 16 mg/kg, i.p.) to the drug-naive mice. CBM at 4 mg/kg significantly decreased the mean overall ambulatory activity. In contrast, 16 mg/kg of CBM significantly increased the activity with stereotyped behaviors such as sniffing, etc. The ambulation-increasing effect attained to the maximum level in 4 hr, and it persisted for longer than 7 hr after the administration.

Figure 4 shows the mean overall ambulatory activity counts for 7 hr after the repeated 9 times administration of CBM (0, 1, 2, 4, 8 and 16 mg/kg) at intervals of 7 days. The repeated administration of saline or CBM at 1 and 2 mg/kg elicited no significant alteration in the ambulatory activity throughout the repeated administration. However, the mice that received CBM at 4 and 8 mg/kg showed a significant increase in the activity counts during the repeated administration, although they demonstrated no significant increase but rather a decrease in the activity in the 1st administration. The ambulation-increasing effect of CBM at 16 mg/kg was also progressively enhanced after the repeated adminis-
Fig. 4. Mean overall ambulatory activity counts with S.E.M. for 7 hr after the repeated 9 times administration of m ergocriptine (CBM, 0: saline with Tween-80; 1, 2, 4, 8 and 16 mg/kg, i.p.) at intervals of 7 days to mice. Number of mice used in each experiment is shown in the parenthesis. *: Significantly different from the value in the 1st administration in each group (P<0.05).

Figure 5 shows the mean overall ambulatory activity counts for 3 hr after the cross-administration of MAP (2 mg/kg, s.c.) to the mice that experienced the repeated 9 times administration of CBM (0, 1, 2, 4, 8 or 16 mg/kg) and to the drug-naive but age-adjusted mice. The repeated administration of saline induced an increase in the sensitivity to MAP. The MAP sensitivities of mice that had experienced CBM at 1 and 2 mg/kg were significantly lower than that of the saline-treated control mice. In contrast, the mice that had experienced CBM at 16 mg/kg tended to show a higher sensitivity to MAP than the control mice.

MAP (2 mg/kg) increased the ambulatory activity of mice, and the effect was progressively enhanced by the repeated administration of the drug. The mean ambulatory activity counts in the 5th MAP administration was about 2 times as high as that in the 1st administration (data not shown).

Figure 6 shows the mean overall ambulatory activity counts for 7 hr after administration of CBM (0, 1, 2, 4, 8 and 16 mg/kg) to the mice that exhibited a reverse tolerance to MAP. The data of the drug-naive mice were the same as those presented in Fig. 3. The repeated administration of MAP elicited a slight, but significant increase in the activity after administration of saline. The sensitivity of CBM at 1 and 2 mg/kg was scarcely modified by MAP. On the other hand, MAP-treated mice demonstrated a marked enhancement in their sensitivity to the ambulation-
Fig. 5. Mean overall ambulatory activity counts with S.E.M. for 3 hr after administration of methamphetamine (2 mg/kg, s.c.) to the drug-naive mice and the mice that experienced the repeated 9 times administration of mergocriptine (CBM, 0: saline with Tween-80; 1, 2, 4, 8 or 16 mg/kg, i.p.). Methamphetamine was administered 7 days after the final (9th) administration of CBM. The drug-naive mice (N=16) were age-matched to the CBM-experienced mice. Numbers of the CBM-experienced mice were the same as those shown in Fig. 5. * and #: Significantly different from the saline-treated mice and the drug-naive mice, respectively (P<0.05).

Fig. 6. Mean overall ambulatory activity counts with S.E.M. after administration of mergocriptine (CBM, 0: saline with Tween-80; 2, 4, 8 and 16 mg/kg, i.p.) to the mice that had experienced 5 times administration of methamphetamine (2 mg/kg, s.c.) at intervals of 3-4 days. CBM was administered 4 days after the final methamphetamine administration. The mean activity counts in the drug-naive mice are the same as those shown in Fig. 3. Numbers of the mice that experienced the repeated methamphetamine (2 mg/kg) administration were as follows: saline group (16); CBM: 2 mg/kg group (N=15), 4 mg/kg group (N=19), 8 mg/kg group (N=18) and 16 mg/kg group (N=20). *: Significantly different as compared with the corresponding activity counts of the drug-naive mice after the administration of the same dose of CBM (P<0.05).
increasing effect of CBM at 8 and 16 mg/kg. Figure 7 shows the mean overall ambulatory activity counts for 3 hr after administration of MAP (2 mg/kg) to the mice that received the repeated 5 times administration of CBM (0, 2, 4, 8 and 16 mg/kg, i.p.) after the establishment of the reverse tolerance to MAP. The repeated administration of CBM at 8 and 16 mg/kg, but not of saline or CBM at 2 and 4 mg/kg, elicited a further enhancement in the sensitivity to MAP.

Discussion

The present experiment demonstrated that CBM shows both depressant and stimulant effects depending on the doses administered. Thus, 4 mg/kg and 16 mg/kg of CBM decreased and increased, respectively, the ambulatory activity of the drug-naive mouse. A similar profile of the dose-effect relationship has been reported after administration of apomorphine (6, 7), and bromocriptine (4, 6). The suppressive effect of low doses of CBM, as well as apomorphine and bromocriptine, might be due to an agonistic action on presynaptic dopamine autoreceptors (1-3), which results in an inhibition of synthesis and release of dopamine. In contrast, the ambulation-increasing effect of the comparatively higher doses of CBM should be due to a direct stimulation of the postsynaptic dopamine receptors, similar to the behavioral properties of apomorphine (8-10). Thus, the turning point of the depressant and stimulant effects of CBM is considered to be around 8 mg/kg in the drug-naive mice. Furthermore, the ambulation-increasing effect of 16 mg/kg of CBM persisted for longer than 7 hr. Such a long-acting property is almost identical with that of bromocriptine (4, 7).

When CBM was repeatedly administered, the dose-effect relationship was different from that in the drug-naive mice. Thus, up to 4 mg/kg of CBM was effective for increasing the mouse’s ambulatory activity. Furthermore, the ambulation-increasing effect of 16 mg/kg of CBM was enhanced when it was repeatedly administered, i.e., eliciting a reverse tolerance.
to the ambulation-increasing effect. However, it is unlikely that such a change in the drug effects can be attributable to an accumulation of CBM after the repeated administration. The half-life of CBM was estimated to be 51 hr (p.o. administration) and 58 hr (i.v. administration) in rats (11). The administration intervals of 7 days might be sufficient to eliminate the previously-administered drug. A similar alteration of the effects has also been demonstrated after the repeated administration of bromocriptine (4) and apomorphine (7, 12).

We have reported that the repeated administration of comparatively higher doses of apomorphine (12) and bromocriptine (4) produced an increase in the sensitivity to MAP, i.e., an induction of cross reverse tolerance. However, the present experiment also demonstrated an increased sensitivity to MAP in the saline-treated mice. It has been demonstrated that there is no significant change in the methamphetamine sensitivity when mice were treated with 5 times repetition of 6 hr exposure in the activity cage after saline administration (4) and 10 times repetition of 3 hr exposure in the activity cage after saline administration (13). These results suggest that repetition of the saline injection is effective for enhancing the sensitivity to MAP when each injection is followed by a long-lasting exposure of the mouse in the activity cage. In this respect, it is notable that the repeated CBM, at 1 and 2 mg/kg, protected against such an enhancement of the sensitivity to MAP. In contrast, the repeated administration of CBM an 16 mg/kg elicited a further enhancement of the MAP sensitivity. The dose-effect relationship of the cross interaction between CBM and MAP was qualitatively similar to that between bromocriptine and MAP (4). The repeated administration of MAP was effective for enhancing the sensitivity to higher doses of CBM as well as those of bromocriptine (4). It has been demonstrated that CBM and bromocriptine act differently on the dopamine D1 receptor (1–3) and the cholinergic and serotonergic systems (1), although these two drugs showed agonistic action on dopamine D2 receptors. However, the present results indicate that in terms of the mouse's ambulatory activity, the behavioral effects of CBM and bromocriptine are very similar. Thus, it is considered that the effects of CBM and bromocriptine on the mouse's ambulatory activity appear mainly through dopamine D2 receptors.

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