ENHANCEMENT OF MOTOR-ACCELERATING EFFECT INDUCED BY REPEATED ADMINISTRATION OF METHAMPHETAMINE IN MICE: INVOLVEMENT OF ENVIRONMENTAL FACTORS

Mohammed Rabiul ALAM
Behavior Research Institute, School of Medicine, Gunma University, 3-39-22 Showa-machi, Maebashi 371, Japan
Accepted June 11, 1981

Abstract—Adult male mice were given 2.0 mg/kg of methamphetamine hydrochloride (MAM) for 6 times at 3–4 day intervals. The acute effects of MAM on locomotor activity of each mouse were investigated for 180 min after each administration. The motor-accelerating effect of MAM was progressively enhanced in parallel to the number of administrations. However, the enhancement of effect was not observed after repeated administration of MAM, when the mice were placed individually in narrow jars, which thereby perfectly inhibited ambulation during 180 min following each administration. The other mice were trained under the discriminated shuttle-type avoidance schedule (intertrial interval = 25 sec, CS presentation = 5 sec and 1 session = 1 hr). A slight influence of training on locomotor activity was detected after 2.0 mg/kg of MAM in the activity cage. A marked enhancement of the motor-accelerating effect was detected in the avoidance-trained mice, when 2.0 mg/kg of MAM was given for 5 times at 3–4 day intervals in the avoidance situation. These results suggest that the conditioned drug effect in association with environmental factors plays an important role in the production of enhancement.

Recent investigations in our laboratory demonstrated that the motor-accelerating effects of d-amphetamine, methamphetamine, cocaine and morphine were progressively enhanced when the drugs were given to the mice at certain intervals (1–5). Furthermore, marked enhancement of the effects was observed only when the mice were placed repeatedly in the activity cages where they were able to move freely during the stage of acute effects of the drugs (6–8).

In the shuttle-type avoidance situation, the animal is forced to move from one side to the other in order to avoid an electric foot shock, even without the drug effects. Furthermore, the properties of the movement in the shuttle-type avoidance situation are clearly different from those in the activity cage. It is meaningful to study the effect of drugs given repeatedly, not only on the avoidance response, but also on the motor-acceleration in an activity cage after the drug has been given in the shuttle-type avoidance situation. The purpose of the present work was to investigate the involvement of environmental factors, in which the animals experienced drug effects, and to assess the development of the enhancing effect on motor activity produced by repeated administration of methamphetamine.
MATERIALS AND METHODS

Experimental animals: Adult male ddN strain mice were obtained from the breeding colony of Gunma University Medical School and they were moved to the breeding room of our laboratory immediately after weaning at three weeks of age. Groups of 6–8 mice were housed in aluminum cages of 32(W) × 25(D) × 10(H) cm with a sawdust floor, and were given a solid diet of MF (Oriental Yeast Co., Tokyo) and tap water ad libitum, except during the time of the experiment. The experiments were initiated when the mice were 10 weeks of age weighed 30–35 g.

Apparatus: The motor activity of a mouse was measured as an ambulation by a basin-type apparatus, a kind of round tilting cage which was devised by Tadokoro and Takano (9). The principle and properties for the measurement were published in detail by Hirabayashi et al. (10). Sixteen activity cages of the same type with a field 25 cm in diameter and 13 cm in height were used simultaneously.

For the pretreatment with methamphetamine, glass jars 5 cm in diameter and a two-way shuttle-type avoidance chamber were used. The shuttle-type avoidance chamber was made of acrylfiber and aluminum boards of 50(W) × 16(D) × 18(H) cm, as shown in Fig. 1. The floor grid consisted of stainless steel rods which were electro-wired to pass an electric current of 100 V, 0.2 mA and 50 Hz AC to give a foot shock. Three photo-cells were set on a side wall in order to control and record movements. The height of the two outer photo-beams was 2.5 cm, and the central one was 5 cm over the floor. A hurdle 2 cm high was set in the center of the floor. The ceiling was equipped with a pilot lamp and a small speaker to give conditioned stimuli (CS). The avoidance schedule consisted of intertrial interval (ITI) = 25 sec, and CS presentation period = 5 sec. When the mouse cut the central beam and then the lateral beam of the opposite side (response) during the CS presentation, the electric foot shock for one trial could be avoided. The maximum duration of the shock delivered was 10 sec, so an escape contingency was considered in the schedule programming. The responses during ITI were not effective for the mice to avoid the shocks. The training session consisted of 1 hr per day and was held every other day.

Procedure and the experimental conditions: The drug used was methamphetamine hydrochloride (MAM, Philopon, Dainippon Pharmaceutical Co.). The test dose given was fixed at 2.0 mg/kg and was administered in the experimental schedules shown in Table 1. The dose was expressed in the salt form. Room temperature was maintained at 22±2°C throughout the experiment, but the humidity

Fig. 1. Schematic diagram of a two-way shuttle-type avoidance chamber for the measurement of running responses in mice.
was not controlled.

In the 1st experiment, the motor activities of 43 naive mice were measured after 6 successive administrations of MAM. The mice were placed individually in the activity cages, and MAM 2.0 mg/kg was given s.c. after an adaptation period of 30 min. Then, the ambulatory counts in each mouse were recorded at 10 min intervals for 180 min. This treatment was repeated for 6 times at 3–4 day intervals.

In the 2nd experiment, 44 naive mice were randomly divided into two subgroups of 21 and 23 each, and were given MAM 2.0 mg/kg and physiological saline vehicle s.c., respectively, and then were placed individually for 180 min in glass jars covered with wire net to avoid increases in the temperature. The ambulation of the animals could be thus perfectly inhibited. The same treatment was repeated 5 times at 3–4 day intervals.

In the 3rd experiment, 26 mice were trained to avoid an electric shock under the discriminated shuttle-type avoidance schedule for 20 sessions. Then, these mice were given MAM 2.0 mg/kg s.c. and the motor activities in the activity cages were measured for 180 min in order to investigate the influence of training. In the next step, the animals were divided into 2 subgroups of 14 and 12, and MAM 2.0 mg/kg and saline vehicle s.c., respectively, were given 5 successive times at 3–4 day intervals in the shuttle-type avoidance situation. Effects of MAM on the avoidance response were observed for 90 min after each administration. On the 6th administration following 5 of these pretreatments in the shuttle-type avoidance situation, all mice were given again 2.0 mg/kg s.c. of MAM and then the motor activities in the activity cages were measured for 180 min. All the experiments were performed from 10:00 to 16:00 under conditions of lighting of over 100 Lux.

**Statistical evaluation:** Significant differences were statistically estimated by the Student’s *t*-test, and the P values required were less than 0.05.

**RESULTS**

The motor activity was markedly accelerated after MAM administration, showing a parabola-like pattern with a peak at 40–50 min. This effect was progressively enhanced according to the number of repetitions. The peak values were significantly elevated and duration of effects was prolonged after the 2nd administration, when compared with that in the 1st administration. Figure 2 represents the temporal changes in the mean counts of motor activity on the same coordinate after repeated administration of

<table>
<thead>
<tr>
<th>Situations of pretreatments</th>
<th>Doses of methamphetamine</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pretreatment</td>
<td>2.0 mg/kg × 6</td>
<td>43</td>
</tr>
<tr>
<td>Placement in glass jars*</td>
<td>2.0 mg/kg × 6</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Saline × 6</td>
<td>23</td>
</tr>
<tr>
<td>Shuttle-type avoidance situation*</td>
<td>2.0 mg/kg × 5</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Saline × 5</td>
<td>12</td>
</tr>
</tbody>
</table>

*Locomotor activities of all mice were measured after 2.0 mg/kg of MAM (test dose) in the final administration.
MAM 2.0 mg/kg s.c. at 3–4 day intervals. The figures given near each curve denote the ordinal numbers of administration.

In contrast, the pattern was completely different after pretreatment with MAM in the glass jars. Figure 3 represents the temporal changes in the motor activities after MAM 2.0 mg/kg s.c. in the mice pretreated with MAM 2.0 mg/kg and saline s.c., 5 times at 3–4 day intervals in glass jars where repeated rearing was observed and the ambulation was completely inhibited. No marked difference was observed between MAM- and saline-pretreated groups on the motor-accelerating effect at the peak time. However, the activity counts were significantly higher at 10 min and lower at 140–160 min after MAM in the MAM-pretreated group, as compared to the saline-pretreated group. The pattern of motor-accelerating effect was similar to that of the first administration, as shown in Fig. 2, even though the drug was given for 6 times.

Figure 4 represents the acquisition process of discriminated shuttle-type avoidance response in mice. The abscissa denotes the number of training sessions and the ordinate the mean avoidance and response rates. The avoidance conditioning was usually established within 10 sessions of the training and thereafter the stable base-line was maintained and showed an average response and avoidance rates of 2.6/min and 95%, respectively.

Figure 5 represents the comparison of the motor-accelerating effects of MAM between before- and after-training of the shuttle-type avoidance response. Several points of values at the beginning and terminal period of the measurement were higher in the after-training group than those in the before-
training one. These differences may be produced due to repeated handling by an experimenter (11, 12). However, the general patterns of the motor-accelerating effect between the two groups were evidently similar, and no marked difference was observed at the peak time.

There was no significant change in the avoidance rate, but the shuttle movement (response) was markedly accelerated after MAM administration in the shuttle-type avoidance situation. Figure 6 represents the temporal changes in the response rate after repeated administration of MAM 2.0 mg/kg. The response-increasing effect of MAM was not always enhanced by repeated administration. The data after repeated administration of saline was not presented in this

Fig. 4. Acquisition process of discriminated shuttle-type avoidance performance as expressed by means of the avoidance and response rates, with standard error. Each point represents mean counts per 1 hr session.

Fig. 5. Comparison of the motor-accelerating effects of MAM 2.0 mg/kg s.c. between before- and after-training of the shuttle-type avoidance response. Each point represents the mean counts per 10 min with standard error.

Fig. 6. Temporal changes in mean response rates after repeated administration of 2.0 mg/kg of MAM in the shuttle-type avoidance situation, 5 times at 3–4 day intervals. The figures given at each curve denote the ordinal numbers of administration. — ○ —— represents a single administration of saline.
Fig. 7. The mean patterns of locomotor activity after 2.0 mg/kg of MAM in mice which were pretreated with 2.0 mg/kg of MAM and saline in the shuttle-type avoidance situation, 5 times at 3–4 day intervals. — Closed circles indicate significant differences from the corresponding saline-pretreated group.

**DISCUSSION**

In the present experiments, the motor-accelerating effect of MAM was progressively enhanced in parallel to the number of administrations, when the drug was given repeatedly in an activity cage at 3–4 day intervals. Hirabayashi et al. (13) in our laboratory reported that the test dose of MAM 2.0 mg/kg was considered as the optimum dose to observe the motor-accelerating effect, without eliciting stereotyped behaviors. The 3–4 day intervals of repetition was also suitable to produce enhancement of the effect (7, 8). However, the enhancement of the effect was completely prevented when the mice were repeatedly placed in glass jars and their ambulation was inhibited while the drug effect appeared. According to Mesaki et al. (6), a wide round space at least 16 cm in diameter where the mice were able to move freely, was required to produce the enhancement. These results suggest that a kind of conditioning, in which the drug effect is connected with environmental factors, plays an important role in production of the enhancement. Similar results of conditioning drug effects have been stated by different groups of researchers (14–19). Hayashi et al. (14) also reported that the enhancement of effect on motor activity of mice was elicited by a small dose (0.25 mg/kg) of d-amphetamine associated with the presentation of a flickering light as a conditioned stimulus.

Although the response-increasing effect was not enhanced after the repeated administration of MAM in the shuttle-type avoidance situation, the ambulatory activities in the activity cage after MAM were significantly higher in mice pretreated with MAM than in the mice pretreated with saline in the shuttle-type avoidance situation. These results suggest that the ambulation of the mouse during the period of acute drug effect in both activity cage and shuttle-type avoidance situations is one of the most important factor in the production of the effect enhancement, though the properties of the ambulation are...
different, e.g. spontaneous and forced, respectively. However, whether the mechanism of the enhancing effect can be explained only by conditioning is open to question.

Neurochemical changes in active substances or irreversible alterations of receptor sensitivities in the brain have often been emphasized. It is considered that the effects of amphetamine derivatives are induced through stimulation of release and inhibition of reuptake of norepinephrine and dopamine at synaptic sites in the brain (20–25). Many investigators reported a decrease in catecholamine content in the brain after repeated administration of d-amphetamine or methamphetamine to rats or mice (20, 24). But the doses of the drugs in general were very large e.g. over 10 mg/kg, when compared with 2.0 mg/kg used in the present experiment. The stereotyped behavior tended to be more prominent than the motor-acceleration when the dose of amphetamines was increased (13). Furthermore, this behavior showed a competitive relation to the motor activity (13, 18). Smith (20) reported that a small dose (3.0 mg/kg) of d-amphetamine increased the dopamine level, while a large dose (10–100 mg/kg) decreased both dopamine and norepinephrine, and increased serotonin in the brain of mice. Short and Shuster (21) noted a marked decrease in norepinephrine levels in the brain when 10 mg/kg of d-amphetamine was given, but norepinephrine levels fluctuated markedly when 5.0 mg/kg of d-amphetamine was given to mice which were sensitized by repeated administration. Other investigators (25–30) emphasized the possible role of supersensitivity of dopamine receptors in the enhancement of effects produced by repeated administration of d-amphetamine.

Data acquired in our laboratory showed that the enhancement of the effect on motor-acceleration and the attenuation of conditioned suppression were observed not only after repeated administration of drugs which were related to dopamine agonists or antagonists, but also could be seen after morphine (31) or diazepam (32). It is also difficult to explain the marked influence of environmental factors on the enhancement phenomenon only on the basis of neurochemical changes in the brain.

Investigations on behavior performed in association with neurochemical observation are required.

Acknowledgements: I am grateful to Prof. S. Tadokoro for guidance throughout this work and to Ms. M. Hirabayashi, T. Mesaki and M. Iizuka for pertinent advice.

REFERENCES

8) Tadokoro, S., Hirabayashi, M. and Iizuka, M.: Pharmacological properties of enhanced effect on ambulatory activity produced by repeated
administrations of central stimulants in mice. Neuroscience 6, Supp. 34P (1978)


