Reverse Tolerance to the Swimming Time Prolonging Effect of d-Amphetamine in Mice

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Accepted January 31, 1986

Abstract—Development and disappearance of reverse tolerance to the swimming time prolonging effect of d-amphetamine (AMP) was studied in mice in comparison with that to the ambulation accelerating effect. The swimming time prolonging effect was progressively enhanced by daily administration of 2 mg/kg AMP. The development of reverse tolerance to the effect was more rapid than that to the ambulation accelerating effect and reached its maximal level by 5–6 repetitions. Repetition at a daily interval was more effective than at the interval of 3–4 days, and administration at a weekly interval failed to develop the reverse tolerance. Restriction of swimming space or immobilization in a small box after administration of AMP blocked the development of reverse tolerance. Reverse tolerance to the swimming time prolonging effect disappeared faster than that to the ambulation accelerating effect, but the enhancement was well maintained after 30 days of withdrawal. Thus, many factors affect the development of reverse tolerance to the various effects of AMP; however, the swimming time prolonging effect is a simple, sensitive, and reproducible index for the study of this phenomenon.

Tatum and Seevers (1) first reported an enhancement of the motor accelerating effect of cocaine after repeated administration in dogs. Similar enhancement of the ambulation accelerating effect by repeated administration have been demonstrated with many other drugs such as d-amphetamine (2, 3), methamphetamine (4–6), methylphenidate (7), lisuride (8), morphine (2, 9, 10); and the phenomenon is called reverse tolerance. Once the reverse tolerance is formed, the state persists for a long period, almost irreversibly, and a challenge of the same drug after a long pause acutely recurs the enhanced effect (11). The acute recurrence is similar to the flashback phenomenon which is observed after a long withdrawal from the drug by a psychic stress or sometimes occurs naturally in amphetamine (12) and psychedelic drug users (12, 13).

The formation of reverse tolerance to the ambulatory activity is greatly influenced by the experimental conditions such as the apparatus for measuring motor activity (5), surrounding circumstances in which the animals are placed (5, 6, 7, 10) or by the interval (6, 7, 10) and duration of drug administration (6).

Here, we report a very simple but highly reproducible method for the development of reverse tolerance in mice using d-amphetamine as a drug model, and a comparison was made with the usual method with an ambulometer.

Materials and Methods

Animals: Male mice of the dd-strain weighing 18 to 20 g were purchased and housed as a group of 10 animals in a plastic cage at an ambient temperature of 22±1°C. In the cage, they were given normal laboratory diet and tap water ad libitum. After reaching 23 to 27 g, they were used for the experiments.

Drug: d-Amphetamine-HCl (AMP) was dissolved in physiological saline and administered i.p. so as to contain the dose in a volume of 0.1 ml/10 g of body weight. The
dose was expressed in terms of the salt.

**Measurement of swimming time:** Immediately after drug administration, animals was put into the water bath (length 24 cm, width 17 cm, height 18 cm and water depth 10 cm) maintained at 20°C. After a period of vigorous swimming activity which lasted about 5 min, animals began to swim slowly and constantly. The time until the animal ceased to swim altogether making only those movements necessary to keep his head above water, and maintained this state more than 30 sec, was measured.

In other experiments, to restrict the swimming space, the test was performed in a narrow glass jar, 7.5 cm in diameter, 18 cm in height and 12 cm of water depth. Under this condition, the animals could only tread water.

**Measurement of ambulation:** Ambulatory activity was measured using an Ambulometer (O'Hara & Co., Ltd.), a round tilting cage of 20 cm in diameter, 18 cm in height. Each animal was placed in the activity cage and was injected with drug or saline after an adaptation period of 30 min. Then, the ambulatory counts of each animal was recorded automatically for 120 min.

**Immobilization of animals:** In order to restrict the movement induced by AMP, the animal was immobilized in a small plastic box, length 7.5 cm, width 2.5 cm, height 2.3 cm, for 30 min immediately after drug administration and returned to the home cage. After 5 daily treatments, measurement was performed in the usual water bath or in an activity cage on the 6th day.

**Acute recurrence test:** In order to detect the retention of reverse tolerance, acute recurrence of the enhancement by readministered AMP was tested after a 30 and 90 days drug-free period.

**Statistical analysis:** Statistical significance of the difference between mean values of the measurements was evaluated by Student’s t-test.

**Results**

**Development of reverse tolerance and its acute recurrence:** d-Amphetamine prolonged the swimming time and increased the ambulatory activity of naive animals in a dose-dependent manner. These effects were progressively enhanced by the daily repetition of the treatment. The increment was more rapid in swimming time than in ambulatory counts. On the 6th day, the swimming time increased 5–7 times of the initial level; on the other hand, the ambulatory counts increased 2–3 times after repeated treatment for 10 days. Repetitions with saline did not produce a significant change in swimming time and ambulatory activity.

After a 30 days drug-free period, in the groups treated with 2 mg/kg of AMP, the enhancement tended to attenuate but still maintained significantly higher level of the activities than that at the initial test with naive animals (corresponded to the level of 2–3 and 5–6 repetitions in swimming time and ambulation, respectively); but 90 days after withdrawal, the reverse tolerance disappeared, and the effect of AMP returned to the control level. In the animals treated with 1 mg/kg of AMP, 30 days after withdrawal, the enhancement still remained in the swimming test, but was completely lost in the ambulation test. The enhancement in the swimming test also disappeared after a 90 days drug-free period (Fig. 1).

**Interval of AMP administration and the development of reverse tolerance:** The swimming time prolonging effect of 2 mg/kg of AMP was progressively enhanced in the daily treated group. However, in the groups treated at 3–4 days interval, the effect of AMP progressed until the 3rd test and was maintained at this level thereafter. When the administration of AMP was repeated weekly, no enhancement of the swimming time prolonging effect was observed during 6 repetitions of the tests (Fig. 2).

**Restriction of swimming space and development of reverse tolerance:** Restriction of swimming space in a narrow glass jar completely blocked the development of reverse tolerance to the swimming time prolonging effect of AMP, both in 1 or 2 mg/kg injection groups, during 5 daily repetitions. On the 6th administration of 2 mg/kg of AMP, the swimming time in a wide water bath was prolonged slightly, and significant difference was observed in the activities between 5th and 6th test; however, the
effect was much less than that observed in the group in which the tests were repeated in the wide water bath (shown in Fig. 1). Nearly the same level of activities were maintained after a 30 days drug-free period (Fig. 3).

**Immobilization and development of reverse tolerance:** Daily repetition of AMP administration developed reverse tolerance to its swimming time prolonging effect on the 6th day, and the enhancement partially remained even after 30 days withdrawal from the drug (these data are also shown in Fig. 1). On the other hand, development of...
reverse tolerance during 5 daily repetitions was completely inhibited in the animals which showed immobilization of their movements after every AMP administration. No significant difference was obtained in the swimming time between naive and immobilized groups after a 30 days drug-free period (Table 1). Similarly, immobilization of the animals completely blocked the development of reverse tolerance to the ambulation accelerating effect of AMP (Table 2).

**Discussion**

Reverse tolerance is readily developed to the swimming time prolonging effect of AMP by daily repetition of the test. The enhancement in swimming time prolonging effect was much more evident than that observed in ambulation accelerating effect. Swimming time increased to the almost maximal level by 5-6 repetitions and further repetitions of the test resulted in weakening and loss of body weight of the animals.

It is well-known that the development of reverse tolerance is greatly influenced by the experimental conditions. As to the intervals of the treatment, the most greatest increment in ambulation accelerating effect of methamphetamine was obtained by 3-4 days intervals, andequipotent or a little less enhancement was attained by weekly repetition, but daily repetition produced least

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**Table 1.** Effect of immobilization on the development of reverse tolerance to the swimming time prolonging effect of d-amphetamine (AMP)

| Dose (mg/kg) | Swimming time (min, mean±S.E.) |
|-------------|-------------------------------|
|             | Non-immobilized<sup>a</sup> | immobilized<sup>b</sup> |
| 1st day     |                               |                             |
| AMP 1       | 5.6±0.9                       | –                            |
| AMP 2       | 10.7±1.1                      | –                            |
|             | 22.8±9.8                      | –                            |
| 6th day     |                               |                             |
| AMP 1       | 5.4±1.0                       | 6.2±0.5                      |
| AMP 2       | 45.4±8.8**                    | 13.4±2.8                     |
|             | 139.2±9.9**                   | 24.0±12.5                    |
| 36th day<sup>c</sup> (Withdrawal) | 52.6±9.7**       | 11.9±1.7                     |
| AMP 1       | 74.5±9.5**                    | 45.6±13.9                    |

<sup>a</sup>: Swimming test was repeated daily in a wide water bath.  
<sup>b</sup>: Animal was immobilized in a plastic box for 30 min after AMP administration during 5 daily treatments, and the swimming test was performed in a wide water bath on the 6th and 36th day.  
<sup>c</sup>: Acute recurrence test after 30 days withdrawal from AMP. Each value represents the mean±S.E. of 15 animals. Significantly different from the corresponding test on 1st day: **P<0.01. Significantly different from the corresponding test on the 6th day: #P<0.01.

**Table 2.** Effect of immobilization on the development of reverse tolerance to the ambulatory activity accelerating effect of d-amphetamine (AMP)

| Dose (mg/kg) | Ambulatory counts (mean±S.E.) |
|-------------|-------------------------------|
|             | Non-immobilized | immobilized |
| 1st day     |                  |             |
| AMP 1       | 122±42.0                 | –           |
| AMP 2       | 302±132.1                 | –           |
|             | 841±187.3                 | –           |
| 6th day     |                  |             |
| AMP 1       | 45±17.6                   | 86±14.0     |
| AMP 2       | 466±240.9                 | 417±128.8   |
|             | 1941±209.8**              | 612±229.7   |

Significantly different from the corresponding test on the 1st day: **P<0.01. For other details, refer to the footnote of Table 1.
effect (6). However, in the case of the swimming time prolonging effect, 3–4 days interval was less effective than daily repetition; and by weekly interval, reverse tolerance was not developed during 6 repetitions.

Alam (5) and Hirabayashi & Alam (6) reported that reverse tolerance to the ambulation accelerating effect of methamphetamine did not develop in the mice kept in a small glass jar to restrict free movement during the presence of the acute drug effect. Thus, they claimed that a repeated exposure to the activity cage was required to induce the reverse tolerance to the ambulation-increasing effect of the drug.

In the present experiments, we confirmed their results with AMP in the ambulation accelerating effect. Similarly, reverse tolerance was found only in the mice which swam in a wide water bath after AMP administration, but not in the animals that treaded water in a narrow glass jar or that had their movements completely restricted in a small plastic box. In the animals exposed to the narrow glass jar during 5 daily repetitions of AMP, the swimming time prolonging effect was enhanced when tested in a wide water bath on the 6th day. The enhancement may be due to the incomplete restriction of the movement after every AMP injection.

In the experiments reported by Alam (5) and Hirabayashi and Alam (6), they placed the mice in narrow jars for 180 min to restrict the ambulation which was elicited as an acute effect of the drug. In our study, mice were confined in a small box for 30 min, but the enhancement of the swimming time prolonging effect of AMP was completely inhibited. Thus, partial suppression of the movement after drug administration is sufficient to inhibit the development of reverse tolerance. On the other hand, Hirabayashi et al. (7) reported that the enhancement of the ambulation-increasing effect of methylphenidate was not evident when the drug administration was repeated in the home cage and the test was done in the activity cage. These results suggest that the experimental conditions and also environmental factors play an important role in the development of reverse tolerance.

The ambulation accelerating effect of methamphetamine was well maintained after a 2 months drug-free period (6). In the present study, the enhancement in the ambulatory counts after the 10th administration of 2 mg/kg of AMP at a daily interval persist for 30 days, and the test on the 11th administration produced an enhancement corresponding to that after 5–6 repetitions. The enhancement of the swimming time prolonging effect after 6 repetitions of 2 mg/kg of AMP also remained after 30 days of withdrawal, but the level was below 50% of the maximal enhancement. The rapid loss of the reverse tolerance to the swimming time prolonging effect of AMP is partially attributable to the limited number of AMP treatments; however, the maximal enhancement was attained by 5–6 repetitions. The fundamental mechanisms of the swimming time prolonging effect and the ambulation accelerating effect may be different from each other, but the results obtained in the present experiments seem to suggest that the reverse tolerance to the former effect develops and disappears more easily than that to the latter effect.

The method presented in the present paper, the swimming time prolonging effect as an index, is simple, needs no special equipment, and is highly reproducible for demonstrating the development of reverse tolerance to AMP, and further applications of the method to other drugs are under investigation.

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