Methamphetamine-Induced Behavioral Alterations Following Repeated Administration of Methamphetamine

Yasumitsu YAMANAKA, Ritsuko TAKANO and Toru EGASHIRA
Department of Pharmacology, Medical College of Oita, Hazama-cho, Oita 879-56, Japan
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Abstract—Repeated administration of a large dose of methamphetamine (MA) (25 mg/kg, i.p. twice daily for 4 days) to mice enhanced locomotor activity and decreased stereotyped behavior following a subsequent injection of MA. Simultaneous determinations of catecholamines revealed a depletion of brain dopamine. The moderate doses of haloperidol significantly enhanced MA-induced locomotor activity in mice. A significant enhancement of MA-induced locomotor activity was observed in the rats pretreated with 6-hydroxydopamine into the striatum, and this effect correlated negatively with the striatal dopamine level. These results suggest that hypofunction of striatal dopaminergic neuron systems induced by repeated administration of MA may be one of possible mechanisms of the enhancement of MA-induced locomotor activity due to the decrease of stereotyped behavior.

Repeated administration of amphetamines has been shown to alter sensitivity to subsequent doses of amphetamines. These alterations produced by repeated administration of amphetamines, however, depend to a large extent on the frequency of administration, the dose, route of administration and the behavior in question.

Because amphetamine psychosis is most frequently observed in addicts who have consumed enormous amounts of the drug over prolonged periods (1), development of this psychosis may require a more sustained level of amphetamine intoxication. In this regard, although many methods have been reported to sustain the level of the intoxication, a dosage schedule of 25 mg/kg of methamphetamine (MA) twice daily for 4 days to the mouse was chosen, which may be selectively neurotoxic on dopamine terminals in the rat striatum, as suggested by Wagner et al. (2).

Behavioral effects and dopaminergic mechanisms of amphetamines in the mouse have been demonstrated to be similar to those in the rat (3, 4). Therefore, we herein report the effects of this dosage schedule of MA on the sensitivity to the behavioral effects of subsequent doses of MA in the mouse and the effects of several neuropharmacological drugs on MA-induced behavioral effects in the mouse or rat.

Materials and Methods

Animals: Male ddY strain mice weighing initially approx. 20 g and male Wistar strain rats weighing initially approx. 180 g were used.

Drugs: Methamphetamine hydrochloride (MA) was obtained from Dainippon Seiyaku Co. 6-Hydroxydopamine hydrochloride (6OHDA) was purchased from Sigma Chemical Co. Haloperidol (Serenase Injection) and levomepromazine hydrochloride (Levotomin Injection) were obtained from Dainippon Seiyaku Co. and Yoshitomi Seiyaku Co., respectively.

Repeated administration of MA: Mice were pretreated with MA (25 mg/kg, i.p.) or saline twice daily at 9:00 and 17:00 for 4 consecutive days.

Behavioral rating: The behavior of each mouse was rated by the method of Peachey et al. (3) over a 4 hr period following the
i.p. injections of the challenge doses of 2.5, 5 and 10 mg/kg of MA 18 hr after the last injection. Mice were placed in individual activity cages 1 hr before MA treatment. The following 2 behavioral parameters were quantitated: activity (locomotor activity in any direction) and stereotyped behavior (gnawing, licking or sniffing). Ten ratings were made over a 90-sec period at exactly 10-sec intervals. Each rating consisted of noting the presence or absence of each type of behavior using a score of 1 or 0, respectively. The maximum possible rating score for each parameter was 10.

**Measurement of activity:** The mouse was individually placed in an activity cage, and activity was recorded for 5 min beginning 1 min after placing the animal in the activity cage each hour after drug treatment using an Automex II 2SD.

**Catecholamine assay:** Brain NE and DA concentrations were determined fluorometrically after high performance liquid chromatography. The detailed procedures were described in the previous paper (5).

**Single administration of haloperidol or levomepromazine:** At 30 min after administration of 0.05, 0.1 and 0.2 mg/kg of haloperidol or 0.2, 0.5 and 1 mg/kg of levomepromazine to drug naive mice, the animals were given a challenge dose of 10 mg/kg of MA, and activity was recorded over 3 hr.

**Repeated administration of haloperidol or levomepromazine:** Drug naive mice were given 1 mg/kg of haloperidol or 5 mg/kg of levomepromazine twice daily (9:00 and 17:00) for 6 consecutive days. After 2 day-drug withdrawal, the mice were given the challenge doses of 2.5 and 10 mg/kg of MA, and activity was recorded over 4 hr.

**Striatal injection of 6OHDA in the rat:** Under pentobarbital anesthesia (40 mg/kg, i.p.), each rat was positioned in a stereotaxic apparatus 30 min after pretreatment of 50 mg/kg of pargyline, and 8 μg of 6OHDA in 2 μl vehicle was infused bilaterally into the region of the corpus striatum over a 1-min period in each region. Sham-operated rats were injected similarly with vehicle. The stereotaxic coordinates were A: 8.0, L: 2.5 and V: +1.2, according to the atlas of Pellegrino (6). After 3 weeks, the rats were injected with the challenge doses of 2.5 and 10 mg/kg of MA, and activity was recorded. The striatal DA levels were then determined 1 week after the MA injection.

**Results**

**Changes in MA sensitivity following repeated administration of MA:** Figure 1 shows the time course of behavioral effects of MA in saline control mice and MA-treated mice. The effects of MA in the saline control mice were almost identical to those described in the previous paper (5). Briefly, 2.5 mg/kg of MA markedly enhanced locomotor activity over 2 hr, while stereotyped behavior was slightly observed. The behavioral effect of 5.0 mg/kg of MA was of the mixed type consisting of increased locomotor activity and stereotyped behavior. At 10 mg/kg of MA, locomotor activity markedly increased at the first 15 min, gradually diminished till 1 hr, and then increased from 2 hr. Stereotyped behavior reached a maximum at 30 min, lasted for 2 hr and then gradually subsided. Accordingly, the characteristic behavioral effects of MA were enhanced locomotor activity at 2.5 mg/kg of MA and stereotyped behavior at 10 mg/kg of MA.

The time course changes in the behaviors after administration of subsequent doses of 2.5 and 5 mg/kg of MA were similar between MA-treated and saline control mice, while the rating scores of locomotor activity in the MA-treated mice tended to decline earlier than those in the saline control mice. In the case of 10 mg/kg of MA, however, a characteristic change in the behavior was observed in the MA-treated mice. Locomotor activity increased at the first 15 min with the peak points of 5.6±1.7, diminished to 2.9±1.8 points at 30 min and then gradually increased, reaching 8.9±0.7 points at 2 hr. At that period, the rating score of locomotor activity in the saline control mice was still 0 points. The rating score of stereotyped behavior in the MA-treated mice reached a maximum of 8.6±1.0 points at 30 min and gradually decreased to 2.0±1.5 at 2 hr. During this period, the maximum points of stereotyped behavior in the saline control mice
mice was still observed. Accordingly, the
marked increase in locomotor activity at 2 hr
in the MA-treated mice seemed to be due to
early subsidence of stereotyped behavior.

Figure 2 shows the activity counts
measured by an Automex II 2SD. At 2.5 mg/

Fig. 1. MA-induced behavioral effects in mice following repeated administration of MA. ●● saline control mice, ○○ MA-treated mice. Mice were placed in individual cages, and MA-induced behaviors were scored as described in Methods. Each point represents the mean±S.E. of 7 animals. MA was given in a dose of 25 mg/kg twice daily for 4 consecutive days. Subsequent doses of MA were 2.5, 5 and 10 mg/kg.

Fig. 2. MA-induced activity in mice following repeated administration of MA. △△ saline to naive mice, ●● MA to saline control mice, ○○ MA to MA-treated mice. A mouse was placed in an activity cage, and activity was recorded using an Automex II 2SD as described in Methods. Each point represents the mean±S.E. of 8 animals. MA was given in a dose of 25 mg/kg twice daily for 4 consecutive days. Subsequent doses of MA were 2.5, 5 and 10 mg/kg. *Significantly different from the control at P<0.01.
kg of MA, activity in the saline control mice was enhanced at 1 and 2 hr and returned to normal at 3 hr. This time course is almost identical to the results estimated by the rating system. At 5 and 10 mg/kg of MA, however, the activity counts did not always coincide with the results estimated by the rating system, i.e., activity counts were inconsistently recorded even when scores of locomotor activity and stereotyped behavior was 0 and 10 points, respectively. These counts, however, were not significantly different from the counts at zero time. Therefore, activity counts seemed to reflect mainly locomotor activity. Following repeated administration of MA, activity was markedly enhanced at any challenge dose of MA. Significant differences in activity between the MA-treated group and the saline control group were demonstrated at 1 and 2 hr in doses of 5 and 10 mg/kg of MA (P<0.01), although there was no significant difference at 2.5 mg/kg of MA.

Effects of MA on NE and DA levels following repeated administration of MA: The time course of the steady-state levels of NE and DA over 3 hr is shown in Table 1. Initial levels of NE were identical between the saline control mice and the MA-treated mice. There was no significant difference in the time course changes in the NE level between the saline control mice and the MA-treated mice, although 5 and 10 mg/kg of MA tended to deplete NE in both groups. The initial level of DA in the MA-treated mice was significantly lower than that in the saline control mice. The time course of the DA levels in the MA-treated mice after injection of 2.5, 5 and 10 mg/kg of MA was not significantly different from that in the saline control mice.

Effects of single administration of haloperidol or levomepromazine on MA-induced activity: Activity induced by 10 mg/kg of MA was recorded over 3 hr in the mice pretreated with haloperidol or levomepromazine. The results are shown in Fig. 3. When haloperidol was given to mice in doses of 0.05 and 0.1 mg/kg, MA-induced activity was significantly enhanced as compared with that in the saline control mice. Haloperidol in a dose of 0.2 mg/kg, however, did not enhance MA-induced activity. When levomepromazine in doses of 0.5 and 1 mg/kg was given to mice, the early stage of increase in MA-induced activity at 30 min was inhibited.

Effects of repeated administration of haloperidol or levomepromazine on MA-induced activity: When mice were repeatedly given haloperidol, an enhancement of activity induced by 2.5 mg/kg of MA was observed, and locomotor activity induced by 10 mg/kg of MA was decreased because of possible enhanced stereotypy. Repeated administration of levomepromazine, however, did not alter activity induced by 2.5 or 10 mg/kg of MA (Fig. 4).

Effects of striatal injections of 6OHDA on MA-induced activity: Eight μg of 6OHDA
Table 1. Effects of MA on the levels of NE and DA in mouse brains following repeated administration of MA

|                   | NE level |          |          |          |          |          |
|-------------------|----------|----------|----------|----------|----------|----------|
|                   |          | 0 min    | 15 min   | 30 min   | 1 hr     | 2 hr     | 3 hr     |
|                   | MA       |          |          |          |          |          |
| 2.5 mg/kg         | 0.426±0.057 | 0.445±0.037 | 0.450±0.039 | 0.440±0.030 | 0.412±0.028 |
| Control           | 0.434±0.018 | 0.368±0.018 | 0.458±0.015 | 0.415±0.037 | 0.423±0.022 | 0.359±0.028 |
| 5 mg/kg           | 0.464±0.048 | 0.467±0.035 | 0.443±0.019 | 0.408±0.030 | 0.370±0.026 |
| 10 mg/kg          | 0.478±0.053 | 0.420±0.043 | 0.391±0.024 | 0.365±0.039 | 0.360±0.045 |
| MA-treated        | 0.433±0.019 | 0.454±0.021 | 0.457±0.017 | 0.440±0.014 | 0.412±0.015 | 0.393±0.027 |
| 5 mg/kg           | 0.516±0.037 | 0.444±0.026 | 0.404±0.045 | 0.358±0.019 | 0.319±0.019 |
| 10 mg/kg          |          |          |          |          |          |          |

|                   | DA level |          |          |          |          |          |
|-------------------|----------|----------|----------|----------|----------|----------|
|                   |          | 0 min    | 15 min   | 30 min   | 1 hr     | 2 hr     | 3 hr     |
|                   | MA       |          |          |          |          |          |
| 2.5 mg/kg         | 1.193±0.149 | 1.259±0.135 | 1.303±0.077 | 1.344±0.081 | 1.206±0.086 |
| Control           | 1.126±0.047 | 1.028±0.076 | 1.287±0.092 | 1.210±0.116 | 1.286±0.091 | 1.139±0.069 |
| 5 mg/kg           | 1.286±0.089 | 1.322±0.105 | 1.312±0.074 | 1.367±0.086 | 1.284±0.094 |
| 10 mg/kg          | 0.918±0.082 | 0.980±0.162 | 0.876±0.050 | 0.758±0.073 | 0.789±0.092 |
| MA-treated        | 0.862±0.054* | 1.003±0.050 | 1.010±0.160 | 0.917±0.068 | 0.881±0.075 | 0.878±0.058 |
| 5 mg/kg           | 0.995±0.104 | 0.920±0.095 | 0.829±0.058 | 0.850±0.069 | 0.837±0.080 |
| 10 mg/kg          |          |          |          |          |          |          |

Values are expressed as µg/g brain tissue. Each value represents the mean±S.E. of 6 determinations except for values at 0 min. Values at 0 min represent the mean±S.E. of 18 determinations. *Significantly different from the control at P<0.01. MA was given in a dose of 25 mg/kg twice daily for 4 consecutive days. Subsequent doses of MA were 2.5, 5 and 10 mg/kg.
injected bilaterally into the striatum of the rats resulted in approx. 50% depletion of endogenous DA; i.e., striatal DA levels were 3.52±0.21 μg/g in the vehicle control rats and 1.84±0.27 μg/g in the 6OHDA-treated rats. Locomotor activity induced by 2.5 mg/kg of MA in the 6OHDA-treated rats was similar to that in the vehicle control rats. However, locomotor activity induced by 10 mg/kg of MA in the 6OHDA-treated rats was significantly enhanced at 30 min as compared with that in the vehicle control rats (P<0.01), as shown in Fig. 5. Regression analysis revealed the negative correlation between striatal DA levels and counts of activity induced by 10 mg/kg of MA in the 6OHDA-treated rats (Fig. 6).

Discussion

The present results demonstrated that
repeated administration of a large dose of MA (25 mg/kg, i.p., twice daily for 4 days) to mice enhanced the early stage of locomotor activity and decreased the stereotyped behavior following a subsequent injection of MA. Simultaneous determinations of catecholamines revealed a depletion of brain dopamine, as reported by Wagner et al. (2) in the rat.

Stimulation of locomotor activity and stereotyped behavior by amphetamine is proposed to be associated with dopaminergic nerve activity of the nucleus accumbens and the striatum, respectively (7). Several authors have reported that repeated administration of moderate amounts of amphetamine or MA produced enhancement of behavioral effects induced by these drugs following the subsequent administration, i.e., reverse tolerance (8–12). However, it is suggested that this effect is not mediated by alteration in the sensitivity of pre- or post-synaptic dopaminergic receptors in the striatum (13).

On the other hand, an interesting report has appeared (9). Rats administered amphetamine in a continuous regime using silicone pellets were hyperactive when initially tested with amphetamine and subsensitive to apomorphine. Following repeated daily injections of an amount of amphetamine equivalent to that released by a pellet, however, rats showed enhanced motor stereotypies when injected with amphetamine. It is also demonstrated that continuous treatment with amphetamine can induce long-lasting structural and biochemical alterations in dopaminergic terminals in the caudate nucleus (14).

Under the present experimental conditions, we demonstrated the production of dopamine depletion in whole mouse brains, and Wagner et al. (2) demonstrated the production of long-term decreases in dopamine levels and decrease in the number of dopamine uptake sites in the rat striatum. From these observations, we hypothesized that hypothesis of striatal dopaminergic neuron systems might produce the enhanced locomotor activity and decreased stereotyped behavior observed in the present experiment. We used two neuroleptics, haloperidol and levomepromazine; the former is a potent amphetamine antagonist and the latter has a potent sedative effect and mild amphetamine antagonism (15). The moderate doses of haloperidol significantly enhanced locomotor activity induced by 10 mg/kg of MA, possibly because stereotypy may be effectively inhibited. A high dose did not enhance locomotor activity, possibly because most of the dopaminergic receptors were inhibited. Chronic neuroleptic treatment increased sensitivity to dopaminergic agents (16–18). Moreover, single neuroleptic treatment has been reported to increase postsynaptic sensitivity (19). After repeated administration of haloperidol to mice, locomotor activity induced by 2.5 mg/kg of MA was increased, and locomotor activity induced by 10 mg/kg of MA was decreased because of possible enhanced stereotypy. When eight µg of 6OHDA was injected bilaterally into the striatum of the rat, locomotor activity induced by 10 mg/kg of MA was significantly enhanced, and this effect correlated negatively with striatal DA levels.

These results may indicate that locomotor activity was enhanced if dopaminergic neuron systems in the nucleus accumbens were intact and those in the striatum were affected either pre- or post-synaptically, because enhanced locomotor activity is masked by predominant stereotypy. Although we demonstrated the depletion of dopamine only in whole mouse brains following repeated administration of MA, Morgan and Gibb (20) demonstrated that tyrosine hydroxylase activity and dopamine levels were significantly decreased in the neostriatum but not altered in the nucleus accumbens after 15 mg/kg of MA were administered to the rat every 6 hr for 5 doses. Lucot et al. (21) reported that treatment of rats with 100 mg/kg/day of MA for 4 days produced long-lasting depletion of brain levels of dopamine. They also reported that this treatment attenuated the ability of MA and apomorphine to produce increases in locomotor activity, but the mechanisms for this were obscure. One of the possible mechanisms may be the depletion of caudate dopamine and the rest of brain dopamine, as they reported.
In conclusion, our results suggest that hypofunction of striatal dopaminergic neuron systems induced by repeated administration of MA may be one of the possible mechanisms for the enhancement of MA-induced locomotor activity due to the decrease in stereotyped behavior.

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