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Suppression of Play Fighting by Amphetamine: Effects of Catecholamine Antagonists, Agonists and Synthesis Inhibitors

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BEATTY, W. W., K. B. COSTELLO AND S. L. BERRY. *Suppression of play fighting by amphetamine: Effects of catecholamine antagonists, agonists and synthesis inhibitors.* PHARMACOL BIOCHEM BEHAV **20**(5) 747–755, 1984.—Moderate doses of amphetamine and methylphenidate profoundly depress play fighting in juvenile rats. To test the idea that this behavioral effect was dependent on the release of catecholamines (CAs) we administered haloperidol (0.05–0.8 mg/kg), chlorpromazine (0.5–5 mg/kg), phenoxybenzamine (0.5–20 mg/kg) or propranolol (0.5–20 mg/kg) alone or in combination with 0.5 or 1 mg/kg d-amphetamine sulfate. None of these CA antagonists reversed the suppression of play fighting (indexed by pinning) caused by amphetamine, but at higher doses haloperidol, chlorpromazine and phenoxybenzamine depressed both pinning and rearing. The presynaptic NE agonist clonidine (0.05–0.2 mg/kg) also failed to block the effects of amphetamine on play; instead it too depressed both pinning and rearing. Finally the CA synthesis inhibitor, α-methyltyrosine (total dose: 100 mg/kg) did not attenuate the suppression of play by amphetamine. Ephedrine (10–80 mg/kg) mimicked the effects of amphetamine on pinning, but apomorphine did not. At doses from 0.125–0.5 mg/kg apomorphine stimulated pinning while 1 mg/kg had no effect. The present findings confirm earlier reports that amphetamine suppresses play fighting but the mechanism of action remains obscure.

Play fighting Amphetamine Haloperidol Phenoxybenzamine Propranolol Clonidine Chlorpromazine Ephedrine Alpha-methyltyrosine Apomorphine

PLAY fighting is a component of the social behavior of young rats and other mammals [1,9] which is profoundly depressed by moderate doses of amphetamine or methylphenidate [4]. Neither adrenal demedullation nor peripheral administration of 6-hydroxydopamine, alone or in combination, alter the effects of amphetamine on play. Further, in intact animals, amphetamine depresses play at much lower doses than 4-OH-amphetamine which penetrates the CNS rather poorly [3]. These observations suggest that peripheral sympathomimetic effects of amphetamine are probably not responsible for its effects on play. Because many of the behavioral effects of amphetamine result from its action as an indirect CA agonist in the CNS it seemed reasonable to suppose that this action might account for its effect on play. In the initial experiment we attempted to test this hypothesis by examining the effects on play fighting of CA antagonists, administered alone or in combination with amphetamine.

EXPERIMENT 1

METHOD

Animals

Subjects were male albino rats obtained from the Holtzman Co., Madison, WI at 21 days of age. Different groups of animals were used in the three phases of the study in which the effects of haloperidol (N=45 pairs), phenoxybenzamine (N=49 pairs) and propranolol (N=32 pairs) were examined. Both rats in each pair received a single dose of a particular drug. The rats were caged singly with free access to food and water in an air-conditioned animal room (22±3°C) that was illuminated from 0700–1900. Relative humidity ranged from 20–35%.

Procedure

All testing occurred during the light phase of the LD cycle. At 25 days of age the rats were habituated individually to the testing apparatus, a 51×32×47 cm high box made of plywood and clear plastic (see [3] for details). Test pairs were then formed and for the following 6 days each pair received a 10 minute long test. The number of rearing (either rat raises its forepaws at least one cm off the floor) and pinning (one rat rolls the other onto its dorsal surface and stands over it) responses made by the pair were recorded for each session. Rearing was included as a concurrent measure of gross motor activation, although it is recognized that this behavior also reflects exploratory activity and probably other processes as well [2]. In addition, the last 4 sessions were videotaped and the duration of play fighting (tail-

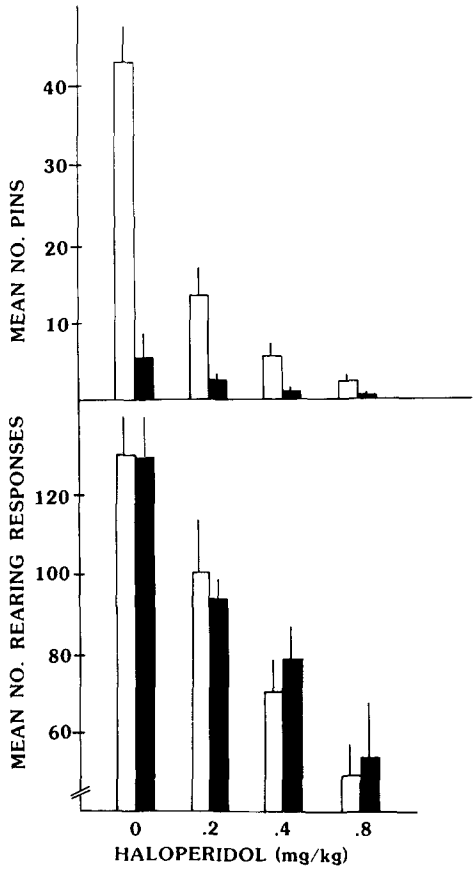

FIG. 1. Mean frequency of pins and rears per session at varying doses of haloperidol. Black bars indicate amphetamine treatment. White bars denote saline treatment. (±SEM.)

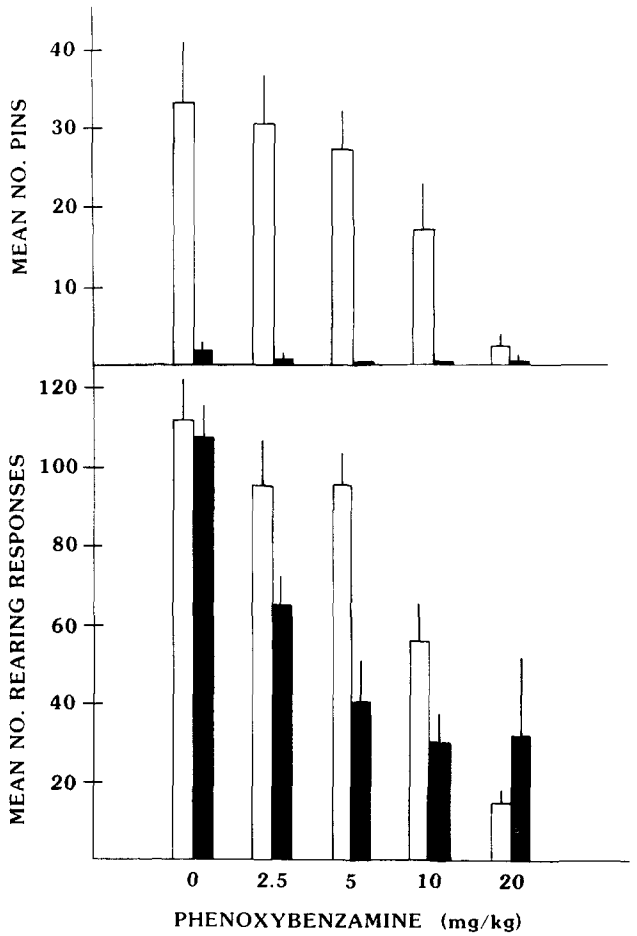

FIG. 2. Mean frequency of pins and rears per session at varying doses of phenoxybenzamine. Black bars indicate amphetamine treatment. White bars denote saline treatment. (±SEM.)

pulling, chasing, boxing, wrestling, pinning and aggressive grooming) was scored. Behaviors were scored from the videotapes. Interrater reliabilities were high (r=0.94 for pins and 0.92 for rears). Although drug treatments for particular pairs were not noted on the videotapes the raters also tested the subjects so we cannot be sure that the scoring was completely blind. Drug treatments were administered on the last 3 days. Test pairs were assigned to drug treatment conditions so as to minimize differences among groups on the pinning measure on pre-drug baseline days. Both members of a test pair received the same combination of drug treatments and test pairs remained intact throughout the study. As in previous studies [3, 4, 18] the frequency of pinning was highly and positively correlated with the duration of play fighting ($r \geq 0.93$). Drug effects on the 2 measures were also quite similar so only the data on the pinning measure are reported.

All drugs were administered IP in a volume of 1 ml/kg. Haloperidol was prepared by diluting the stock solution (Haldol, McNeil, 5 mg/ml) with saline; other drugs were dissolved in saline. Pairs in the haloperidol phase of the study received 0, 0.2, 0.4 or 0.8 mg/kg haloperidol 30 minutes before testing and 0 or 1 mg/kg d-amphetamine sulfate (Dexedrine, Smith Kline) 20 minutes prior to testing. Rats in the phenoxybenzamine phase received 0, 2.5, 5, 10 or 20 mg/kg phenoxybenzamine HCl (Dibenzyline, Smith Kline) 90 minutes prior to testing and 0 or 1 mg/kg d-amphetamine 20 minutes before testing. In the propranolol phase the animals received 0, 10 or 20 mg/kg propranolol HCl (Inderal, Ayerst) 20 minutes prior to testing and 0 or 1 mg/kg d-amphetamine 20 minutes before the start of testing. Except for haloperidol doses are expressed as the weight of the salt. There were 5–8 pairs in each of the 8 conditions in the haloperidol study, 4–7 pairs in each of the 10 conditions of the phenoxybenzamine study and 5–6 pairs in each of the 6 conditions of the propranolol study. Each pair received the same combination of drugs throughout the study. Since we did not score the behavior of the individual rats separately, each pair contributed a single score for pins and for rears for each test day. Unweighted means ANOVAs were performed on both dependent measures. All pairs completed all tests.

RESULTS

As seen in Fig. 1, both amphetamine, $F(1,37)=37.21$, $p<0.001$, and haloperidol, $F(3,37)=25.34$, $p<0.001$, depressed pinning, but the effect of haloperidol depended on whether or not the animals also received amphetamine (Haloperidol × Amphetamine: $F(3,37)=15.22$, $p<0.001$). Subsequent analysis showed that haloperidol reduced pinning if the pairs did not receive amphetamine, $F(3,19)=26.89$, $p<0.001$. In this condition every haloperidol dose depressed pinning relative to the saline control condition ($Fs \geq 18.53$,

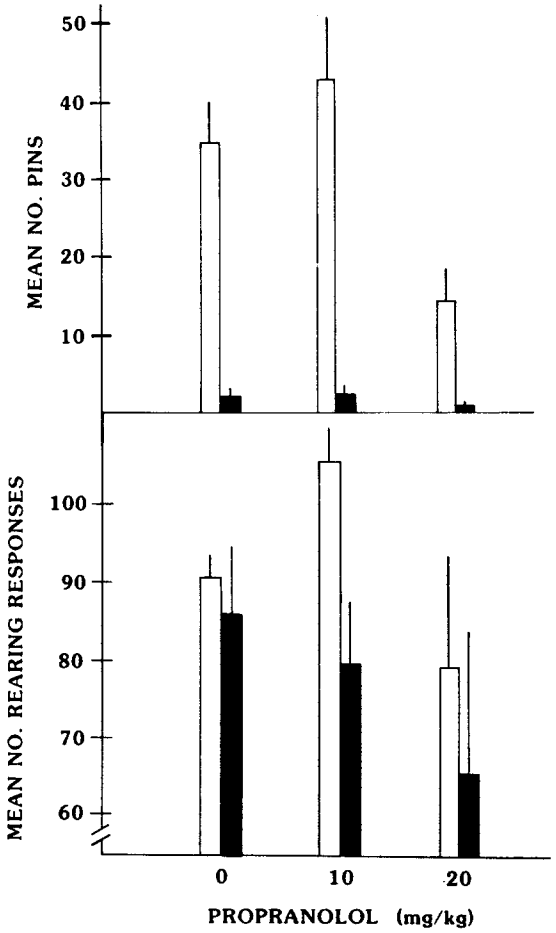

FIG. 3. Mean frequency of pins and rears per session at varying doses of propranolol. Black bars indicate amphetamine treatment. White bars denote saline treatment. (±SEM.)

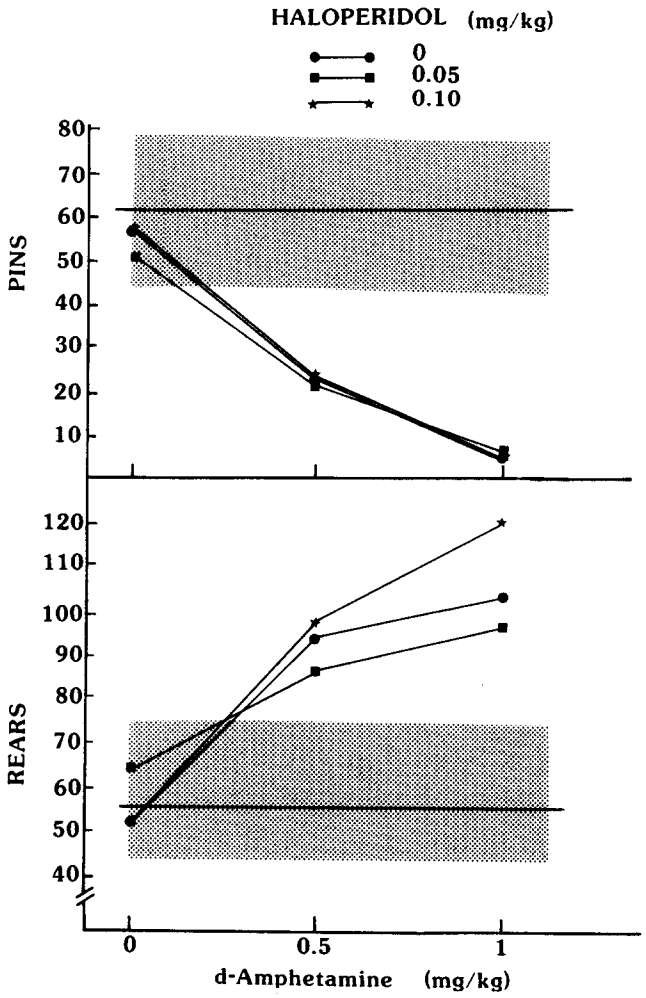

FIG. 4. Mean number of pins and rears at varying doses of amphetamine and haloperidol. Horizontal line denotes the overall mean performance under no treatment; shaded area is the range of means on no treatment test days.

$p<0.001$). In addition the 0.8 mg/kg dose was more effective than the 0.2 mg/kg dose, $F(1,8)=8.64$, $p<0.02$. Other comparisons were not significant. In the pairs that received amphetamine, treatment with haloperidol tended to depress pinning, but the effect was not reliable presumably because of the marked depression in responding caused by amphetamine. Of greater significance was the fact that haloperidol did not reverse the depression in pinning resulting from amphetamine. In fact, amphetamine significantly reduced pinning at every dose of haloperidol. Thus the effects of haloperidol and amphetamine were roughly additive.

Haloperidol also depressed rearing, $F(3,37)=21.19$, $p<0.001$, but amphetamine did not influence this measure ($F<1$) (Fig. 1). The effect of haloperidol was dose-dependent; every dose differed significantly from every other dose ($Fs\geq 5.38, p<0.05$). The magnitude of the haloperidol effect on rearing increased with successive days, suggesting a cumulative effect of the drug on this measure, but the conclusions are in no way altered if only the first day's data are considered. No such cumulative influence was seen on the pinning measure, perhaps because of a floor effect.

Figure 2 describes the effects of phenoxybenzamine and amphetamine on pinning and rearing. Again pinning was depressed by phenoxybenzamine, $F(4,39)=3.62$, $p<0.02$, or amphetamine, $F(1,39)=51.81$, $p<0.001$, and the influence of phenoxybenzamine depended on whether or not the rats also received amphetamine (Phenoxybenzamine × Amphetamine: $F(4,39)=3.14$, $p<0.03$). Subsequent analysis showed that the effect of phenoxybenzamine was reliable, $F(4,20)=3.51, p<0.03$, if the rats also received saline, but not if they received amphetamine ($F=1.52$). Only the highest (20 mg/kg) dose of phenoxybenzamine reliably depressed pinning ($p<0.02$). Again treatment with phenoxybenzamine did not antagonize the depression in pinning caused by amphetamine; amphetamine depressed pinning at every phenoxybenzamine dose and this effect was reliable at all doses except the 20 mg/kg condition.

Phenoxybenzamine also depressed rearing. Surprisingly, the effect was greater if the animals also received amphetamine (Phenoxybenzamine × Amphetamine: $F(4,39)=3.25$, $p<0.05$). Subsequent analyses showed that among saline-treated pairs only the 10 and 20 mg/kg doses of phenoxybenzamine depressed rearing ($F\geq 14.21$, $p<0.001$) while among amphetamine treated rats all phenoxybenzamine doses reduced rearing ($F\geq 14.23$, $p<0.001$). Again

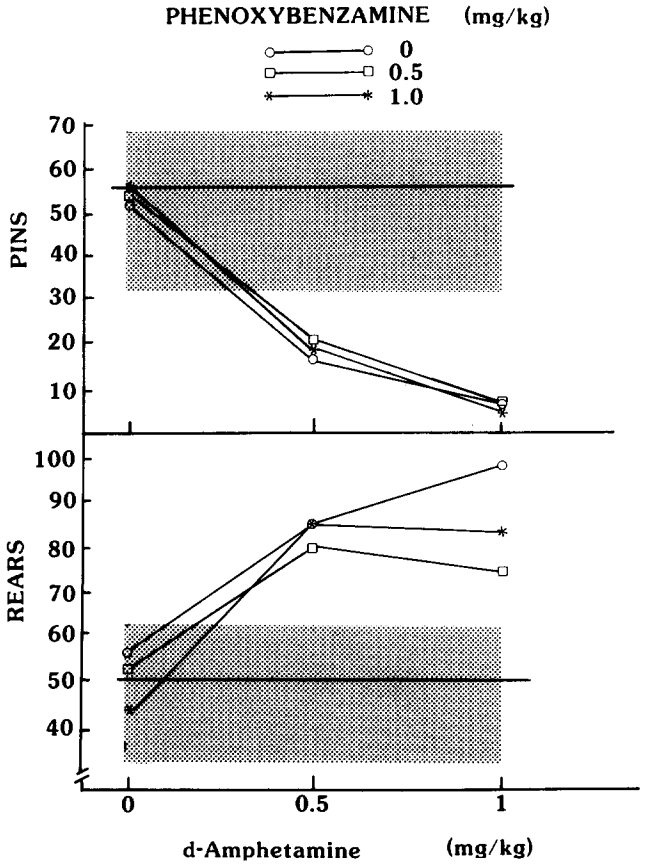

FIG. 5. Mean number of pins and rears at varying doses of amphetamine and phenoxybenzamine. Horizontal line denotes the overall mean performance under no treatment; shaded area is the range of means on no treatment test days.

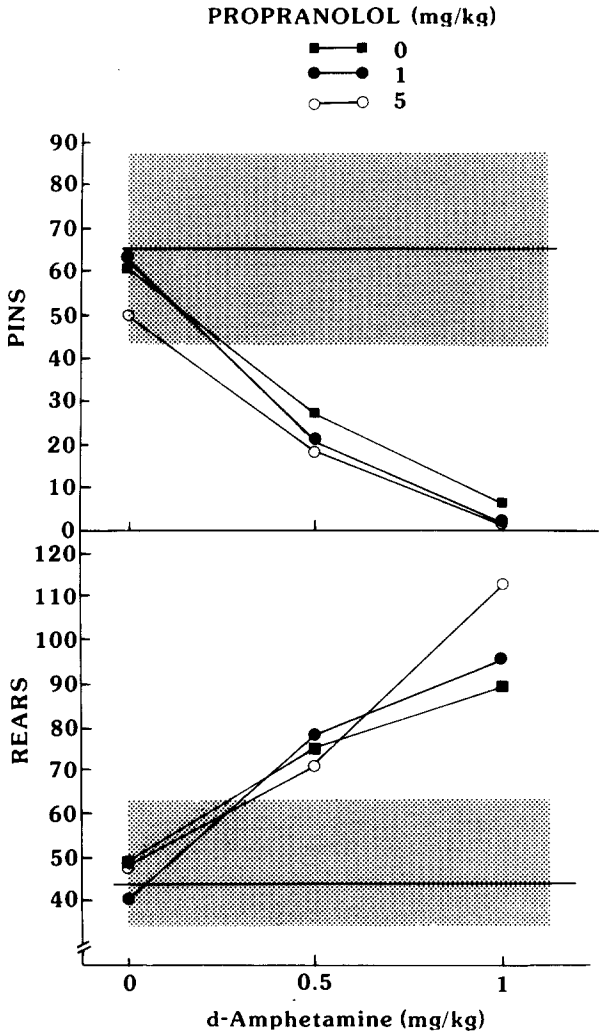

FIG. 6. Mean number of pins and rears at varying doses of amphetamine and propranolol. Horizontal line denotes the overall mean performance under no treatment; shaded area is the range of means on no treatment test days.

the effects of phenoxybenzamine increased with successive days, but the conclusions are not altered if only the first day is considered.

Treatment with propranolol also depressed pinning, $F(2,26)=7.07$, $p<0.01$, but only at the 20 mg/kg dose (see Fig. 3). In this experiment amphetamine also reduced pinning, $F(1,26)=79.96$, $p<0.001$, and the propranolol effect depended on whether or not the rats also received amphetamine (Propranolol × Amphetamine: $F(2,26)=5.94$, $p<0.01$). Among saline-treated pairs propranolol affected pinning, $F(2,13)=6.60$, $p<0.02$, but only at the higher dose. Treatment with 20 mg/kg propranolol depressed pinning ($p<0.05$), but 10 mg/kg propranolol slightly, but insignificantly enhanced performance on this measure. Among amphetamine-treated pairs propranolol did not reliably affect pinning.

Propranolol also failed to antagonize the depression in pinning caused by amphetamine. Amphetamine-treated pairs exhibited significantly fewer pinning responses at each propranolol dose. Neither propranolol nor amphetamine significantly affected rearing (see Fig. 3), although the higher propranolol dose tended to depress rearing.

EXPERIMENT 2

In the previous experiment none of the CA antagonists tested reversed the depression of play caused by 1 mg/kg amphetamine. However, since the doses employed were rather high and only one dose of amphetamine was employed, nonspecific actions of the antagonists might have masked their antagonistic effects on suppression of play. In the present study lower doses of haloperidol, phenoxybenzamine and propranolol were studied and the dose of amphetamine was also varied.

METHOD

Animals

Male albino rats obtained from the Holtzman Co. at 21 days of age were used. Separate groups (N=9 pairs each) were used in the haloperidol, phenoxybenzamine and propranolol phases of the experiment. Maintenance and housing conditions were the same as in the first experiment.

Procedure

At 24 days of age the rats were assigned at random to test pairs. Once formed these pairs remained intact for the dura-

tion of the study. From days 24–26 the animals were habituated to the apparatus for 10 min each day. For the following 17 days each pair was tested for 10 min each day. Pinning and rearing were quantified as in Experiment 1.

Drug doses were delivered IP at a volume of 1 ml. Except for haloperidol all agents were dissolved in saline and doses are expressed as the weight of the salt. Haloperidol was prepared by diluting the 5 mg/ml solution of Haldol with saline. Drugs were administered every other day starting at Day 27. On the intervening days the rats were tested without treatment.

For the haloperidol phase both rats in each pair received 0, 0.05 or 0.1 mg/kg haloperidol 30 min before the test and 0, 0.5 or 1.0 mg/kg d-amphetamine sulfate 20 min before testing. Each pair was tested once at each of the nine treatment combinations; the order of treatments was counterbalanced using a Latin square design.

The experimental procedure was identical for the pairs tested with the phenoxybenzamine and propranolol except that phenoxybenzamine (0, 0.5 or 1 mg/kg) was given 90 min before behavioral testing while propranolol (0, 0.5 or 1.0 mg/kg) was given 20 min before testing. Again each pair received all possible combinations of (0, 0.5 or 1.0 mg/kg) amphetamine and the appropriate antagonist with the order of treatments varied using a Latin square design.

Initial analyses of the data from no treatment days indicated that drug effects on behavior had dissipated within 24 hr and that there were no consistent changes as a function of age or repeated testing. Accordingly the data were analyzed with repeated measures ANOVAs including the factors: amphetamine dose and antagonist dose. Separate analyses were conducted for pinning and rearing for each antagonist.

RESULTS

As seen in Fig. 4 amphetamine reduced the number of pins, $F(2,16)=91.76$, $p<0.001$, and increased the number of rears, $F(2,16)=13.75$, $p<0.001$. By contrast, haloperidol had no effect on either measure ($Fs<1.64$). Of particular importance was the finding that neither haloperidol dose reversed the suppression of pinning caused by either amphetamine dose.

The results with phenoxybenzamine (Fig. 5) were quite similar. Again amphetamine reduced pinning, $F(2,16)=85.62$, $p<0.001$, and increased rearing, $F(2,16)=16.47$, $p<0.001$. Phenoxybenzamine did not affect pinning ($F<1$) and slightly reduced rearing, $F(2,16)=4.29$, $p<0.05$. But there was no indication that this antagonist reversed the suppression of pinning resulting from amphetamine.

Propranolol also did not antagonize the effects of amphetamine on play (Fig. 6). Amphetamine reduced the number of pins, $F(2,16)=54.58$, $p<0.001$, and increased rearing frequency, $F(2,16)=21.59$, $p<0.001$. Propranolol had no effect on rearing ($F<$) and tended to reduce pinning at the higher dose, but the effect was not reliable ($F=3.02$, $p>0.05$).

EXPERIMENT 3

Administering the CA antagonists haloperidol, phenoxybenzamine or propranolol in a wide range of doses failed to reverse the suppression of play fighting (indexed by pinning) caused by amphetamine.

To test the idea that the effect of amphetamine on play fighting might involve both DA and NE systems acting together we assessed the effect of chlorpromazine, a DA antagonist which also blocks α_1 synapses, on the suppression of pinning by amphetamine.

TABLE 1

AMPHETAMINE AND CHLORPROMAZINE EFFECTS ON PINNING AND REARING

Chlorpromazine HCl (mg/kg)	Amphetamine Sulfate (mg/kg) 0	0.5	1.0
	Mean Number of Pins		
0	26.4	5.5	1.3
0.5	20.0	3.0	0.3
5.0	0.8	0	0
	Mean Number of Rears		
0	56.5	99.8	93.6
0.5	78.6	108.9	110.1
5.0	47.5	38.4	48.0

METHOD

Nine pairs of male albino rats obtained from Holtzman at 21 days of age were used. Maintenance, adaptation and testing procedures were identical to those employed in Experiment 2. Again drugs were administered every other day starting at 27 days of age. On alternate (no treatment) days the animals were simply tested. Pairs received 0, 0.5 or 5 mg/kg chlorpromazine HCl (Thorazine, Smith Kline) 30 min before the test and 0, 0.5 or 1.0 mg/kg d-amphetamine sulfate 20 min before testing. Amphetamine was dissolved in saline; chlorpromazine HCl was prepared by diluting the stock solution (Thorazine, 25 mg/ml) with saline. All drugs were given IP at 1 ml/kg. In both phases the order of administration was counterbalanced using a Latin square design such that each pair received each combination of drug treatments once and only once. One rat died leaving 8 pairs.

RESULTS

Chlorpromazine failed to reverse the effect of amphetamine on pinning as seen in Table 1. Again amphetamine depressed pinning, $F(2,14)=24.87$, $p<0.001$, and increased rearing, $F(2,14)=6.64$, $p<0.01$. Chlorpromazine depressed both measures, $Fs(2,14)>12.73$, $p<0.001$. Subsequent analyses showed that only the 5 mg/kg dose affected pinning or rearing relative to the appropriate control (all $p<0.05$). The only exception was that chlorpromazine had no reliable effect on pinning when the rats also received 1 mg/kg amphetamine. However, this dose of amphetamine reduced pinning to 0 in 6 of the 8 pairs.

EXPERIMENT 4

In this experiment we examined the effect of three CA agonists: apomorphine, a relatively selective DA agonist, ephedrine, an indirect CA agonist which also directly stimulates α_1 and β receptors and clonidine, which acts preferentially as an α_2 agonist. Since clonidine is known to inhibit release of NE by virtue of its effects on presynaptic receptors, we anticipated that it might reverse the effect of am-

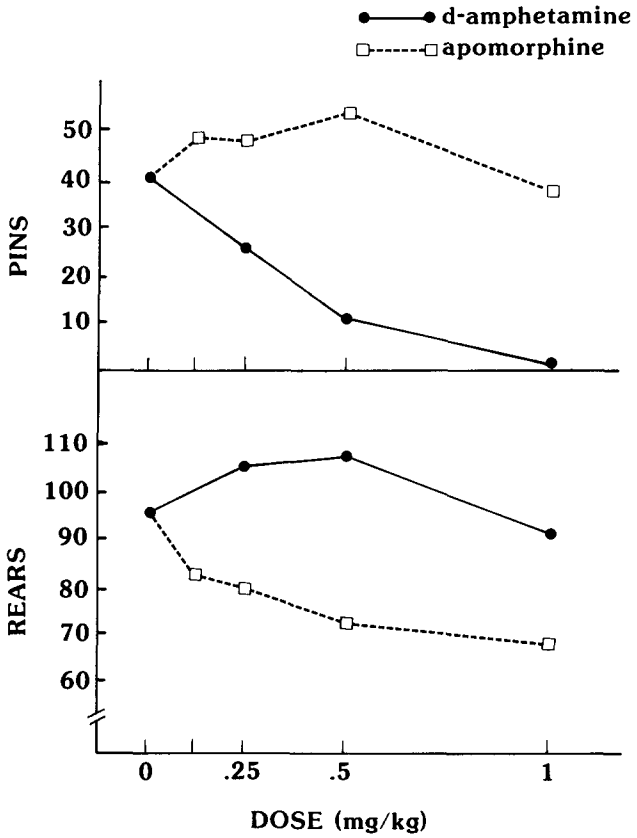

FIG. 7. Mean number of pins and rears at varying doses of amphetamine and apomorphine.

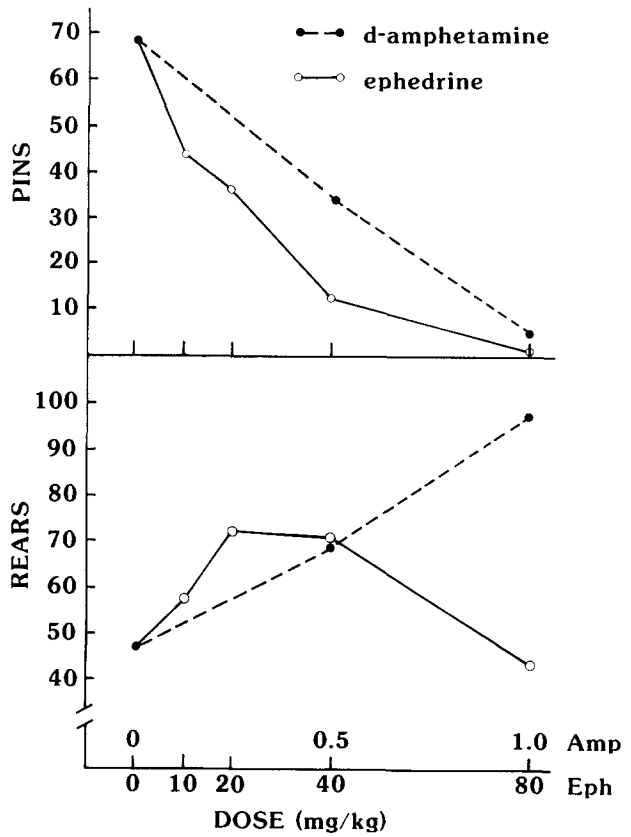

FIG. 8. Mean number of pins and rears at varying doses of amphetamine and ephedrine.

phetamine on play fighting. Further, if CAs mediate the effect of amphetamine on play either apomorphine or ephedrine or both would be expected to mimic the behavioral action of amphetamine.

METHOD

Separate groups of juvenile rats obtained from Holtzman were used in the apomorphine (N=16 pairs), ephedrine (N=11 pairs), and clonidine (N=9 pairs) phases of the study. General maintenance, adaptation and testing procedures were the same as in the preceding experiments.

Animals in the apomorphine experiment received either 0, 0.25, 0.5 or 1.0 mg/kg d-amphetamine sulfate or 0.125, 0.25, 0.5 or 1.0 mg/kg apomorphine HCl (Lilly) 20 min before testing. Each pair received each treatment only once. Drugs were administered every other day and the order of treatments was counterbalanced. The rats were not tested on the intervening days.

Pairs in the ephedrine study received either 0, 0.5 or 1.0 mg/kg d-amphetamine sulfate or 10, 20, 40 or 80 mg/kg ephedrine HCl (Sigma) 20 min before testing. Each pair received each treatment only once. Drugs were given on alternate days and the order of treatments was counterbalanced. On the intervening days the rats were tested in the usual way without drug treatment. Since there was no difference between the no treatment and saline tests, data from the no treatment condition are not reported.

Animals in the clonidine experiment received either 0, 0.5 or 0.2 mg/kg clonidine HCl (Catapres, Roeringer) 60 min prior to testing and 0, 0.5 or 1.0 mg/kg d-amphetamine sulfate 20 min before the tests. The order of treatments was varied according to a Latin square design. Drugs were administered every other day. On alternate days the rats were simply tested without drug treatment. All drugs were dissolved in saline and administered IP. Amphetamine, apomorphine and ephedrine were given at 1 ml/kg; clonidine was given at 2 ml/kg.

For both pins and rears data from the apomorphine and ephedrine studies were analyzed using repeated measures ANOVAs which evaluated the effects of amphetamine and either apomorphine or ephedrine separately. Data from the clonidine experiment were analysed using a within-subjects factorial design that evaluated the effects of varying doses of clonidine and amphetamine.

RESULTS

The effects of apomorphine and amphetamine are compared in Fig. 7. Amphetamine decreased the number of pins, $F(3,45)=38.17$, $p<0.001$, and tended to increase the number of rears although the latter effect was not reliable. By contrast, apomorphine increased the number of pins, $F(4,60)=3.27$, $p<0.02$, and reduced the number of rears, $F(4,60)=3.70$, $p<0.01$. Subsequent analyses of the apomorphine effects revealed that doses of apomorphine from 0.125–0.5 mg/kg increased the number of pins ($p<0.05$), but the 1 mg/kg dose was without effect. All apomorphine doses 0.25 mg/kg or greater reduced rearing reliably ($p<0.05$).

TABLE 2

AMPHETAMINE AND CLONIDINE EFFECTS ON PINNING AND REARING

Clonidine HCl (mg/kg)	Amphetamine Sulfate (mg/kg) 0	0.5	1.0
	Mean Number of Pins		
0	41.2	13.3	0.9
0.05	0	0	0
0.2	2.0	0	0
	Mean Number of Rears		
0	45.8	69.3	69.8
0.05	18.9	35.3	28.9
0.2	14.3	10.9	25.7

As seen in Fig. 8 amphetamine and ephedrine had qualitatively similar effects on pinning and rearing. Both agents suppressed pinning, $F(2,20)=29.64$, $p<0.001$ for amphetamine and $F(4,40)=25.32$, $p<0.001$ for ephedrine, and increased rearing, $F(2,20)=14.86$, $p<0.001$ for amphetamine and $F(4,40)=5.20$, $p<0.01$ for ephedrine. Subsequent analyses showed that all doses of both drugs depressed pinning ($p<0.05$). Both amphetamine doses as well as the 20 and 40 mg/kg ephedrine doses increased the number of rearing responses ($p<0.05$).

As seen in Table 2 both doses of clonidine profoundly depressed rearing, $F(2,14)=27.87$, $p<0.001$, and virtually eliminated pinning, $F(2,16)=26.64$, $p<0.001$. These effects were transient and behavior recovered to normal by the following day. Amphetamine reduced pinning, $F(2,16)=13.53$, $p<0.001$, and increased rearing, $F(2,16)=7.37$, $p<0.01$.

EXPERIMENT 5

Treatment with agents that inhibit the synthesis of CAs attenuates or blocks the stimulatory effects of amphetamine on locomotor activity [19,22], suggesting that this action of amphetamine depends on the release of CAs from the newly synthesized pool. In this experiment we examined the effect of the CA synthesis inhibitor, l-α-methyltyrosine (AMT) on the suppression of play fighting by amphetamine.

METHOD

Behavioral data were obtained on 26 pairs of juvenile male rats obtained from Holtzman at 21 days of age. General maintenance, housing and testing procedures were the same as in the preceding studies.

At 25 days of age the rats were randomly assigned to pairs which remained intact for the duration of the experiment. All pairs were tested for 8 days, 10 min each day. On the last 2 days pins and rears were scored. On the basis of these days 6 groups were formed that were approximately matched for average number of pins. On the following day 13 pairs received 50 mg/kg AMT (Metyrosine, Merck) suspended in 0.5% carboxymethylcellulose (CMC) 6 and again 3 hr before testing. The remaining 13 pairs were injected with 1 ml/kg of the CMC vehicle at the same times relative to testing as AMT was given to the other rats. Twenty min prior to testing the animals received 0.5 or 1.0 mg/kg injections of d-amphetamine sulfate or the saline vehicle (1 ml/kg). The number of pairs in each treatment group is given in Table 3. All drugs were administered IP.

RESULTS

Table 3 summarizes data for all treatment conditions, both for baseline days (base) and test days. The groups were well matched in terms of the baseline rates of pinning and there were no reliable differences. Analysis of the number of pins on the test day revealed only a main effect of amphetamine, $F(2,20)=38.76$, $p<0.001$. Neither the main effect of AMT nor the AMT × AMP interaction were reliable (both $F<2.2$). Thus amphetamine caused comparable depression of pinning in pairs treated with the CA synthesis inhibitor or the vehicle. This conclusion was confirmed by subsequent analyses which revealed reliable amphetamine effects on pinning in both the CMC vehicle and AMT conditions, $F(2,10)=32.70$ and 10.57, $p<0.001$ and 0.005 respectively.

Interpretation of the drug effects on rearing is complicated by the fact that the groups that subsequently received amphetamine differed reliably at baseline. While the data suggest that AMT attenuated the stimulatory effect of amphetamine on rearing, an analysis of difference scores (i.e., the difference in number of rears on the test day and baseline days) did not reveal reliable effects of amphetamine in either the AMT groups or the CMC controls.

GENERAL DISCUSSION

The present findings confirm earlier reports [3,4] that psychomotor stimulants such as amphetamine and methylphenidate profoundly reduce play fighting at doses that usually stimulate activity and rearing. To the list of effective agents ephedrine can be added. All of the stimulants that have been shown to suppress play are known to release CAs from neurons, a pharmacological action which is believed to be critical to their action as stimulants [6, 19, 22]. However, the present findings lend no support to the hypothesis that amphetamine suppresses play fighting by its action as an indirect CA agonist.

First, treatment with widely varying doses of the CA antagonists haloperidol, phenoxybenzamine, propranolol and chlorpromazine consistently failed to reverse the effects of amphetamine on play. The pattern of the results from these experiments can be summarized simply. At lower doses the antagonists did not affect play or rearing regardless of whether or not amphetamine was also administered. At higher doses these agents depressed play fighting, but usually only at doses that also depressed rearing. In general, the behavioral effects of the higher doses of the antagonists probably reflect relatively non-specific depression of motor activity or other toxic effects and are of interest only in that these data confirm the absence of antagonism of the effect of amphetamine on play seen at lower doses. While CA antagonists are potent blockers of the stimulating effects of amphetamine on activity and stereotyped behavior Miczek has recently reported that CA antagonists do not reverse the depressing effects of amphetamine on agonistic behavior in mice [17].

The failure of CA antagonists to block the effects of amphetamine on pinning would be more easily interpreted if we had shown that the same antagonists blocked the action of amphetamine on some other behavior. For this reason we

TABLE 3
MEAN NO. PINS AND REARS PER PAIR (±SEM)

	d-Amphetamine mg/kg	N	Pins		Rears	
			Base	Test	Base	Test
AMT	0	5	40.5 ± 4.5	24.6 ± 5.6	55.3 ± 9.9	34.6 ± 6.9
	0.5	4	42.0 ± 2.8	15.0 ± 2.1	64.8 ± 5.4	68.0 ± 12.8
	1.0	4	41.8 ± 3.1	0.5 ± 0.3	41.8 ± 8.4	44.5 ± 10.9
CMC	0	5	40.8 ± 5.6	37.4 ± 5.0	65.3 ± 7.0	54.8 ± 5.8
	0.5	3	46.2 ± 9.0	15.7 ± 2.3	65.8 ± 12.1	84.7 ± 7.3
	1.0	5	41.1 ± 3.2	0 ± 0	41.0 ± 3.8	61.0 ± 12.8

measured rearing but the effects of amphetamine on this behavior were inconsistent. In Experiment 1 using a between subjects design and a shorter overall testing sequence we observed no effect of amphetamine. In subsequent experiments more sensitive within-subjects designs were used and generally the rats in these studies were given more tests. Amphetamine consistently increased rearing.

Although amphetamine and methamphetamine generally stimulate rearing when given in doses of 2–4 mg/kg [11, 13, 14], these doses may elicit stereotyped rearing. The effects of lower doses (1 mg/kg or less) are less consistent [11, 13, 14]. This inconsistency may arise because rearing is a form of exploratory behavior as well as an active motor response and amphetamine and methamphetamine reduce other measures of exploratory behavior while simultaneously stimulating ambulation [13,20]. If one assumes that the exploratory component of rearing would decline with increasing exposure to the test chamber, then the stimulatory effect of amphetamine on rearing in Experiments 2–4 and its absence in Experiment 1 could be explained since the exploratory component would be less important in a longer series of tests. This idea is supported by the fact that rearing scores under control conditions were appreciably higher in Experiment 1 than in subsequent experiments.

The general failure of CA antagonists to block the stimulatory effect of amphetamine on rearing in Experiment 2 is probably the result of insufficiently high doses. This interpretation is supported by the fact that in Experiment 3, 5 mg/kg chlorpromazine did block the stimulatory effect of amphetamine on rearing, but the lower 0.5 mg/kg dose was ineffective.

The findings with clonidine, which preferentially stimulates α_2 receptors, reducing the release of norepinephrine (NE) from presynaptic terminals, pose a second problem for the idea that amphetamine suppresses play through its action as an indirect CA agonist. Assuming that NE is involved in the effect of amphetamine on play then clonidine should have attenuated the suppression of pinning caused by amphetamine. Instead clonidine virtually abolished pinning and profoundly depressed rearing as well. Its effects on both measures were surprisingly large considering the effects of similar doses on avoidance behavior in adult rats [15].

AMT blocks the stimulating effect of amphetamine on motor activity [6, 19, 22] indicating that the release of newly synthesized CAs is responsible for the enhancement of activity. The failure of AMT to influence the effect of amphetamine on play presents a third problem for the idea that amphetamine suppresses play by acting as an indirect CA agonist. To be sure we used only a single dose of AMT and did not confirm its effectiveness biochemically. The particular treatment regimen was selected because we wanted to avoid the risk of acute toxic effects that can occur when higher doses of AMT are given in a single injection. Our procedure of giving 2 50 mg/kg doses of AMT spaced 3 hours apart was adapted from Rech *et al.* [19]. Based on their work as well as that of others [22], a reduction of 50% or more in brain CA concentrations might be expected from the AMT treatment we employed.

If one supposes that the release of dopamine (DA) is important to the behavioral effects of amphetamine, then the marked differences in the effects of amphetamine and apomorphine on pinning may present a fourth difficulty for the notion that amphetamine depresses play by its action as an indirect CA agonist. In fact, these data are somewhat equivocal since lower doses of apomorphine significantly increased pinning. At low doses apomorphine appears to preferentially affect presynaptic DA receptors [8], reducing the release of DA, so that it might be argued that DA normally inhibits play fighting. If this is the case, then it is surprising that 1 mg/kg treatment with apomorphine had no effect on play since at this dose apomorphine stimulates postsynaptic receptors. We have tested a few pairs of rats with 2 mg/kg doses of apomorphine. At this dose the drug clearly reduced play, but also elicited substantial amounts of stereotyped behavior, precluding a meaningful interpretation of the data. At present we cannot dismiss the possibility that release of DA contributes to the effect of amphetamine on play, but given our data with haloperidol, it is difficult to argue that this mechanism is of major importance.

Several years ago Weiner [21] speculated that some of the effects of amphetamine might arise because of its action as a direct or indirect 5 HT agonist. Although amphetamine does appear to release 5HT in striatal tissue, rather high doses are required [10]. In a preliminary study we administered 1 or 5 mg/kg methysergide alone or in combination with 0.5 or 1 mg/kg d-amphetamine. Neither methysergide dose reversed the suppression of play fighting by amphetamine; in fact, methysergide weakly potentiated the effect of amphetamine on play fighting. Thus, it seems unlikely that the effects of amphetamine on play are mediated by its actions on 5HT neurons.

The suppression of play fighting could be explained by supposing that amphetamine acts by leading to the accumulation of a false transmitter in CA neurons. One attraction of

this hypothesis is that it could explain why the effects of amphetamine and CA antagonists are more or less additive rather than cancelling. It has been shown that p-hydroxynorephedrine does accumulate in noradrenergic neurons following amphetamine treatment [7], and this metabolite is released in response to nerve stimulation. Although plausible, this false transmitter hypothesis encounters two difficulties. First, the accumulation of p-hydroxynorephedrine occurs slowly while the suppression of play fighting by amphetamine is readily observed within 20 min after a single IP injection. Second, AMT apparently blocks the formation of p-hydroxynorephedrine [7], but does not block the suppression of play fighting by amphetamine. If a false transmitter is responsible for the effect of amphetamine on play fighting, it must be rapidly formed from a precursor that is present even when tyrosine hydroxylase is inhibited.

In conclusion, psychomotor stimulants exert a powerful suppressant effect on play fighting by juvenile rats, but the mechanism by which this effect occurs is not clear. None of the accepted neuropharmacological actions on CA neurotransmitter systems of these stimulants provide a very satisfactory account of their effects on play fighting. Recently it has been reported that some CA neurons also contain peptides that are thought to act as neurotransmitters [12,16]. For example, cholecystokinin (CCK) and dopamine are colocalized in neurons of the mesolimbic pathway and infusion of CCK into the nucleus accumbens potentiates the effect of low doses of apomorphine on stereotyped behavior [5]. If we assume that drugs like amphetamine increase the release of both the amine and the peptide transmitter and both transmitters act to depress play, then it might not be surprising that treatments with CA antagonists or synthesis inhibitors fail to reverse the effect of amphetamine. This analysis is obviously speculative, but it seems clear from the present work that the possibility that the effects of psychomotor stimulants on play involve drug actions on multiple transmitter systems needs to be considered.

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