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Circadian Variation in Methamphetamine- and Apomorphine-Induced Increase in Ambulatory Activity in Mice

HISASHI KURIBARA AND SAKUTARO TADOKORO

Division for Behavior Analysis, Behavior Research Institute, School of Medicine, Gunma University 3-39-22 Showa-machi, Maebashi 371, Japan

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KURIBARA, H. AND S. TADOKORO. *Circadian variation in methamphetamine- and apomorphine-induced increase in ambulatory activity in mice.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1251–1256, 1982.—The existence of circadian variation in methamphetamine- and apomorphine-induced change in ambulatory activity in mice was investigated. Adult male mice of dd strain, which had been housed on a 12 hr light-dark schedule (light period; 6:00–18:00) for 4 weeks, received injections of either methamphetamine HCl 1 or 2 mg/kg SC at one of six times of day (3:00, 7:00, 11:00, 15:00, 19:00 and 23:00), or apomorphine HCl 0.5 or 1 mg/kg SC at one of six times of day (3:30, 7:30, 11:30, 15:30, 19:30 and 23:30). The control animals were administered a physiological saline vehicle alone at the corresponding times of day. The ambulatory activity of each mouse was measured by a tilting-type activity cage for 3 hr after methamphetamine, and for 1 hr after apomorphine. A circadian variation in the ambulatory activity was observed after the administration of the saline, methamphetamine and apomorphine. Here, the highest activity counts were found when the saline, methamphetamine and apomorphine were administered during the late dark period (3:00 or 3:30), while the lowest activity counts were found when the saline and apomorphine 1 mg/kg were administered during the mid light period (11:00 or 11:30), and methamphetamine 1 and 2 mg/kg and apomorphine 1 mg/kg were administered during the late light period (15:00 or 15:30). The circadian variation in methamphetamine-induced increase in the activity was abolished by a pretreatment with reserpine 2 mg/kg SC 4 hr before, but that of apomorphine was maintained even by the pretreatment with reserpine. The present results suggest that the methamphetamine- and apomorphine-induced increase in the ambulatory activity in mice is dependent on the time-of-day of the drug administration, and the occurrence is mainly due to a circadian variation in activity of the catecholaminergic systems in the brain.

Ambulatory activity Circadian variation Methamphetamine Apomorphine Mice

IT has been documented that many behaviors in rodents show a nocturnal pattern with a higher activity during the dark period and a lower activity during the light period [2, 9, 14, 18]. Many investigators also reported that the drug-induced changes in behaviors of rodents showed a circadian fluctuation [4, 7, 8, 11, 12, 13, 14, 16, 19, 20, 21]. However, there are few studies on circadian variations in the methamphetamine- and apomorphine- induced increase in the ambulatory activity in mice.

Recently, the members of our department [5,6] assembled a simple device for measurement of ambulatory activity in mice, and reported various activity patterns after administration of d-amphetamine, methamphetamine, cocaine and morphine.

The purpose of this experiment was to study the existence of circadian variation on the increase in ambulatory activity in mice after administration of methamphetamine and apomorphine.

METHOD

Animals

The experimental animals were adult male mice of the dd strain, which were provided by the breeding colony of Gunma University Medical School at an age of 3 weeks. Groups of 8 mice were housed in aluminium cages of 20 (W) × 30 (D) × 10 (H) cm with a free access to a solid diet (MF: Oriental Yeast Co., Tokyo) and tap water. The breeding room was artificially illuminated with fluorescent lamps on a 12-hr light-dark schedule (light period; 6:00–18:00). The room temperature was regulated to 23±2°C. The humidity was not controlled. When the mice were 7 weeks of age and weighed 28–32 g, they were used for the drug tests. Eighteen groups of 32 mice each was used for the test of methamphetamine and its control experiment and eighteen groups of 16 mice each were used for the test of apomorphine and its control experiment. Another twelve groups of 16 mice each were used for the tests of combined effects of methamphetamine or apomorphine with reserpine. Repeated use of the same animals was avoided.

Apparatus and Procedure

Sixteen tilting-type round activity cages of 25 cm in diameter and in 13 cm in height were used for the test of methamphetamine. Since the apomorphine-induced increase in the

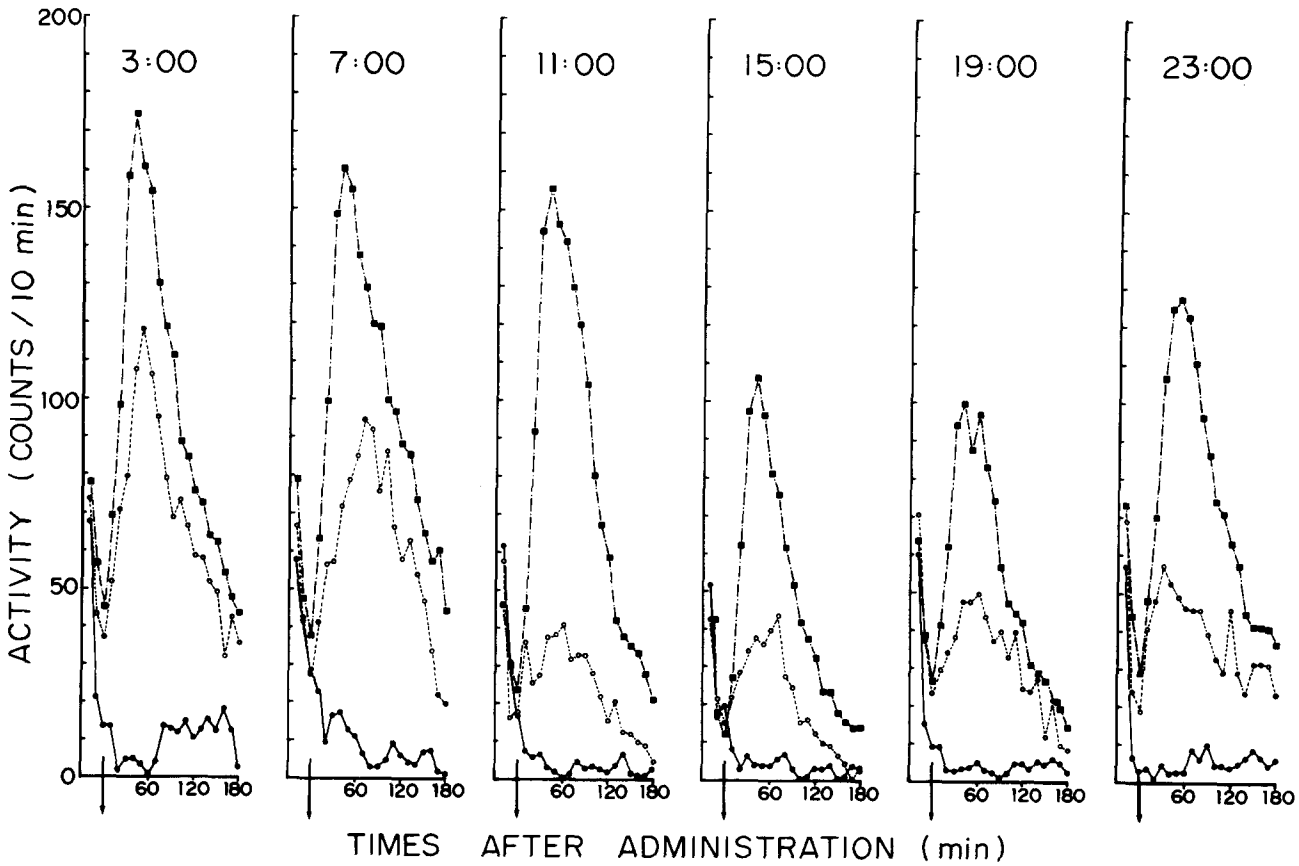

FIG. 1. Temporal changes in mean ambulatory activity counts of the mice given a physiological saline or methamphetamine 1 and 2 mg/kg SC at one of six times of day (3:00, 7:00, 11:00, 15:00, 19:00 and 23:00). The saline or methamphetamine was administered after an adaptation period of 30 min for the tilting-type round activity cage and the cumulative activity counts during 10 min segments were measured for 180 min thereafter. ●——●: Results after the administration of the saline. ○---○. Results after the administration methamphetamine 1 mg/kg SC. ■— - —■: Results after the administration of methamphetamine 2 mg/kg SC. Thirty-two drug naive mice were used for each test.

ambulatory activity was lower than that after methamphetamine, an increase in sensitivity of the apparatus was required. The other 8 activity cages of 20 cm in diameter and 18 cm in height were used for the test of apomorphine. The principles of the device and the method for measurement of ambulatory activity in the mouse were reported in detail by Hirabayashi *et al.* [5,6]. Briefly, each tilt of the activity cage according to the movement of the mouse was recorded through a microswitch attached to the cage.

A mouse was put into each activity cage, and the cumulative activity counts during 10 min segments were recorded for 30 min before the drug administration, and 180 min and 60 min after the administration of methamphetamine and apomorphine, respectively. The movements of the reserpinized mice ceased after 60 min following methamphetamine therefore, in the test of combined administration of methamphetamine or apomorphine with reserpine, the ambulatory activity counts following were recorded for 60 min after the administration of the drugs.

The intensity of illumination in the activity cages was about 500 and 1 Lx during the light and dark periods, respectively.

Drugs

The drugs were methamphetamine HCl (Philopon; Dainippon), apomorphine HCl (Sigma) and reserpine (Reserpine Inj.; Fuso). Since more than 4 mg/kg SC of methamphetamine and more than 2 mg/kg SC of apomorphine induced stereotyped behaviors, such as head-twitching, sniffing, licking, gnawing, etc., and elicited an irregular pattern of ambulatory activity, the doses given in the present experiment were 1 and 2 mg/kg SC for methamphetamine, and 0.5 and 1 mg/kg SC for apomorphine. Reserpine was administered 2 mg/kg SC. The drugs were dissolved or diluted in a physiological saline vehicle, and the doses were expressed in the salt form. The dose volume administered was fixed to 10 ml/kg.

Methamphetamine was administered at one of six times of day (3:00, 7:00, 11:00, 15:00, 19:00 and 23:00). Apomorphine was administered at one of six times of day (3:30, 7:30, 11:30, 15:30, 19:30 and 23:30). The control animals received administration of the saline vehicle alone at the corresponding times of day. In the test of combined administration of methamphetamine or apomorphine with reserpine, reserpine

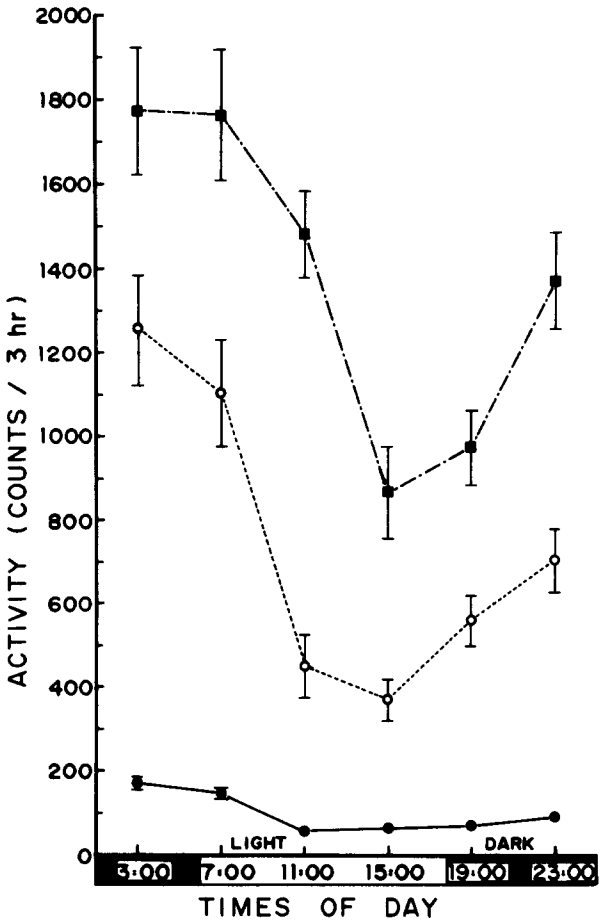

FIG. 2. Circadian variation in mean overall ambulatory activity counts for 3 hr after the administration of physiological saline or methamphetamine 1 and 2 mg/kg SC at one of six times of day (3:00, 7:00, 11:00, 15:00, 19:00 and 23:00). ●——●: Results after the administration of the saline. ○----○: Results after the administration of methamphetamine 1 mg/kg SC. ■— - —■: Results after the administration of methamphetamine 2 mg/kg SC. Vertical bar attached to each symbol indicates the standard error of the mean value of the 32 mice.

was administered 4 hr before the administration of methamphetamine or apomorphine. Methamphetamine or apomorphine was administered at one of six times of day (3:30, 7:30, 11:30, 15:30, 19:30 and 23:30).

Statistical Analysis

The data were statistically analyzed by a two-factor analysis of variance (ANOVA). The first factor was time-of-day (6 levels), and the second one was the doses (3 levels including the saline as dose 0).

RESULTS

Figure 1 shows temporal changes in mean ambulatory activity counts of 32 mice after the administration of the saline or methamphetamine 1 and 2 mg/kg SC at 3:00, 7:00, 11:00, 15:00, 19:00 and 23:00. Figure 2 shows mean overall activity counts during the 3 hr sessions. Methamphetamine induced a dose-dependent increase in the ambulatory activity at any time of drug administration. Moreover, a clear circadian variation in the activity counts was found after the administration of both the saline and methamphetamine. ANOVA revealed significant time-of-day, $F(5,558)=105.73$, $p<0.001$, and dose-dependent, $F(2,558)=136.19$, $p<0.001$, changes in the ambulatory activity. The highest activity counts were observed after the administration of both the saline and methamphetamine at 3:00. The lowest activity counts were observed after the administration of the saline and methamphetamine at 11:00 and 15:00, respectively.

Figure 3 shows temporal changes in mean ambulatory activity counts of 16 mice after the administration of the saline or apomorphine 0.5 and 1 mg/kg SC at 3:30, 7:30, 11:30, 15:30, 19:30 and 23:30. Figure 4 shows mean overall activity counts during the 1 hr sessions. Apomorphine induced an increase in the ambulatory activity. However, a clear dose-effect relation was seen only after the administration at 3:30, 15:30, 19:30, and 23:30. A circadian variation in the apomorphine-induced increase in the ambulatory activity was also found. ANOVA revealed significant time-of-day dependent, $F(5,270)=51.85$, $p<0.001$, and dose-dependent, $F(2,270)=35.79$, $p<0.001$, changes in the activity counts. The highest activity counts were found after the administration of the saline and apomorphine at 3:30. The lowest activity counts were found after the administration of the saline and apomorphine 1 mg/kg at 11:30, and apomorphine 0.5 mg/kg at 15:30.

Figure 5 shows mean overall activity counts during the 1 hr sessions after the administration of methamphetamine 2 mg/kg and apomorphine 0.5 mg/kg to 16 mice pretreated with reserpine 2 mg/kg 4 hr before. The reserpinized mice did not show any ambulation. The data of the animals were not shown in the figure. Reserpine pretreatment induced a decrease in the sensitivity of the mice to methamphetamine, but induced a marked increase in the sensitivity to apomorphine. A clear circadian variation in the ambulatory activity was not found after methamphetamine given to the reserpinized mice. However, a circadian variation in the activity was found after apomorphine given to the reserpinized mice, $F(5,270)=5.37$, $p<0.001$. The highest activity count was found after the administration of apomorphine at 3:30. The lowest activity count was found after the administration at 15:30. This circadian variation in the apomorphine-induced increase in the activity of reserpinized mice was almost identical with that of non-reserpinized mice.

DISCUSSION

The ambulatory activity counts observed after the single administration of the saline exhibited a circadian variation with the highest during the late dark period (administration at 3:00 or 3:30), and the lowest during the mid light period (administration at 11:00 or 11:30). This result may reflect the nocturnal habit of the mice [2,14].

Both methamphetamine and apomorphine induced a dose-dependent increase in the ambulatory activity in mice. Moreover, a clear circadian variation in the methamphetamine- and apomorphine-induced changes was demonstrated in the present experiment.

For the production of circadian fluctuation in the drug effects, the drug-metabolizing enzyme activities in the liver, the drug-excretion activity of the kidney, neurotransmitter levels and the sensitivities of the receptors in the brain are considered to be involved mainly. In fact, Holcslaw *et al.*

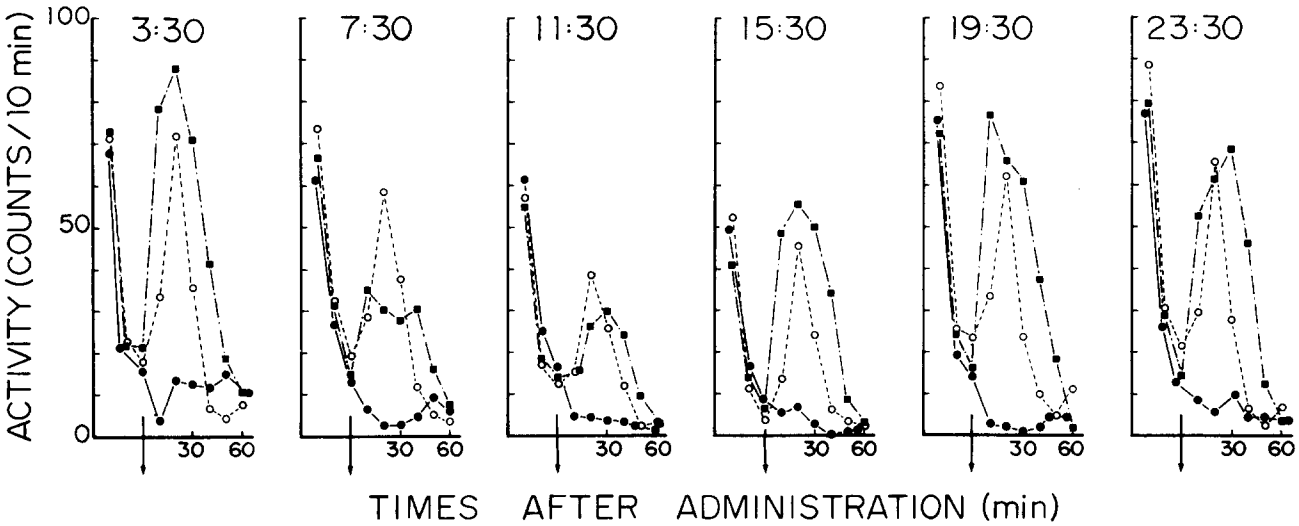

FIG. 3. Temporal changes in mean ambulatory activity counts of the mice given a physiological saline or apomorphine 0.5 and 1 mg/kg SC at one of six times of day (3:30, 7:30, 11:30, 15:30, 19:30 and 23:30). The saline or apomorphine was administered after an adaptation period of 30 min for the tilting-type round activity cage and the cumulative activity counts during 10 min segments were measured for 60 min thereafter. ●——●: Results after the administration of the saline. ○---○: Results after the administration of apomorphine 0.5 mg/kg SC. ■— - —■: Results after the administration of apomorphine 1 mg/kg SC. Sixteen drug naive mice were used for each test.

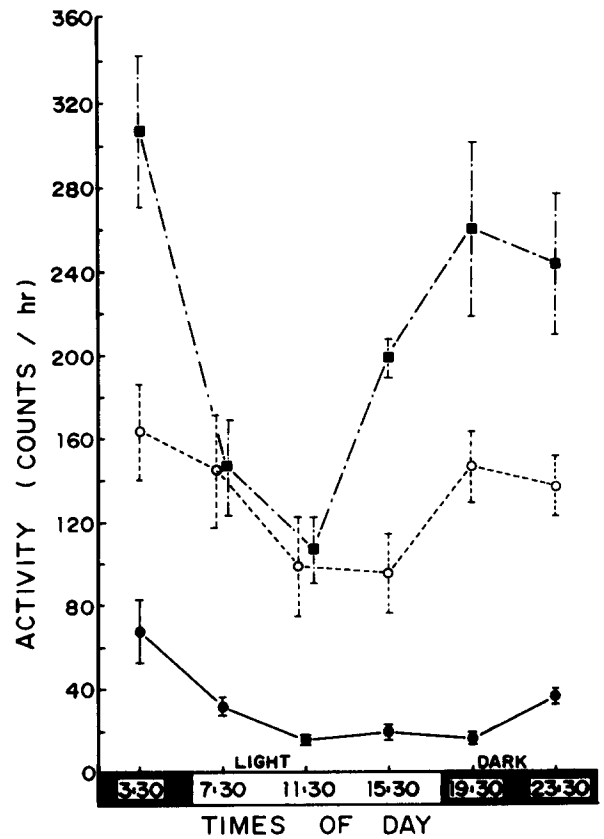

FIG. 4. Circadian variation in mean overall ambulatory activity counts for 1 hr after the administration of a physiological saline or apomorphine 0.5 and 1 mg/kg SC at one of six times of day (3:30, 7:30, 11:30, 15:30, 19:30 and 23:30). ●——●: Results after the administration of the saline. ○---○: Results after the administration of apomorphine 0.5 mg/kg SC. ■— - —■: Results after the administration of apomorphine 1 mg/kg SC. Vertical bar attached to each symbol indicates the standard error of the mean value of the 16 mice.

[7], Koukkari *et al.* [8], Nakano *et al.* [12], and Radzialowski and Bousquet [15] reported circadian variations in the effects of hexobarbital, imipramine, apomorphine and aminopyrine, that the highest response to these drugs were found during the late light period when administered to rats and mice. They then emphasized that the circadian variation in the drug-metabolizing enzyme activities in the liver was the main factor for the production of the circadian variation in the drug effects.

The role of the circadian variation in the drug-metabolizing enzyme activities in the liver and drug-excretion activity of the kidney for the effects of methamphetamine and apomorphine are not known. However, the ambulatory activity counts after methamphetamine and apomorphine observed in the present experiment were the highest during the late dark period and the lowest during the mid to late light period. The present result seen after methamphetamine is almost identical with those reported by Evans *et al.* [4], Scheving *et al.* [16], Urba-Holmgren *et al.* [20], and Wolfe *et al.* [21] that the highest behavioral and lethal effects of d-amphetamine and methamphetamine were found during the dark period in the rats. However, the result seen after apomorphine in the present experiment is inconsistent with those reported by Nagayama *et al.* [11], and Nakano *et al.* [12] in rats. Nagayama *et al.* reported that the duration of the apomorphine-induced stereotyped behaviors was the longest during the late dark to early light period and the shortest during the mid dark period. Nakano *et al.* demonstrated that the degree of the stereotyped behaviors was the maximum during the late light period and the minimum during the late dark period.

Methamphetamine stimulates the animal behaviors through the central catecholaminergic systems with an increase in norepinephrine and dopamine release, and decrease in the reuptake of these neuro-transmitters. Therefore, the daily variation in the brain norepinephrine and dopamine

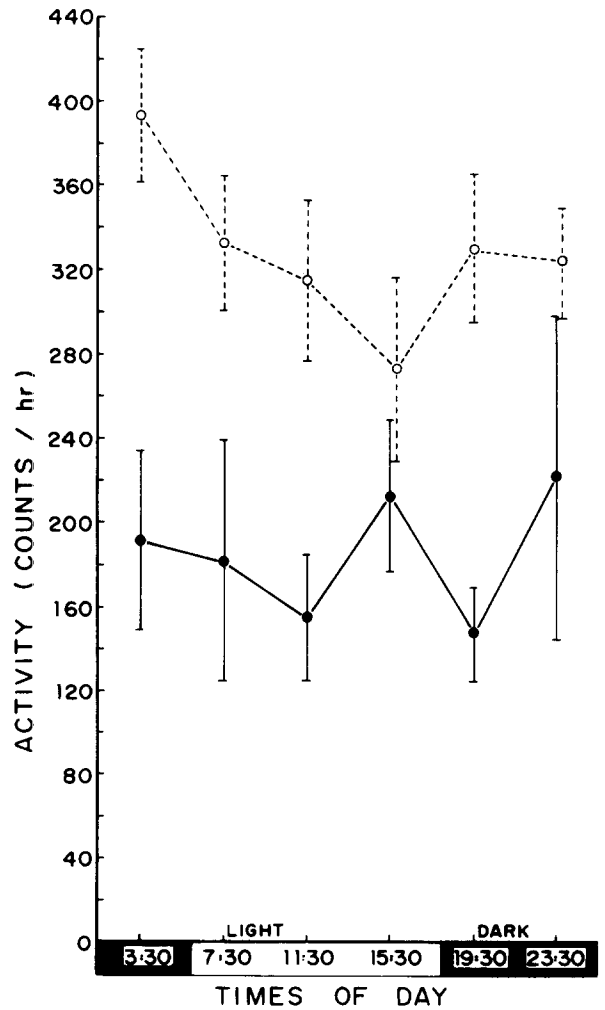

FIG. 5. Circadian variation in mean overall ambulatory activity counts for 1 hr after the administration of methamphetamine 2 mg/kg SC and apomorphine 0.5 mg/kg SC to the reserpinized mice (2 mg/kg SC 4 hr before the administration of the drugs) at one of six times of day (3:30, 7:30, 11:30, 15:30, 19:30 and 23:30). ●——●: Results after the administration of methamphetamine 2 mg/kg SC. ○---○: Results after the administration of apomorphine 0.5 mg/kg SC. Vertical bar attached to each symbol indicates the standard error of the mean value of the 16 mice.

levels as well as the sensitivities of the receptors also affect the effect of methamphetamine. Tapp and Holloway [19] reported a failure of α-methyl-p-tyrosine-induced impairment of the avoidance learning in rats after administration of the drug during the late dark to early light period, while a marked impairment after the administration during the other periods. They concluded that the brain catecholamine levels were involved to the production of the circadian variation in the α-methyl-p-tyrosine-induced impairment of the avoidance learning. Lew [10] also showed that the norepinephrine levels in the rat hypothalamus and medial brainstem were highest during the late dark to early light period. However, Scheving *et al.* [17] demonstrated an "ultradian" pattern of the norepinephrine and dopamine levels in the rat brain. The correlation between the catecholamine levels in the brain and the effect of methamphetamine on the ambulatory activity in mice must be studied in detail.

On the other hand, daily variation in the effect of apomorphine, a direct dopamine agonist [1,3], on the ambulatory activity in mice also exhibited a circadian pattern with the peak during the late dark period and the lowest during the mid to late light period. This result suggests that the circadian variation in the activities of catecholaminergic systems plays an important role for the effects of methamphetamine and apomorphine. However, Romero *et al.* [15] demonstrated that the number of β-adrenergic receptors in the rat pineal body was the maximum during the late light to early dark period.

Reserpine depletes the storage of monoamines in the granules and induces an increase in the receptor sensitivities. The present experiment demonstrated that the pretreatment with reserpine induced a decrease in the response to methamphetamine, and abolished the circadian variation in the activity-increasing effect of methamphetamine. In contrast, the pretreatment with reserpine induced an increase in the response to apomorphine. Moreover, the circadian variation in the activity-increasing effect of apomorphine was still maintained even after the pretreatment with reserpine. Therefore, the circadian variations in the methamphetamine- and apomorphine-induced increase in the ambulatory activity in mice may be due to mainly the daily fluctuation in the receptor sensitivities of catecholaminergic, in particular dopaminergic, systems in the brain.

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