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Circadian Variation in Susceptibility to Methamphetamine After Repeated Administration in Mice

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KURIBARA, H. AND S. TADOKORO. *Circadian variation in susceptibility to methamphetamine after repeated administration in mice.* PHARMACOL BIOCHEM BEHAV 20(2) 247–250, 1984.—Since repeated administration of methamphetamine sometimes induces an augmentation in susceptibility, i.e., a reverse tolerance, to the stimulant drug effect in animals, the circadian variation in susceptiblity to the ambulation-increasing effect of methamphetamine after repeated administration was investigated in mice. The ambulatory activity of each mouse was measured by a tilting-type round activity cage of 25 cm in diameter. Mice, which had been housed under a 12 hr light-dark schedule (light period; 6:00–18:00) for 5 weeks, were administered methamphetamine 1 or 2 mg/kg SC at one of 6 times of day (3:00, 7:00, 11:00, 15:00, 19:00 and 23:00) for 5 times at intervals of 7 days, and their ambulatory activities were measured for 3 hr after each administration. The repeated administration of methamphetamine induced a reverse tolerance to the ambulation-increasing effect of the drug, and the mean overall ambulatory activity counts on the 5th session were estimated to be 2–4 times as high as the corresponding activity counts on the 1st session. However, the circadian variation in susceptibility, which was at maximum during the late dark period (administration at 3:00) and at minimum during the late light period (administration at 15:00), was well maintained even after the repeated administration. When the times of day of the drug administration were changed by 12 hr on the 6th session, a marked increase in the activity counts was observed in the mice changed from 15:00 to 3:00, while a marked decrease was observed in the mice changed from 3:00 to 15:00. The present results suggest that repeated administration of methamphetamine induces a reverse tolerance in mice to the ambulation-increasing effect. However, the circadian variation in susceptibility to the stimulant drug effect is not affected by the repeated administration.

Circadian variation Methamphetamine Ambulation-increasing effect Repeated administration
Reverse tolerance Mice

IN a previous report, we [8] demonstrated that the susceptibility to the ambulation-increasing effect of methamphetamine in mice showed a clear circadian rhythm with the maximum during the late dark period and the minimum during the late light period, and that the rhythm was mainly elicited by a circadian variation in the receptor sensitivities of the catecholaminergic, in particular dopaminergic, systems in the brain. Evans *et al.* [2], Scheving *et al.* [9], Urba-Holmgren *et al.* [13] and Wolfe *et al.* [14] also reported similar circadian variations in susceptibility to the behavioral and lethal effects of d-amphetamine in rats. However, these results were obtained after a single administration of methamphetamine or d-amphetamine.

On the other hand, Alam [1] and Hirabayashi and Alam [4] reported that a reverse tolerance, i.e., an augmentation in the susceptibility, to the ambulation-increasing effect of methamphetamine was induced in mice by repeated administration of the drug when they were put into activity cages with a sufficient space for free movement during a presence of the acute drug effect after each administration. Tilson and Rech [12] also demonstrated a similar result after a repeated administration of d-amphetamine in rats. In these respects, there is a question whether or not the circadian variation in susceptibility to methamphetamine is affected by repeated administration.

The purpose of this experiment was to investigate the change in circadian variation in susceptibility to the ambulation-increasing effect of methamphetamine after repeated administration in mice.

METHOD

Animals

The experimental animals were 384 male mice of the dd strain, which were provided by Institute of Experimental Animal Research, Gunma University School of Medicine at the age of 3 weeks. The mice had been housed in groups of 8 in aluminum cages of 20 (W) × 30 (D) × 10 (H) cm with wooden-flake floor mat, and were freely given solid diet (MF: Oriental Yeast Co., Tokyo) and tap water except during the sessions of the experiment. The breeding room was artificially illuminated with fluorescent lamps on a 12 hr light-dark schedule (light on at 6:00 and light off at 18:00) throughout the breeding and the experimental periods. The intensities of the illumination were about 500 Lx during the light period and less than 0.5 Lx during the dark period. The

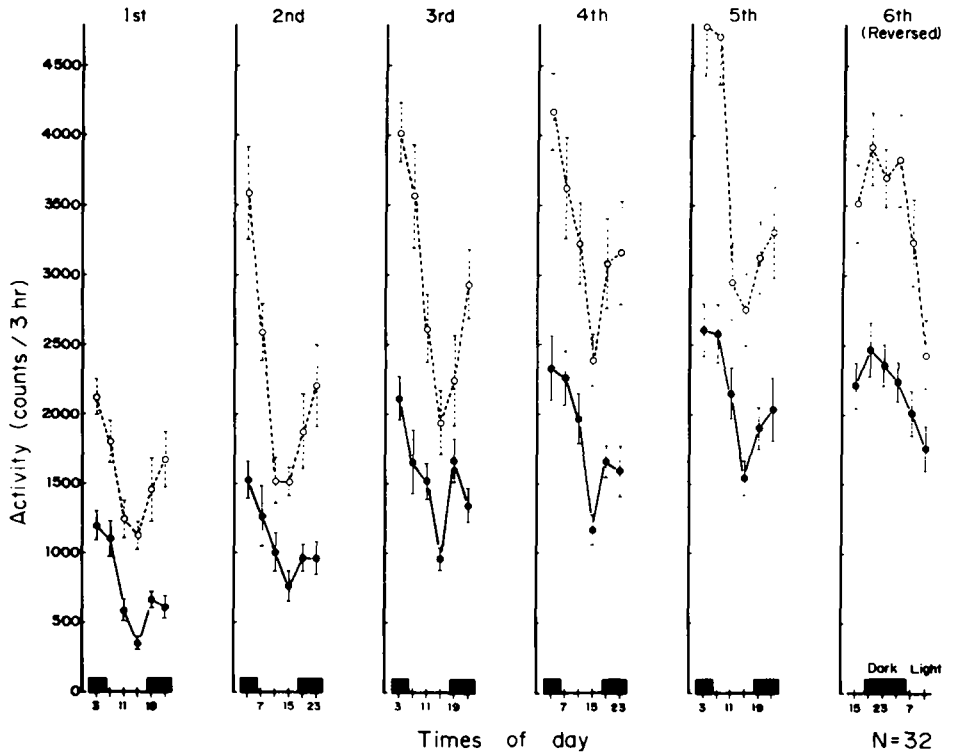

FIG. 1. Circadian variation in mean overall ambulatory activity counts for 3 hr after repeated administration of methamphetamine 1 and 2 mg/kg SC for 6 times at intervals of 7 days. Each group of 32 mice was administered methamphetamine 1 or 2 mg/kg at one of 6 times of day (3:00, 7:00, 11:00, 15:00, 19:00 and 23:00) for 5 times, and on the 6th administration day, the time of administration was changed by 12 hr. ●—●: Results after the administration of methamphetamine 1 mg/kg SC. ○— —○: Results after the administration of methamphetamine 2 mg/kg SC. Each vertical bar attached to each symbol indicates the standard error of mean value. Statistical comparisons are presented in the text.

room temperature was regulated to 22±2°C. However, the humidity was not controlled. When the mice achieved the age of 8 weeks and weighed 30–32 g, they were divided into 12 groups of 32 mice each and were used for the experiment described below.

Apparatus and Procedure

Sixteen tilting-type round activity cages of 25 cm in diameter and 13 cm in height for measurement of ambulatory activity of mice were the same with those used in the previous experiment [8]. The principle of the device and the method for the measurement of ambulatory activity were reported in detail by Hirabayashi *et al.* [3].

A mouse was put into each activity cage, and the cumulative ambulatory activity counts during 10 min segments were recorded for 30 min before the drug administration and for 180 min after the administration of methamphetamine. The time course changes in the ambulatory activity counts after methamphetamine 1 and 2 mg/kg SC had already been shown in the previous paper [8]. Therefore, the overall ambulatory activity counts for 3 hr after the administration were used in the calculation of mean values.

Drug and Administration Schedules

The drug used was methamphetamine HCl (Philopon: Dainippon Pharm. Co., Osaka). The doses tested (1 and 2 mg/kg SC) were the same as those tested in the previous experiment [8]. The drug was dissolved in a physiological saline vehicle, and the doses were expressed in the salt form. Each dose volume administered was fixed to 1 ml per 100 g body weight.

Each group of 32 mice was administered methamphetamine 1 or 2 mg/kg at one of 6 times of day (3:00, 7:00, 11:00, 15:00, 19:00 and 23:00). The same treatment was carried out for 5 times at intervals of 7 days. However, on the 6th session of drug administration, which was carried out 7 days after the 5th, the times of day of the drug administration were changed by 12 hr.

Statistical Analysis

The data obtained were statistically analyzed by an analysis of variance (ANOVA).

RESULTS

Figure 1 shows changes in the mean overall ambulatory activity counts during the 3 hr sessions of each group of 32 mice after the repeated administration of methamphetamine 1 or 2 mg/kg SC for 5 times at intervals of 7 days at one of 6 times of day (3:00, 7:00, 11:00, 15:00, 19:00 and 23:00). Figure 1 also shows the results on the 6th session which was carried out 7 days after the 5th session. The times of day of the drug administration on the 6th session were changed by 12 hr.

The 1st administration of methamphetamine 1 and 2

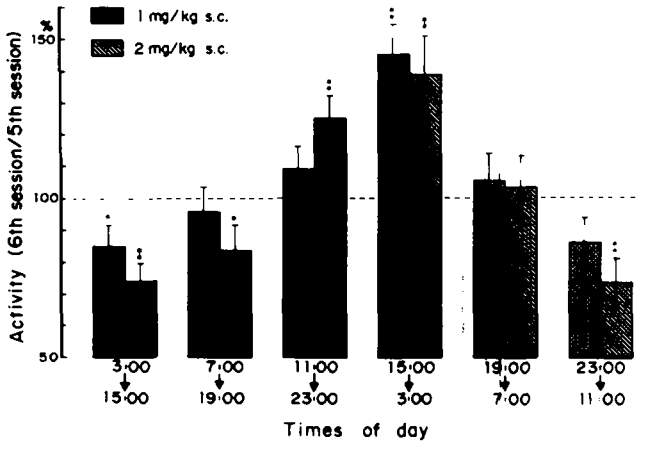

FIG. 2. Circadian variations in % activities between the 6th and 5th sessions within the same groups of mice after administration of methamphetamine 1 and 2 mg/kg SC. Each group of 32 mice had been administered methamphetamine 1 or 2 mg/kg SC at the same time of day (3:00, 7:00, 11:00, 15:00, 19:00 or 23:00) for 5 times at interval of 7 days. On the 6th session the time of day of the drug administration was changed by 12 hr. The times of day with an arrow shown in the bottom of the figure indicate changes in the times of day of the drug administration on the 6th session from those on the 1st–5th sessions. Each vertical line attached to each column indicates the standard error of mean value. *Indicates significant difference as compared with the activity on the 5th session shown by a horizontal broken line at 100% level ($p<0.05$, Student's t-test). **$p<0.01$.

mg/kg SC induced an increase in the mean overall ambulatory activity count of the mice. Moreover, a clear circadian variation in susceptibility to the ambulation-increasing effect of methamphetamine was observed. ANOVA revealed significant time-of-day dependent changes, $F(5,186)=32.69$, $p<0.001$ for 1 mg/kg, and $F(5,186)=47.15$, $p<0.001$ for 2 mg/kg, in the mean overall ambulatory activity counts. The maximum and minimum susceptibilities to the ambulation-increasing effect of methamphetamine were found when the drug was administered at 3:00 (late dark period) and 15:00 (late light period), respectively.

A reverse tolerance, i.e., an augmentation in the susceptibility to the ambulation-increasing effect of methamphetamine, was progressively induced by the repeated administration. ANOVA revealed significant treatment dependent changes, $F(4,930)=73.92$, $p<0.001$ for 1 mg/kg, and $F(4.930)=54.28$, $p<0.001$ for 2 mg/kg, in the mean overall ambulatory activity counts. On the 5th session, the mean overall ambulatory activity counts were 2–4 times as high as the corresponding activity counts on the 1st session.

The circadian variation in susceptibility to the ambulation-increasing effect of methamphetamine was maintained even after the production of reverse tolerance to the drug effect. ANOVA revealed significant time-of-day dependent changes, $F(5,930)=131.27$, $p<0.001$ for 1 mg/kg, and $F(5,930)=82.75$, $p<0.001$ for 2 mg/kg, in the mean overall ambulatory activity counts throughout the 1st–5th sessions.

When the times-of-day of the drug administration were changed by 12 hr on the 6th session, the mean overall ambulatory activity counts increased or decreased dependent on the times-of-day. ANOVA revealed significant time-of-day dependent changes, $F(5,186)=15.72$, $p<0.001$ for 1 mg/kg, and $F(5,186)=12.38$, $p<0.001$ for 2 mg/kg, in the mean overall ambulatory activity counts on the 6th session. The maximum activity counts were found when methamphetamine 1 and 2 mg/kg were administered at 19:00 (early dark period), and the minimum activity counts when the drug was administered at 11:00 (mid light period).

In order to study in detail the changes in the ambulatory activity by the alteration of times-of-day of the drug administration by 12 hr, the ambulatory activity counts on the 6th session were compared with those on the 5th session within the same groups of mice. The results are shown in Fig. 2. Each column indicates activity counts on the 6th session/activity counts on the 5th session (defined as % activity between the 6th and 5th sessions). ANOVA revealed significant time-of-day dependent changes, $F(5,186)=9.18$, $p<0.001$ for 1 mg/kg, and $F(5,186)=13.71$, $p<0.001$ for 2 mg/kg, in the % activity differences between the 6th and 5th sessions. The highest values in the % activity between the 6th and 5th sessions were found when the time of day of the administration of methamphetamine 1 and 2 mg/kg was changed from 15:00 to 3:00, and the lowest values when changed from 3:00 to 15:00.

DISCUSSION

The circadian variation in susceptibility to the ambulation-increasing effect of methamphetamine in mice observed on the 1st session, i.e., administration to drug-naive mice, is consistent with the result obtained in the previous experiment by us [8], that the maximum and minimum ambulatory activity counts appear when the drug is administered at 3:00 and 15:00, respectively. Similar circadian variations in susceptibility in rats to the behavioral and lethal effects of d-amphetamine were reported by many researchers [2, 9, 13, 14]. For the production of the circadian variation in susceptibility to the ambulation-increasing effect of amphetamines, circadian fluctuation in the receptor sensitivities of catecholaminergic, in particular dopaminergic, systems in the brain is considered to be mainly involved [8].

On the other hand, the present experiment demonstrates that the repeated administration of methamphetamine at intervals of 7 days for 5 times induces a reverse tolerance to the ambulation-increasing effect of the drug, and the mean overall ambulatory activity counts on the 5th session are estimated to be 2–4 times as high as the corresponding activity counts on the 1st session. This result is consistent with those reported by Alam [1] and Hirabayashi and Alam [4]. In addition, they [1,4] demonstrated that the phenomenon could be observed only in mice which were exposed to the round activity cages with a sufficient space for free movement. Kuribara [7] and Tilson and Rech [11,12] also reported that the appearance of reverse tolerance to the avoidance-facilitating and motor-stimulating effects of d-amphetamine in rat was dependent on the experimental condition on which the rat was exposed during the presence of the acute drug effect. These results suggest that the production of reverse tolerance to the behavior stimulating effects of methamphetamine and amphetamine is elicited by an interaction between the drug effects and the environmental situation, and that a conditioning to the drug effects is induced after repeated administration.

In these respects, there is a question whether or not an entrainment of the circadian variation in susceptibility to the ambulation-increasing effect of methamphetamine is induced by repeated administration. This is because the mice tested

are administered the drug for 5 times at the same time of day at intervals of 7 days, and they exhibit a marked increase in the ambulatory activity after each drug administration.

However, the present experiment demonstrates that, even though the mice exhibit a reverse tolerance to the ambulation-increasing effect of methamphetamine after the repeated administration, the patterns of the circadian variation in susceptibility on the 2nd–5th sessions are almost identical with that on the 1st session. In addition, when the times of day of the drug administration are changed by 12 hr on the 6th session, a marked increase in the susceptibility to the ambulation-increasing effect of methamphetamine is found in the mice changed from 15:00 to 3:00, while a marked decrease occurred in the mice changed from 3:00 to 15:00. The circadian patterns of % activity between the 6th and 5th sessions are inversely correlated to the patterns of the mean overall ambulatory activity counts on the 1st–5th sessions as shown in Figs. 1 and 2. These results suggest that the circadian variation in susceptibility of mice to the ambulation-increasing effect of methamphetamine is not itself markedly affected by the repeated administration of the drug, even though a reverse tolerance to the drug effect is induced.

We previously reported that a single administration of methamphetamine 1–4 mg/kg SC [6], or repeated administration of 2 mg/kg SC for 5 times at intervals of 3 hr [10] did not induce a marked change in the diurnal patterns of the naive behaviors such as ambulation, eating and drinking in rats, except for one day after the administration. However, Ikeda and Chiba [5] demonstrated that continuous oral intake of d-amphetamine 0.01% solution for more than 100 days, during which the daily intake of d-amphetamine was estimated to be about 10 mg/kg/day, induced a slight change in the diurnal pattern of motor activity in rats.

Therefore, it can be concluded that the circadian variation in susceptibility to the ambulation-increasing effect of methamphetamine in mice is maintained when the drug is administered intermittently. However, the repeated administration induces a reverse tolerance in mice to the drug effect which shifts the entire dose-time response pattern for the circadian variation.

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