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Two Automated Locomotor Activity Tests for Dopamine Autoreceptor Agonists

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SCHREUR, P. J. K. D. AND N. F. NICHOLS. *Two automated locomotor activity tests for dopamine autoreceptor agonists.* PHARMACOL BIOCHEM BEHAV **25**(1)255–261, 1986.—In the first test (exploratory activity), pretreated rats explored a novel environment in the dark. The potential autoreceptor agonists apomorphine HCl, N-n-propylnorapomorphine (NPA), and N-n-propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP) and its enantiomers decreased the total distance travelled while at the same time paradoxically increasing the number of discrete movements. This is a very different pattern from that of the typical antipsychotic drugs haloperidol HCl and chlorpromazine HCl, and the atypical antipsychotic drug clozapine, which also decreased the total distance travelled but decreased the number of movements. Both groups decreased the distance/movement. In the second test, rats were habituated to the monitors in the light and then treated with test drug and stimulant (d-amphetamine sulfate or apomorphine HCl). Apomorphine HCl, NPA, and (+)3-PPP antagonized amphetamine-stimulated locomotor behavior (total distance) without antagonizing apomorphine-stimulated behavior, suggesting a presynaptic dopamine autoreceptor agonism. EMD 23448 gave equivocal activity. On the other hand, haloperidol HCl, chlorpromazine HCl, and clozapine decreased both amphetamine- and apomorphine-stimulated behavior, suggesting a postsynaptic dopamine antagonism. 3-PPP and (−)3-PPP showed neither pattern in this test.

Dopamine Autoreceptor agonists Exploratory Locomotor Amphetamine Apomorphine 3-PPP NPA Haloperidol Chlorpromazine Clozapine EMD 23448

ACCORDING to the dopamine hypothesis of schizophrenia, symptoms are caused by excessive activity in dopaminergic pathways [4,5]. Standard antipsychotic drugs act by antagonizing receptors on the postsynaptic cells [2].

Some dopaminergic synapses have receptors for dopamine on presynaptic as well as postsynaptic neurons. Receptors on the presynaptic terminal are called "autoreceptors." When dopamine, released from the presynaptic terminal, acts on these autoreceptors, it decreases the further release of transmitter [3]. Accordingly, there are two mechanisms by which drugs can inhibit transmission across the synapse, by a presynaptic agonism (as autoreceptor agonists inhibiting the release of dopamine) or a postsynaptic antagonism (as standard antipsychotic drugs, for example, blocking the released dopamine from acting at the receptor).

Dopamine and its postsynaptic agonists stimulate locomotor activity. Thus, antagonism of locomotor behavior has been used to screen for drugs with potential antipsychotic activity [6]. Locomotor activity can be activated either by environmental stimuli, such as novelty, or by drugs. Two drugs which stimulate locomotor activity, by different mechanisms, are amphetamine sulfate (AMPHET) and apomorphine HCl (APO). AMPHET causes the release of dopamine from presynaptic terminals and inhibits its reuptake, while APO mimics dopamine at the postsynaptic receptors. Since both presynaptic agonists and postsynaptic antagonists inhibit locomotor activity, special procedures are required to distinguish between them. This presentation describes two such procedures using automated equipment available from Omnitech Electronics Inc.

GENERAL METHOD

In both experiments, male Sprague-Dawley rats (160–260 g) were group housed under 12 hours light/dark, with testing during the light period. Locomotor activity was measured via infrared photobeams in individual 8-beam Digiscan activity monitors (Model RXYZCM). Absorbent paper was snugly fitted on the floor and changed after each rat. Each monitor was situated in a simple, inexpensive plywood shell which permitted testing in the dark (in Experiment 1). Total distance (inches travelled) and number of discrete movements were calculated by the Digiscan computer (Model DCM-8) and printed every 10 min by an Epson printer in a sound-attenuating chamber. Data were analyzed by Student's *t*-test or Wilcoxon's rank sum.

EXPERIMENT 1. EXPLORATORY ACTIVITY

Method

Naive rats (Charles River, 8/group) were injected IP with test drug 10–20 min before placement into the individual chambers (except clozapine, which was given orally 30 min before). Drugs were solutions in 0.9% saline (except clozapine, which was a suspension in 0.25% methylcellulose). Exploratory locomotor activity was measured in the dark for 10 min.

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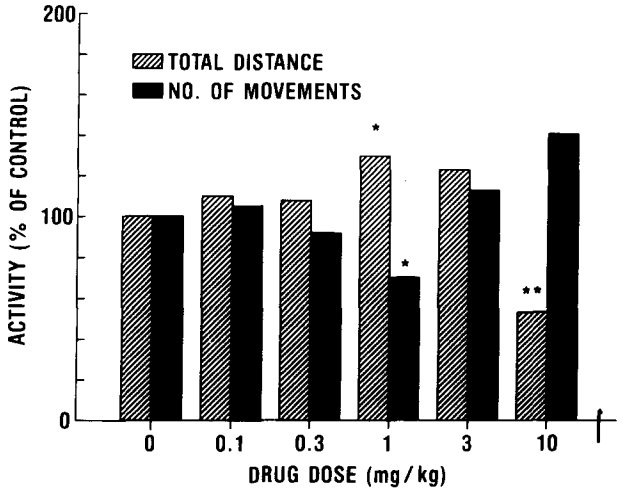

FIG. 1. Effect of d-amphetamine sulfate in the exploratory locomotor activity test (mean total distance and mean number of discrete movements). For all figures, groups are compared to control: $^{*}p<0.05$, $^{**}p<0.01$, $^{+}p<0.001$.

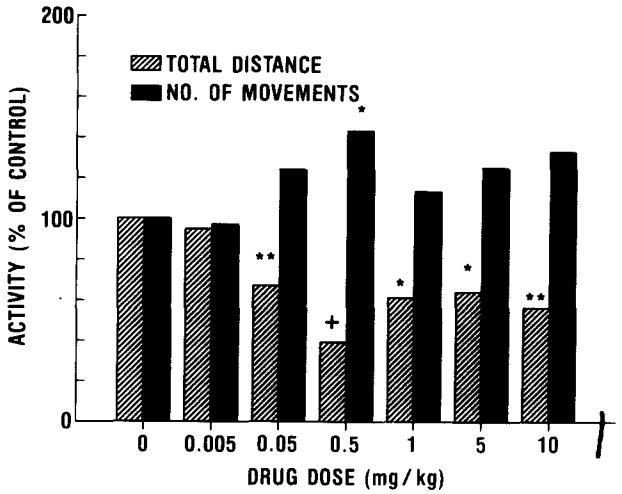

FIG. 2. Effect of apomorphine HCl in the exploratory locomotor activity test.

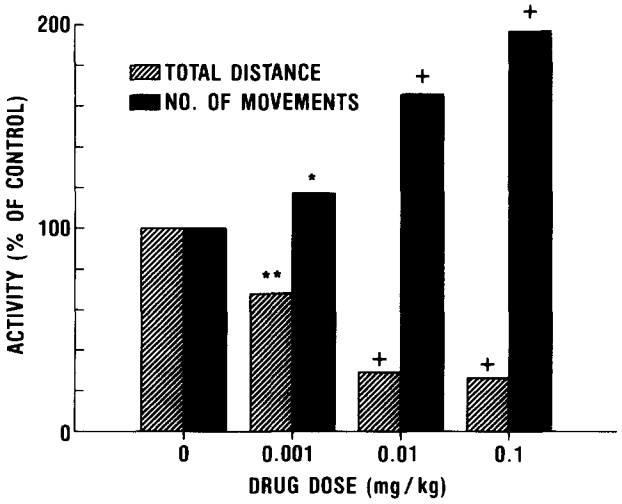

FIG. 3. Effect of NPA in the exploratory locomotor activity test.

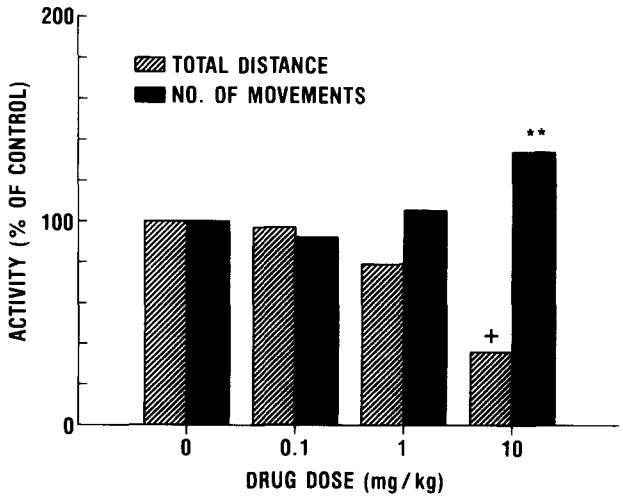

FIG. 4. Effect of 3-PPP in the exploratory locomotor activity test.

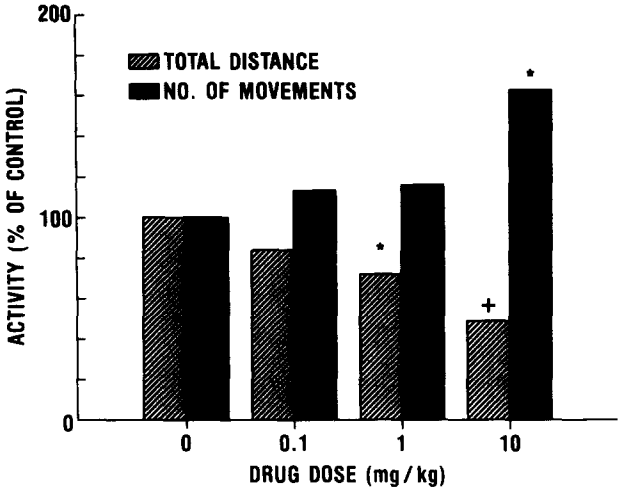

FIG. 5. Effect of (+)3-PPP in the exploratory locomotor activity test.

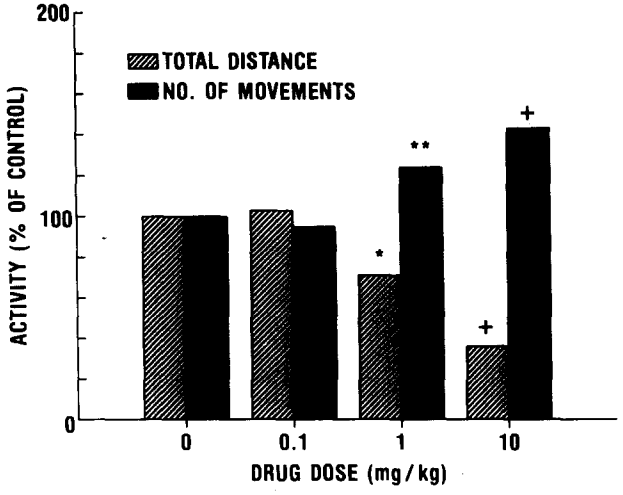

FIG. 6. Effect of (−)3-PPP in the exploratory locomotor activity test.

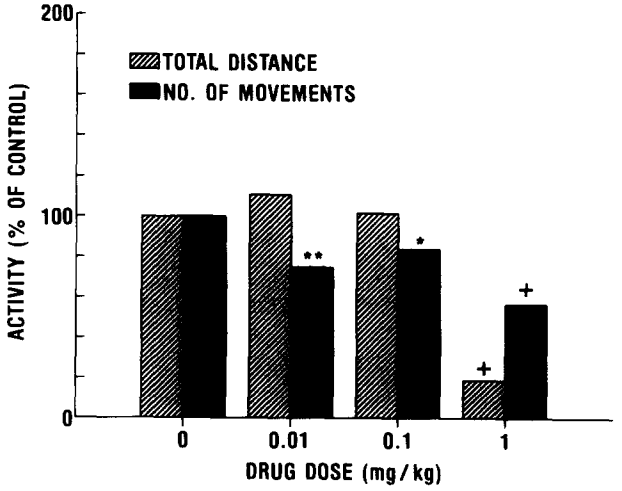

FIG. 7. Effect of haloperidol HCl in the exploratory locomotor activity test.

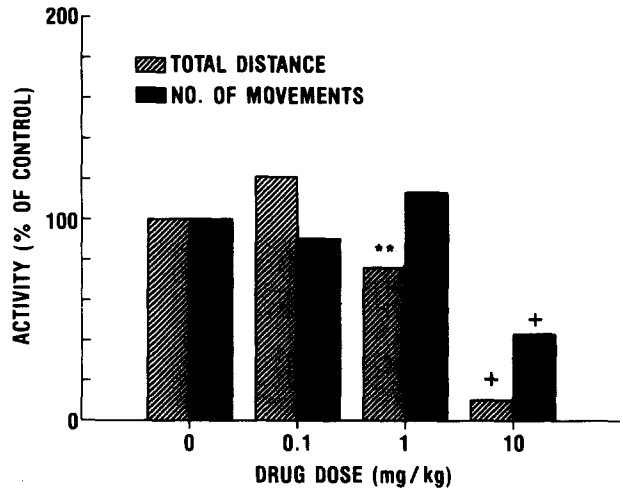

FIG. 8. Effect of chlorpromazine HCl in the exploratory locomotor activity test.

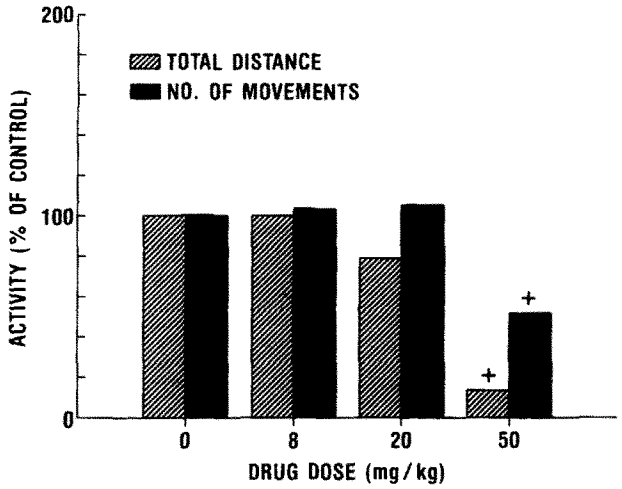

FIG. 9. Effect of clozapine in the exploratory locomotor activity test.

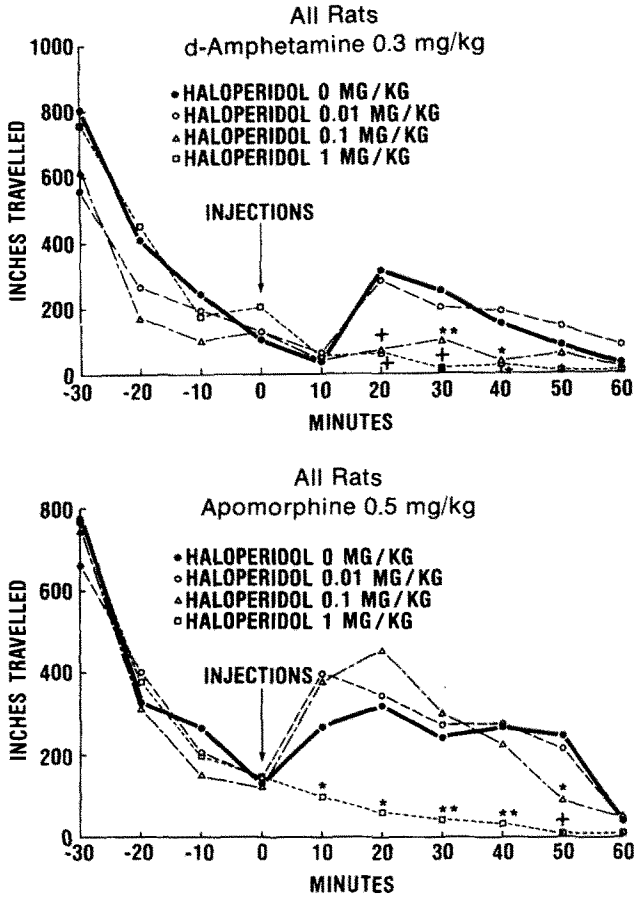

FIG. 10. Top: Effect of haloperidol HCl on locomotor activity stimulated by d-amphetamine sulfate. Bottom: Effect of haloperidol HCl on activity stimulated by apomorphine HCl.

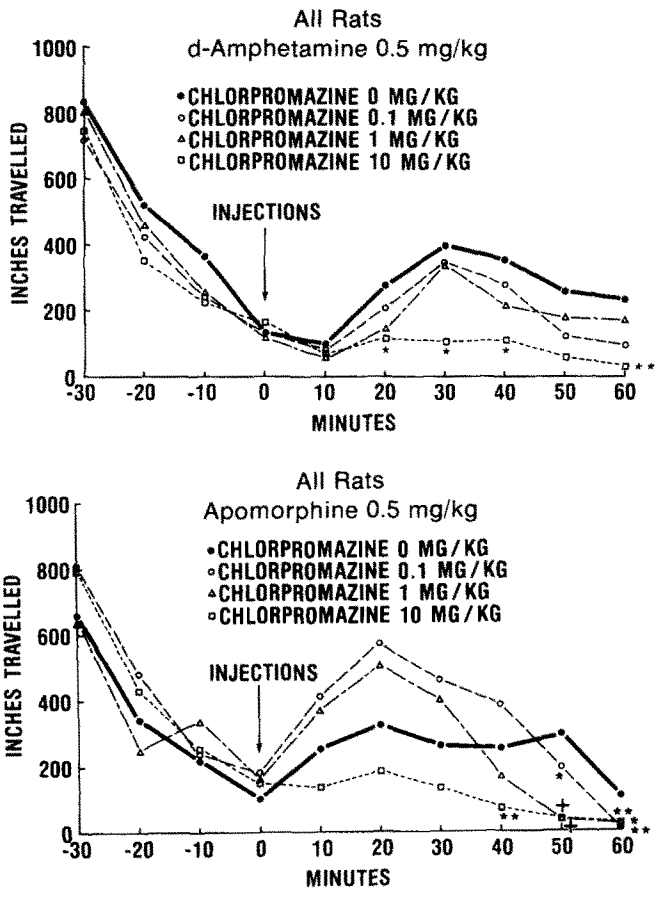

FIG. 11. Top: Effect of chlorpromazine HCl on locomotor activity stimulated by d-amphetamine sulfate. Bottom: Effect of chlorpromazine HCl on activity stimulated by apomorphine HCl.

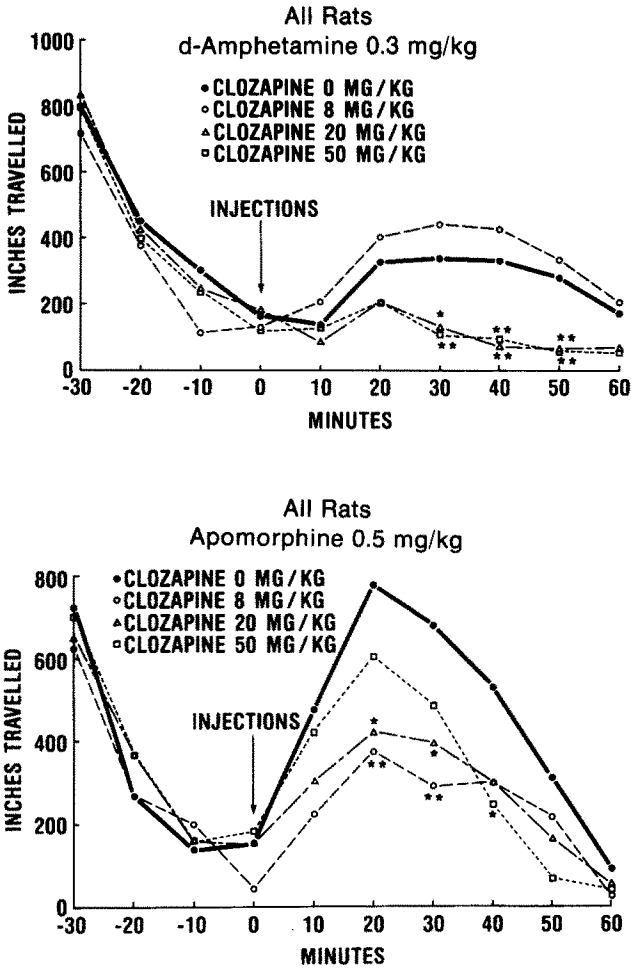

FIG. 12. Top: Effect of clozapine on locomotor activity stimulated by d-amphetamine sulfate. Bottom: Effect of clozapine on activity stimulated by apomorphine HCl.

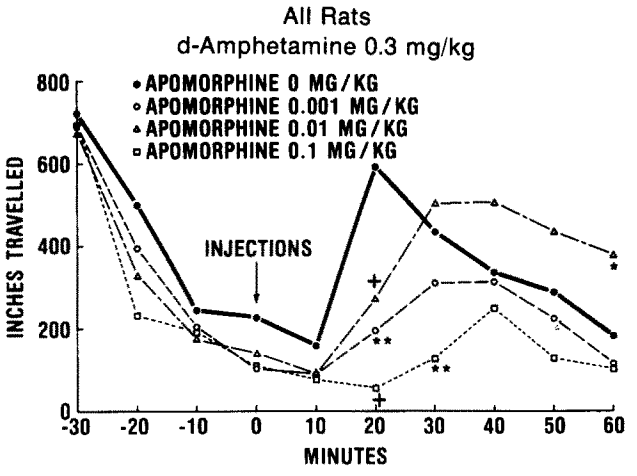

FIG. 13. Effect of apomorphine HCl on locomotor activity stimulated by d-amphetamine sulfate.

Results

Naive rats placed into a dark, novel environment are highly stimulated by the handling and injections, the darkness, and the novelty. In 10 min, saline-injected rats travelled 987±36 (SE) inches and made 39±1 (SE) discrete movements. Further stimulation was very difficult: AMPHET was able to increase total distance only modestly and at only one dose (1 mg/kg) (Fig. 1), and APO also was unable to stimulate rats further (Fig. 2). At high doses, the locomotor activity was inhibited by the competing stereotypical behavior.

The following potential autoreceptor agonists decreased the total distance travelled by exploring rats while at the same time paradoxically increasing the number of discrete movements: APO (Fig. 2), N-n-propylnorapomorphine (NPA) (Fig. 3), and N-n-propyl-3-(3-hydroxyphenyl)-piperidine (3-PPP) and its enantiomers (Figs. 4–6).

On the other hand, standard antipsychotic drugs decreased both the distance and the number of movements: the typical neuroleptics haloperidol HCl and chlorpromazine HCl and the atypical antipsychotic clozapine showed this pattern (Figs. 7–9).

Though the autoreceptor agonists increased the number of movements and the postsynaptic antagonists decreased them, both groups decreased total distance and both decreased the distance/movement.

EXPERIMENT 2. DRUG-STIMULATED ACTIVITY

Method

Naive rats (Upjohn-reared, 8–11/group) were habituated in the light to individual chambers for 40 min, after which time locomotor activity was quite low. They were then injected with two drugs: SC stimulant (either 0.3 mg/kg d-amphetamine sulfate, AMPHET, or 0.5 mg/kg apomorphine HCl, APO) and IP test drug (except clozapine, which was given orally). Drugs were solutions in 0.9% saline or water, except for clozapine and EMD 23448, which were suspensions in water, saline, or 0.25% methylcellulose. Immediately after the two injections, rats were replaced into the chambers and locomotor activity was measured for 60 min in the light.

Results

As expected, haloperidol, chlorpromazine, and clozapine decreased both AMPHET-stimulated and APO-stimulated locomotor activity (total distance), suggesting a postsynaptic dopamine antagonism (Figs. 10–12).

Low doses of APO (0.001–0.1 mg/kg) antagonized AMPHET-stimulated locomotor activity (Fig. 13). NPA (0.001 mg/kg), and (+)3-PPP (1 mg/kg) antagonized AMPHET-stimulated locomotor behavior (total distance) without antagonizing APO-stimulated behavior, suggesting a presynaptic dopamine autoreceptor agonism (Figs. 14–15). 3-PPP and (−)3-PPP showed neither pattern in this test (Figs. 16–17). EMD 23448 gave results only suggestive of autoreceptor agonist activity (Fig. 18).

DISCUSSION

The present study demonstrates two methods for differentiating dopamine autoreceptor agonists from postsynaptic

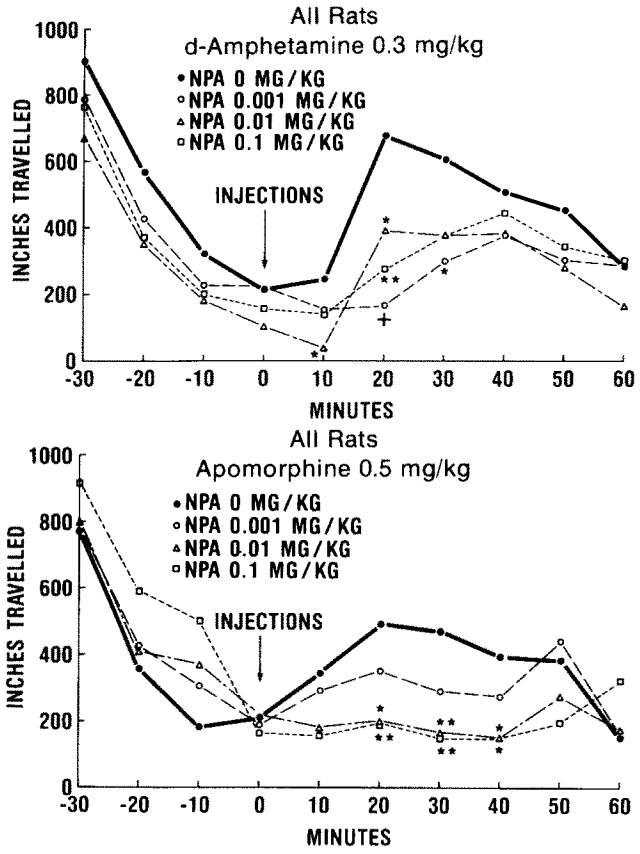

FIG. 14. Top: Effect of NPA on locomotor activity stimulated by d-amphetamine sulfate. Bottom: Effect of NPA on activity stimulated by apomorphine HCl.

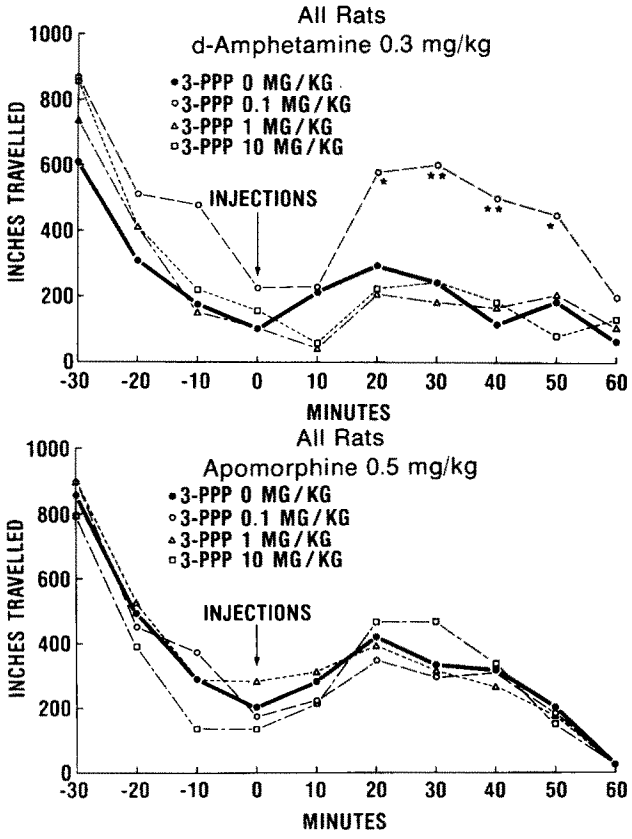

FIG. 16. Top: Effect of 3-PPP on locomotor activity stimulated by d-amphetamine sulfate. Bottom: Effect of 3-PPP on activity stimulated by apomorphine HCL.

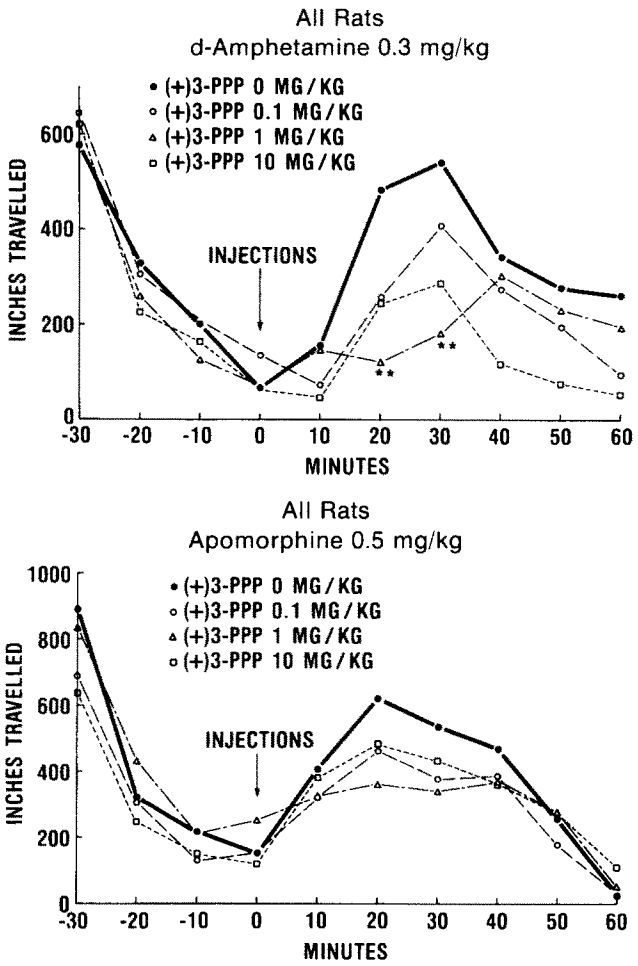

FIG. 15. Top: Effect of (+)3-PPP on locomotor activity stimulated by d-amphetamine sulfate. Bottom: Effect of (+)3-PPP on activity stimulated by apomorphine HCl.

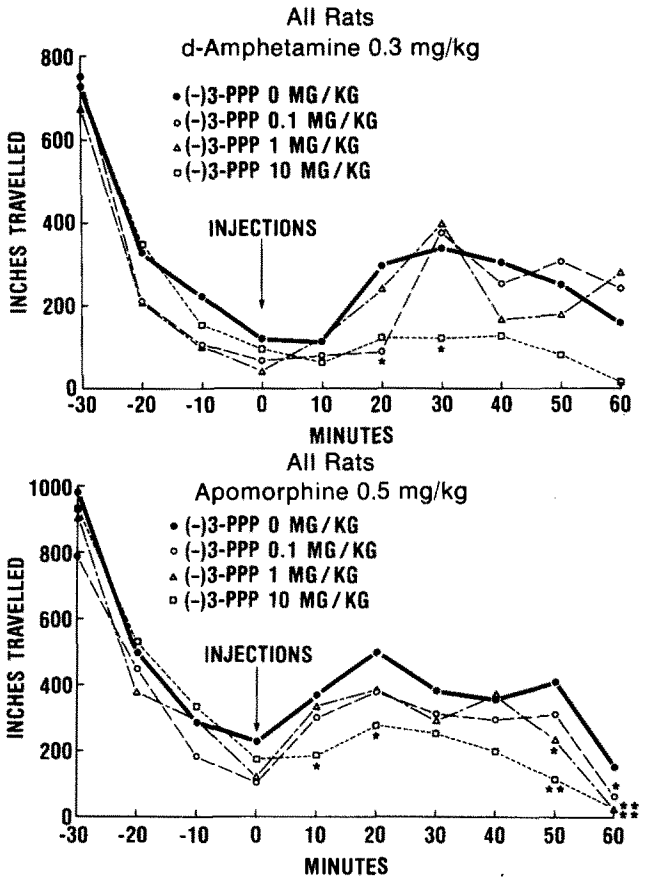

FIG. 17. Top: Effect of (−)3-PPP on locomotor activity stimulated by d-amphetamine sulfate. Bottom: Effect of (−)3-PPP on activity stimulated by apomorphine HCl.

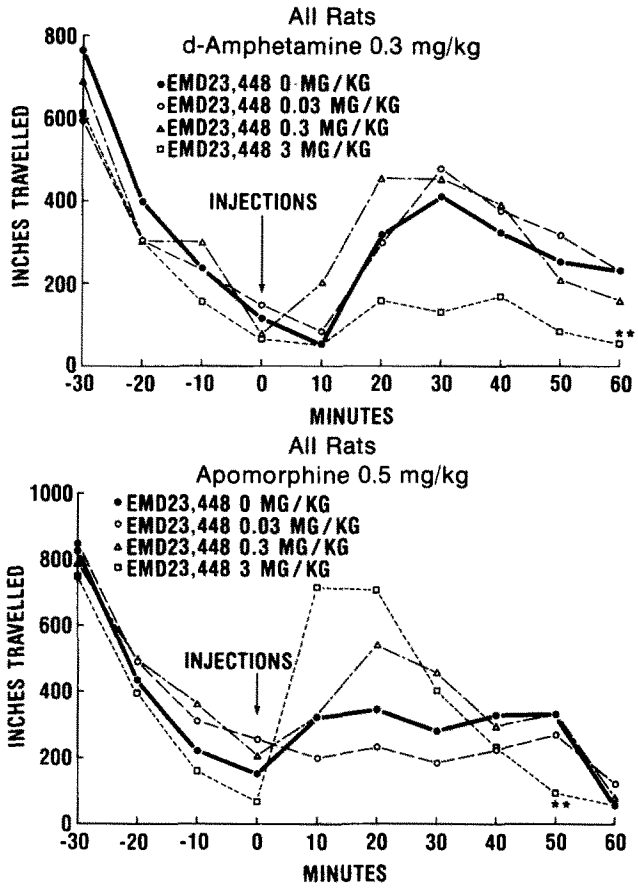

FIG. 18. Top: Effect of EMD 23448 on locomotor activity stimulated by d-amphetamine sulfate. Bottom: Effect of EMD 23448 on activity stimulated by apomorphine HCl.

antagonists by their action on locomotor activity. In exploratory behavior, the putative autoreceptor agonists decreased the total distance travelled while paradoxically increasing the number of discrete movements. The postsynaptic antagonists decreased both the total distance and the number of movements. Both groups decreased the distance per movement.

In drug-stimulated activity the autoreceptor agonists APO, (+)3-PPP, and NPA antagonized AMPHET without antagonizing APO, while the postsynaptic antagonists were active against both stimulants. Surprisingly, 3-PPP and (−)3-PPP showed neither pattern in this test. EMD 23448 has been reported to be a potent autoreceptor agonist in other tests [1]. Its relatively low potency here in comparison to other in vivo models may have been due to its low solubility in the aqueous vehicle used in this test.

ACKNOWLEDGEMENTS

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