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The Effects of Dopaminergic Agents on the Locomotor Activity of Rats After High Doses of Methylamphetamine[1]

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LUCOT, J. B., G. C. WAGNER, C. R. SCHUSTER AND L. S. SEIDEN. *The effects of dopaminergic agents on the locomotor activity of rats after high doses of methylamphetamine.* PHARMAC. BIOCHEM. BEHAV. 13(3) 409–413, 1980.—Treatment of rats with 100 mg/kg/day of methylamphetamine for four days produced long lasting (16 week) depletions of central nervous system levels of dopamine but not of norepinephrine. This methylamphetamine treatment also attenuated the ability of methylamphetamine and apomorphine to produce increases in locomotor activity without shifting the dose-response curve to the right or left. These rats underwent significant decreases in activity after lower doses of haloperidol while the response to L-Dopa was virtually unchanged.

Apomorphine L-Dopa Haloperidol Locomotor activity Methylamphetamine Dopamine depletions

CHRONIC treatment of rats with the amphetamines lowers brains levels of dopamine, decreases the number of dopamine uptake sites ([17], Wagner *et al.*, in press) and decreases tyrosine hydroxylase activity [4] without changing levels of norepinephrine [15,17] or altering the activity of choline acetyltransferase or glutamate decarboxylase [8]. The dopamine depletions have been observed as long as 8 weeks after cessation of the methylamphetamine treatment in rats, suggesting that they are the result of neurotoxicity [17]. The dopamine depletions also occur in guinea pig [15] and rhesus monkey [14]. Chronic treatment with methylamphetamine also attenuates the disruptive effects of methylamphetamine both in rhesus monkeys responding under a DRL schedule of food presentation [6] and in rats responding under a fixed-interval schedule of food presentation [16].

Dopaminergic function is an important determinant of levels of locomotor activity [3, 9, 10]. Thus, the locomotor activity of rats after the administration of dopaminergic agents should provide information on the functional consequences of the methylamphetamine-induced dopamine depletions.

METHOD

Animals

Male Sprague-Dawley rats weighing approximately 250 g were obtained from the Holtzman Co. (Madison, WI) and were housed individually in standard wire cages and had free access to food and water. The rooms were illuminated from 0700 to 1800 and the temperature was maintained at 22 ± 1°C. Rats were injected SC at 0800 and 1700 for 4 days with 2 ml/kg of either physiological saline or 25 mg/ml of methylamphetamine HCl in physiological saline.

Locomotor Activity

Locomotor activity was measured in 12 wire mesh stabilimeters 38×18×20 cm mounted on a pivoting axle. The excursion of the tilt was carefully adjusted to be the minimum excursion necessary to operate a microswitch mounted at the edge of the cage. The cages were enclosed in sound attenuating chambers which were ventilated by a fan and which were illuminated by two 28 V houselights during the one hour sessions [5]. Test sessions were conducted daily between 0700 and 0930. The sessions were timed and the data recorded by a PDP 8/e computer. Daily locomotor activity sessions were begun two weeks after cessation of the methylamphetamine treatment. From the ninth to the eleventh day of testing, the rats were injected with vehicle to familiarize them with the injection procedure.

Four groups were used, 2 receiving the methylamphetamine treatment and 2 receiving the vehicle. The 4 groups were tested in 2 one-hour sessions. In the first session, the first 6 boxes had vehicle treated rats and the second 6 had methylamphetamine treated rats. In the second ses-

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sion, the first 6 boxes had methylamphetamine treated rats and the second 6 had vehicle treated rats. No differences in baseline performance were observed as a function of the chambers or time of testing. Every third day, all rats were injected with one of the doses in the determination of drug dose-response curves. A dose-response curve for methylamphetamine was determined for the rats tested in the first hour, with 2 determinations at doses of 0.5 mg/kg and greater, and 1 determination at lower doses. Subsequently, a dose-response curve for apomorphine was determined with 1 determination at each dose. In the second session, dose-response curves for haloperidol and then for L-Dopa were determined with 1 determination at each dose. Drug injection days were preceded by vehicle injection days, and vehicle injections were randomly administered on days when drug was normally administered.

Neurochemistry

Sixteen weeks after cessation of the methylamphetamine treatment, rats were killed by decapitation, their brains removed quickly and dissected on ice. A transverse cut was made posterior to the olfactory bulbs and another cut was made anterior to the optic chiasm. From this slice the anterior striatum was dissected from the cortex and septum to provide the caudate sample. All remaining tissue was pooled for the rest of brain sample. Dopamine levels were determined by high performance liquid chromatography with electrochemical detection (Lucot *et al.*, submitted), and the norepinephrine levels were determined by alumina absorption with subsequent fluorometric assay [2,13].

Drugs

Methylamphetamine HCl (NIDA) was dissolved in physiological saline and apomorphine HCl (Merck) was dissolved in physiological saline and injected 10 min before the start of the session. Haloperidol (McNeil) was dissolved in a vehicle of 5% glucose with minimal acetic acid and injected 30 min before the start of the session. RO 4-4602 (Hoffman LaRoche) (50 mg/kg) was dissolved in physiological saline and injected 70 min before the start of the session. L-Dopa (Sigma) was dissolved in a solution of physiological saline with 0.001 N HCl and injected 30 min before the start of the session. All injections were IP in a volume of 1 ml/kg, with the exception of L-Dopa which was always in a 10 mg/ml solution.

Statistics

Control values for individual rats were determined by taking the mean of all vehicle injection days during the determination of a dose-response curve. Drug points were calculated as the percent of control values for individual rats as there were small but nonsignificant differences in baselines between vehicle and methylamphetamine treated rats. Tests for significance were made using 2 tailed Wilcoxin and Mann-Whitney U tests for the activity data. Comparisons of the chemical data were made using a two-tailed Student's t-test. Significance was defined as $p<0.05$.

RESULTS

Catecholamine Levels

The methylamphetamine treatment was effective in inducing long-lasting depletions in all but four of the treated rats. As the dopamine values for the 4 unaffected rats were greater than 3 S.D. from the mean of the remaining rats, these 4 rats were omitted from all analyses. Caudate levels of dopamine were significantly reduced to roughly 50% of control values, while the values for the rest of the brain sample were reduced even further to roughly 40% of control (Table 1). Levels of norepinephrine in the rest of brain sample were not significantly affected (data not shown).

TABLE 1

DOPAMINE LEVELS IN VEHICLE AND METHYLAMPHETAMINE TREATED RATS

	n	Caudate DA*	Rest of brain DA
Vehicle	12	8.96 ± 0.44	0.41 ± 0.05
Methylamphetamine	8	4.80 ± 0.20†	0.17 ± 0.01†

*Data are expressed in μg of dopamine per gram of tissue, ± SE.
†Two-tailed Student's t-test, $p<0.05$.

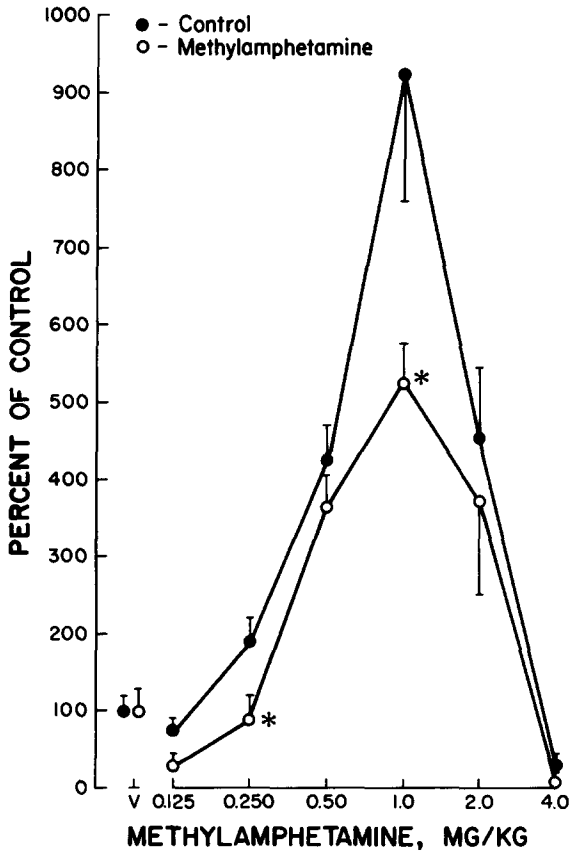

FIG. 1. The effects of methylamphetamine on the locomotor activity of rats previously treated with vehicle or high doses of methylamphetamine. Abscissa: activity counts per hour as percent of control. Ordinate: dose of methylamphetamine in mg/kg. Brackets extend 1 SE. Control, n=6; methylamphetamine, n=4. *$p<0.05$, Wilcoxin test, Mann-Whitney U test.

Locomotor Activity

The dose-response curve for the effects of methylam-

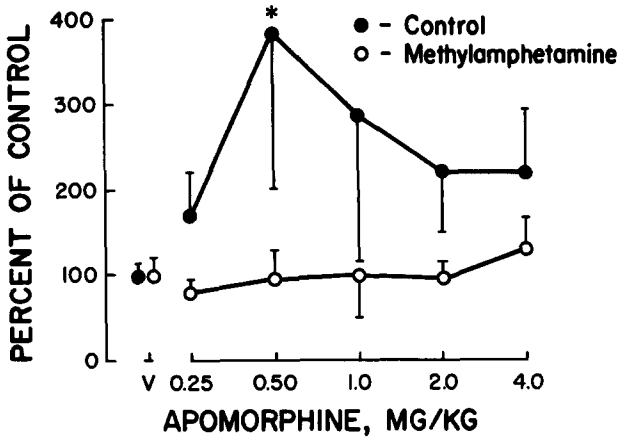

FIG. 2. The effects of apomorphine on the locomotor activity of rats previously treated with vehicle or high doses of methylamphetamine. Abscissa: activity counts per hour as percent of control. Ordinate: dose of apomorphine in mg/kg. Brackets extend 1 SE. Control, n=6; methylamphetamine, n=5. *$p<0.05$, Wilcoxin test.

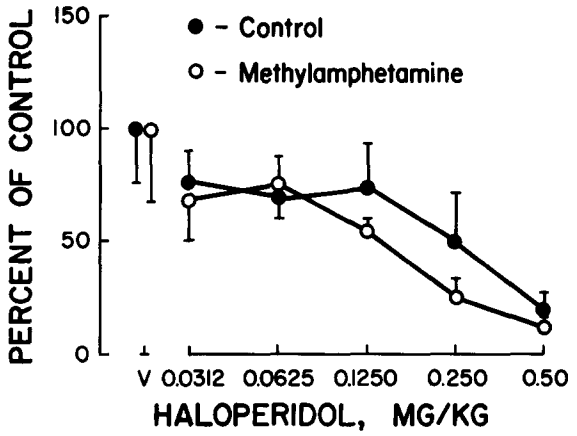

FIG. 3. The effects of haloperidol on the locomotor activity of rats previously treated with vehicle or high doses of methylamphetamine. Abscissa: activity counts per hour as percent of control. Ordinate: dose of haloperidol in mg/kg. Brackets extended 1 SE. Control, n=6, methylamphetamine, n=4.

phetamine on locomotor activity was an inverted "u" shaped function with both groups displaying peak locomotor activity increases at the dose of 1 mg/kg (Fig. 1). However, the rats that received the methylamphetamine treatment did not exhibit increases in locomotor activity equal in magnitude to those seen in control rats. The methylamphetamine treated rats displayed significantly less locomotor activity at the doses of 0.25 and 1 mg/kg (Wilcoxin and Mann-Whitney U tests). The raw data also differed significantly at the doses of 0.25 and 1 mg/kg by the Wilcoxin test.

Apomorphine produced variable results in the control group with 3 rats exhibiting extremely large increases in locomotor activity while the other 3 exhibited small increases or decreases (Fig. 2). The groups differed significantly at the dose of 0.5 mg/kg by the Wilcoxin test. The locomotor activity of the methylamphetamine treated rats was virtually unaffected by any dose of apomorphine.

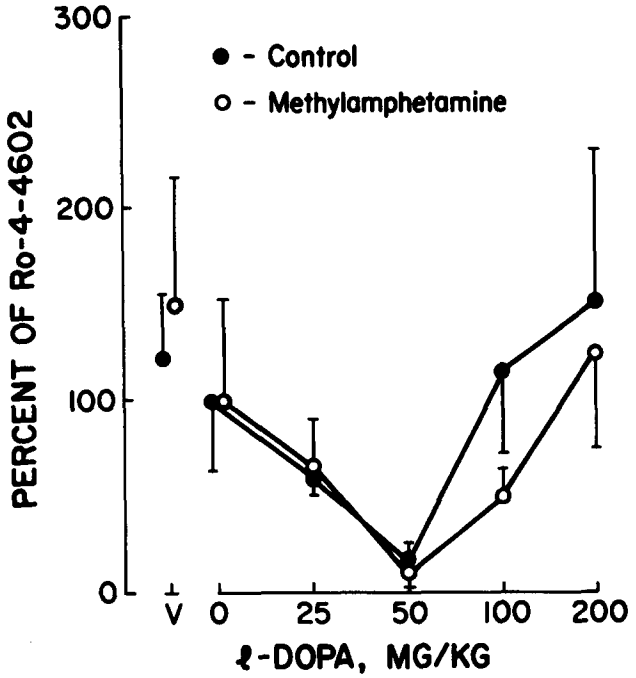

FIG. 4. The effects of l-Dopa plus RO 4-4602 on the locomotor activity of rats previously treated with vehicle or high doses of methylamphetamine. Abscissa: activity counts per hour as percent of the values obtained after RO 4-4602 alone. Ordinate: dose of l-Dopa in mg/kg. Brackets extend 1 SE. Control, n=6; methylamphetamine, n=4.

Haloperidol produced dose-dependent decreases in locomotor activity in both groups (Fig. 3). The methylamphetamine treated rats appeared slightly more sensitive to the locomotor activity decreasing effects of haloperidol, with the doses of 0.125 and 0.25 mg/kg producing roughtly the same (50% and 74%) decrease in locomotor activity as did 0.25 and 0.5 mg, respectively, in the control group. Further, the lowest dose producing a significant decrease in activity was lower in the methylamphetamine treated rats (raw data, paired t-test; percent of control, Wilcoxin and Mann-Whitney U tests), suggesting that the methylamphetamine rats may have been more sensitive to the activity decreasing effects of haloperidol.

L-Dopa produced decreased locomotor activity at the dose of 50 mg/kg and a very small increase at the dose of 200 mg/kg (Fig. 4). Although the dose of 100 mg/kg decreased the locomotor activity of the methylamphetamine treated rats to 50% of control but did not change the activity of the controls, the difference did not reach statistical significance.

DISCUSSION

Treatment of rats with high doses of methylamphetamine for 4 days caused a long lasting decrease in the ability of methylamphetamine and apomorphine to increase locomotor activity. It also made the rats more sensitive to the activity decreasing effects of haloperidol, but produced little change in the effects of L-Dopa in the dose range tested. These effects on the locomotor activity after administration of dopaminergic agents are similar to the effects that treatment

with higher doses of methylamphetamine has on the ability of methylamphetamine to decrease fixed-interval responding in rats [16] and to disrupt DRL responding in rhesus monkeys [6]. The treatment with high doses of methylamphetamine also produced dopamine (but not norepinephrine) depletions lasting 16 weeks, thus extending the time over which the depletions are seen in rats [17].

The attenuation of the increases in activity produced by dopamine agonists is probably related to the decrease in dopamine produced by the methylamphetamine treatment. Permanent depletions of caudate dopamine (to 8% of control) using the neurotoxin 6-hydroxydopamine (6-HDA) also decreases stimulation caused by *d*-amphetamine [12], although smaller lesions of caudate dopamine with 6-HDA (to 46–65%; [3]) or with electrolytic lesions (to 60%; [11]) were without effect. Depletions of non-striatal dopamine (to 30%) also abolished the stimulatory effects of *d*-amphetamine, but enhanced the stimulation of apomorphine [9,10]. Since the noneffective caudate dopamine depletions were roughly the same size as were obtained here, the depletion of non-striatal dopamine in the present study may have caused the attenuation of the locomotor stimulation produced by methylamphetamine. However, in the present study, the non-striatal depletions were only to 40% (Table 1) and the effects of apomorphine were abolished, not enhanced.

Apomorphine produced large increases in the locomotor activity of 3 of the control rats (300–1200%) while producing modest increases or decreases in the other 3 controls. Apomorphine did not produce large changes in any of the methylamphetamine treated rats. There was a significant difference using the Wilcoxin test, but the large variance resulting from the between subject variability in the control group prevented the between group comparisons from reaching statistical significance using the Mann-Whitney U test.

The methylamphetamine treatment produced a one-dose shift to a lower dose in the dose necessary to produce a significant decrease in activity, while the between group comparisons did not reach statistical significance. It is difficult to produce statistically significant decreases in activity when the baseline activity is as low as 20 counts per hour. However this finding is consistent with an enhanced decreased in operant responding after administration of haloperidol both to rhesus monkeys that had received a chronic methylamphetamine treatment (Finnegan *et al.*, in preparation), and in rats responding under a fixed-ratio schedule of food presentation and pretreated with AMPT [1].

There was virtually no change in the effect of l-Dopa after chronic methylamphetamine treatment. The lack of change in the effects could be due to several factors. The dose used did not produce large increases in activity and most of the differences between vehicle and methylamphetamine treated rats in response to the agonists occurred at doses that increased the activity of the vehicle treated group. Another possibility is related to the fact that l-Dopa is also the precursor for norepinephrine, which may also be involved in regulating locomotor activity [7]. As the norepinephrine levels were not altered by the methylamphetamine treatment, l-Dopa may have altered locomotor activity through its action on norepinephrine and this could have masked changes that occurred in the dopaminergic system.

The observed changes in drug effects as a result of the methylamphetamine treatment are probably related to the decrease in dopamine levels. The decreased levels were probably due to neurotoxicity, since this treatment decreases dopamine uptake sites without changing the number or affinity of the binding sites [17]. The lack of shift to the left of the dose-response curves for the agonists here adds further evidence that there is no receptor supersensitivity after this methylamphetamine treatment.

The effects observed were a decrease in the magnitude of the increase in locomotor activity produced by dopaminergic agonists and an increased sensitivity to the decreases produced by haloperidol. The observed attenuation of activity increases could be due to a long term change produced by the methylamphetamine treatment in other (non-dopaminergic) neural systems either as a direct result of the treatment or as a compensatory change induced by the dopamine depletions. Another possibility is a general decrease in the number of dopamine neurons which contribute to maintaining locomotor activity, such that during administration of apomorphine or methylamphetamine, the portion of the locomotor activity due to normal firing of dopamine neurons is attenuated to the point of causing less locomotor activity than would be seen in control rats receiving the same dose. Both of these possibilities are highly speculative, and at this time, the precise mechanism by which the methylamphetamine treatment alters the effects of dopaminergic agents is unknown.

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