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# Alpha$_2$-Adrenergic Antagonists Effect on Amphetamine-Induced Behaviors

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LUTTINGER, D. AND M. E. DURIVAGE. *Alpha$_2$-adrenergic antagonists effect on amphetamine-induced behaviors.* PHARMACOL BIOCHEM BEHAV 25(1) 155–160, 1986.—The effects of alpha$_2$-adrenergic antagonists on amphetamine-induced locomotion and stereotypy were studied in mice. Six alpha$_2$-antagonists (i.e., yohimbine, rauwolscine, piperoxan, tolazoline, RX781094, and RS21361) selectively attenuated amphetamine-induced increases in locomotion at doses which did not effect amphetamine-induced stereotypies. Higher doses of the antagonists which attenuated baseline stereotypies also attenuated amphetamine-induced increases in stereotypies. The effect of the alpha$_2$-antagonists was qualitatively similar to that observed with the atypical antipsychotic clozapine. Furthermore, the *in vivo* relative potency of the alpha$_2$-antagonists in the present study was comparable to that reported in other studies. These results suggest that alpha$_2$-adrenergic receptors may modulate the effects of amphetamine on locomotion in mice.

$\alpha_2$-Adrenergic antagonists Amphetamine Motor activity Mouse Dopamine

DOPAMINE synthesis and metabolism have been shown to be affected by alpha$_2$-adrenergic agonists and antagonists. Anden and Grabowska [2] have demonstrated that yohimbine, an alpha$_2$-antagonist, enhances dopamine synthesis and metabolism. Piperoxan, but not tolazoline, also enhanced dopamine synthesis and metabolism [3]. The reason for this difference between the alpha$_2$-antagonists, piperoxan and tolazoline, is unclear. In addition, Anden *et al.* [3] demonstrated that the alpha$_2$-agonist clonidine had an opposite effect to that of yohimbine. This increase in dopamine metabolism by yohimbine and not tolazoline has been confirmed by Scatton *et al.* [10] who used different methods of estimating dopamine metabolism. These researchers further characterized alpha$_2$-adrenergic antagonist interactions with dopamine by testing the alpha$_2$-antagonists, rauwolscine and RX781094 [11]. Rauwolscine, but not RX781094, also enhanced striatal dopamine metabolism. In a recent paper van Oene *et al.* [13] have generated evidence which suggests that yohimbine may possess direct central dopamine autoreceptor blocking properties *in vivo*. Thus some evidence exists which suggests that some but not all alpha$_2$-antagonists may also have some effects on dopaminergic activity *in vivo* that are similar to dopamine antagonists.

The actions of a variety of dopamine antagonists (antipsychotic) compounds at alpha$_2$-adrenergic receptors have also been studied [8,9]. These investigations indicated a wide range of affinity of the antipsychotic compounds for alpha$_2$-adrenergic receptors. However, there was no readily apparent correlation of alpha$_2$-adrenergic receptor affinity and any clinical manifestation of the anitpsychotic compounds tested.

In the present studies we were interested in determining if the alpha$_2$-adrenergic antagonists would effect dopamine mediated behavior. For this purpose we examined the interactions of various alpha$_2$-antagonists with d-amphetamine-induced increases in locomotion and stereotypy and compared these effects with those observed with antipsychotic compounds (e.g., chlorpromazine and clozapine). It is generally believed that the locomotion and stereotypy induced by d-amphetamine is mediated via dopamine [6]. Dopamine antagonists inhibit these actions of amphetamine, thus inhibition of these behaviors would suggest antidopaminergic activity by the alpha$_2$-adrenergic antagonists.

## METHOD

Male, Swiss-Webster mice (Taconic Farms) weighing between 14 and 25 grams were used. The animals were grouped housed 25/cage with food and water available ad lib until the day of testing. The mice were habituated to a 12 hour light-dark cycle. On the day of testing the subjects were housed 8/cage in Plexiglas cages with bedding material. Food and water were not available during testing.

The test compound was injected subcutaneously (SC) and the mice returned to the Plexiglas cage. Fifteen minutes after the first injection the mice were injected intraperitoneally (IP) with d-amphetamine sulfate (3 mg/kg, free base wt.) or 0.85% saline. In one study investigating the interactions of rauwolscine and amphetamine a lower dose of amphetamine (1.73 mg/kg) was utilized. The doses of the compounds are expressed as free base wt. and were injected in a volume of 10 ml/kg; when possible the compounds were dissolved in 0.85% saline. If the compounds were insoluble in saline they were suspended in 1% gum tragacanth. After the second injection the animals were placed in activity cages, one mouse/cage and allowed to habituate to their environment. The activity meters were opto-varimex (Columbus Instruments) Plexiglas cages measuring 41.4×42.3 cm and were interfaced to an Apple IIe computer. The program supplied by Columbus Instruments is designed to quantify ambulatory

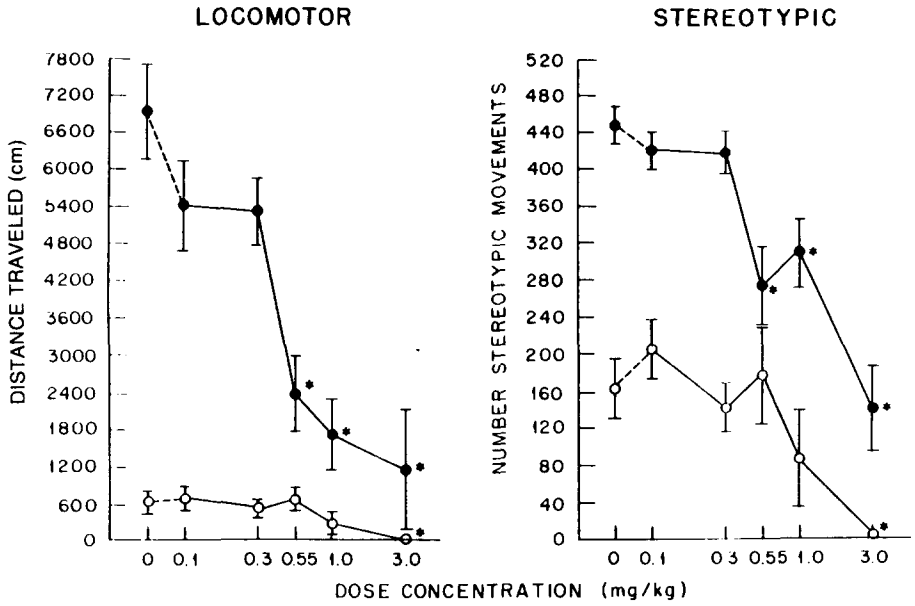


FIG. 1. The effects of chlorpromazine on baseline (○) and amphetamine (●) induced increases in locomotor activity (left panel) and sterotypy (right panel). n=8/group. *$p<0.05$ Dunnetts test for multiple comparisons.

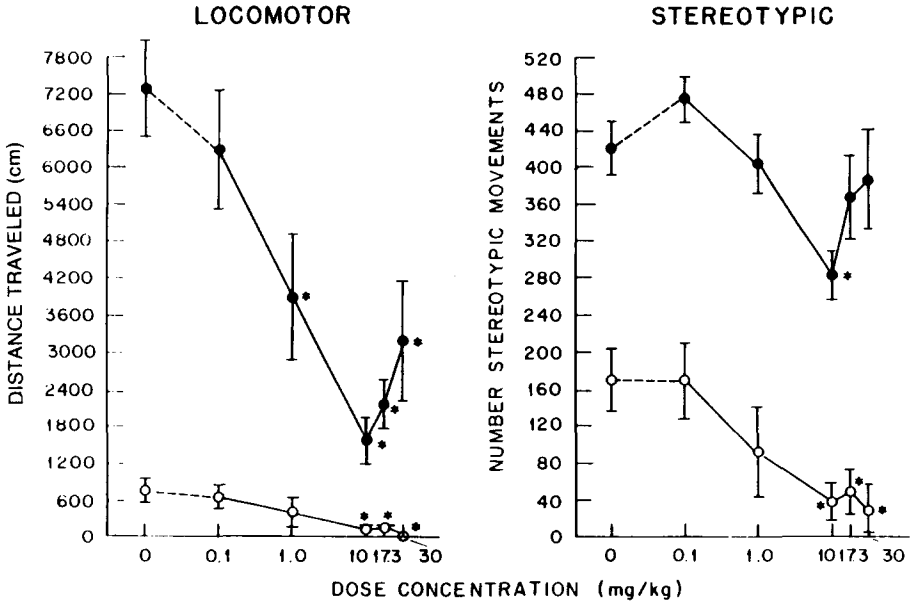


FIG. 2. The effects of clozapine on baseline (○) and amphetamine (●) induced increases in locomotor activity (left panel) and stereotypy (right panel). n=8/group. *$p<0.05$ Dunnetts test for multiple comparisons.

movement as well as the number of small movements. These activity cages contain a 15×15 photocell matrix with the photocells being equally spaced along the sides. Twenty-five minutes after being placed in the activity cage locomotor activity (distance travelled) and the number of stereotypies were collected for the following 10 minutes. These behaviors are programmed as mutually exclusive, thus if locomotion occurs during the 0.25 second sampled period no stereotypy can be recorded during that period. A behavior was considered a stereotypy if the same photocell beam was disrupted during the 0.25 second sample interval. In pilot studies amphetamine was found to produce an inverted U-shaped dose

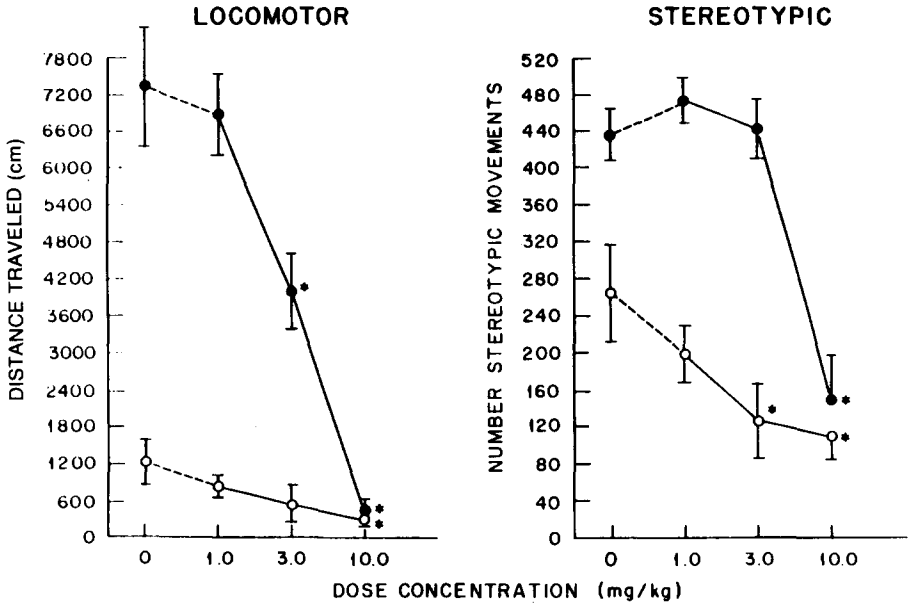


FIG. 3. The effects of yohimbine on baseline (○) and amphetamine (●) induced increases in locomotor activity (left panel) and stereotypy (right panel). n=8/group. *$p<0.05$ Dunnetts test for multiple comparisons.

reponse curve for locomotor activity (0.3–10 mg/kg, IP), in this dose range stereotypy was observed to be a monotonically increasing function. The predominant behaviors recorded as stereotypy in this system were head weaving and grooming.

To characterize the sensitivity of the behavioral system used, two antipsychotic compounds were tested for their ability to antagonize amphetamine-induced behaviors. These compounds were chlorpromazine and the atypical antipsychotic, clozapine.

In a study designed to address duration of action, rauwolscine was injected followed fifteen minutes later by amphetamine 3 mg/kg (IP). The mice were then placed immediately into the activity cages and activity assessed for 2 hours in 10 successive 12 minute intervals.

To further delineate and characterize the effects of the alpha$_2$-adrenergic antagonists, the interactions of rauwolscine with two other stimulants, scopolamine (3 mg/kg) and methylphenidate (3 mg/kg), were assessed.

The data for the alpha$_2$-antagonists and antipsychotic were analyzed by the Dunnetts test for multiple comparisons when comparing the different drug pretreatments to vehicle or amphetamine treated mice. Amphetamine, and other stimulants, were compared to vehicle by Student's $t$-test.

## RESULTS

Amphetamine produced dose-related increases in both the distance travelled and the number of stereotypies. The largest increase in distance travelled was observed at 3 mg/kg IP, higher doses produced less of an increase in distance travelled and a greater increase in stereotypies. A dose of 3 mg/kg d-amphetamine was used for studies directed at assessing the interactions of a compound with amphetamine-induced effects. The typical antipsychotic chlorpromazine significantly reduced amphetamine-induced increases in both distance travelled and number of stereotypies at the same dose, 0.55 mg/kg (Fig. 1). This occurred without chlorpromazine affecting baseline activity. At a higher dose (3 mg/kg), chlorpromazine alone essentially abolished locomotor and stereotypic activity. Haloperidol (1 mg/kg) had similar effects on amphetamine-induced behaviors, i.e., a reduction in amphetamine-induced increases in both locomotion and stereotypy (data not shown). Similar to chlorpromazine, haloperidol attenuated amphetamine-induced increases in locomotion and stereotypy at the same dose, a dose which alone had no effect on baseline stereotypy or locomotion. Clozapine, an atypical antipsychotic, had a slightly different profile (Fig. 2). The minimally effective dose that antagonized amphetamine-induced increases in locomotion was 1 mg/kg. Unlike typical antipsychotic compounds this dose did not affect amphetamine-induced increases in stereotypy. The dose of 10 mg/kg did attenuate amphetamine-induced stereotypy. This effect, however, did not appear to be dose-related as higher doses had no effect on amphetamine-induced stereotypies, despite the fact that these doses (10–30 mg/kg) reduced the baseline number of stereotypies. Thus typical antipsychotics reverse amphetamine-induced increases in both locomotion and stereotypy at comparable doses whereas an atypical antipsychotic preferentially attenuated amphetamine-induced increases in locomotion.

Six alpha$_2$-antagonists were tested and all were observed to attenuate amphetamine-induced increases in locomotor activity in a dose-related fashion. Yohimbine attenuated the effects of amphetamine on locomotor activity, with 3 mg/kg being the minimally effective dose (Fig. 3). Amphetamine-induced increases in stereotypies were also attenuated by yohimbine, however, the dose required to produce a significant effect was larger (i.e., 10 mg/kg). Furthermore, 10 mg/kg of yohimbine reduced the baseline level of stereotypies independent of amphetamine administration.

TABLE 1

MINIMALLY EFFECTIVE DOSE FOR ATTENUATING*

| | Amphetamine-Induced Increases in† | | Baseline | |
|---|---|---|---|---|
| | Locomotion | Stereotypy | Locomotion | Stereotypy |
| Rx781094 | 1 | >10 | >10 | 10 |
| Rauwolscine | 1 | 10 | >10 | 10 |
| Yohimbine | 3 | 10 | 10 | 3 |
| Piperoxan | 3 | 10 | 30 | 30 |
| Tolazoline | 10 | >30 | 3 | 10 |
| RS21361 | 100 | >100 | 100 | 30 |

*Doses are expressed as mg/kg. The minimally effective dose was defined as the dose of the alpha$_2$-antagonist which produced a significant ($p<0.05$) decrease in activity when compared to appropriate controls (i.e., vehicle or amphetamine treated). Dunnetts test for multiple comparisons was used for determining significance. n = 8/group.

†Amphetamine (3 mg/kg, IP) was administered 25 minutes before testing. The alpha$_2$-adrenergic antagonists were administered subcutaneously 15 minutes before amphetamine.

TABLE 2

MINIMALLY EFFECTIVE DOSE FOR ATTENUATING*

| | Baseline | | Drug Induced Increases† | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Amphetamine | | Scopolamine | | Methylphenidate | |
| | locomotor | stereotypy | locomotor | stereotypy | locomotor | stereotypy | locomotor | stereotypy |
| Chlorpromazine | 3 | 3 | 0.55 | 0.55 | 1 | 0.3 | 0.55 | 0.55 |
| Clozapine | 10 | 10 | 1 | 10 | 3 | 3 | 10 | 10 |
| Rauwolscine | >10 | 10 | 1 | 10 | >10 | >10 | 10 | 10 |

*Doses are expressed as mg/kg. The minimally effective dose was defined as the dose of rauwolscine, chlorpromazine, or clozapine which produced a significant ($p<0.05$) decrease in activity when compared to appropriate controls (i.e., vehicle or stimulant treated). Dunnetts test for multiple comparisons was used for determining significance. n = 8/group.

†Amphetamine (3 mg/kg, IP), scopolamine (3 mg/kg, IP) or methylphenidate (3 mg/kg, IP) were administered 25 minutes before testing. Rauwolscine, chlorpromazine or clozapine were administered subcutaneously 15 minutes before amphetamine, scopolamine, methyphenidate or vehicle.

Table 1 summarizes the effects of the six different alpha$_2$-antagonists. As can be seen in the table all six antagonists were effective at attenuating amphetamine-induced increases in locomotion. In addition to attenuating amphetamine-induced locomotion several were effective in blocking amphetamine-induced stereotypy as well. However in all cases the dose required to block stereotypies was larger than that required to attenuate locomotion. Table 1 also illustrates the fact that these compounds were often more potent at attenuating the effects of amphetamine on locomotion than at attenuating baseline activity. Furthermore, there are examples where baseline stereotypy was attenuated at lower doses than amphetamine-induced increases in stereotypy. These observations suggest that the interactions of the alpha$_2$-antagonists with amphetamine are not due to additive effects. Rauwolscine selectively reduced amphetamine-induced increases in locomotion both when amphetamine was injected at 3 mg/kg and at 1.73 mg/kg (data now shown). Thus the decrease in amphetamine-induced locomotion is not due to the reduction of a maximal increase in locomotion, since 1.73 mg/kg of amphetamine produced a submaximal increase in locomotion. Corynanthine, an isomer of rauwolscine, which exhibits relative selectivity for the alpha$_1$-adrenergic receptor was ineffective at attenuating the amphetamine-induced increases. This occurred even though 10 mg/kg reduced the baseline level of stereotypies. The alpha$_2$-agonist clonidine (0.01–3 mg/kg) did not affect amphetamine-induced increases in locomotion. However, amphetamine-induced stereotypies were attenuated by 0.03 and 0.1 mg/kg of clonidine, but not with higher doses (data not shown). When locomotor activity and stereotypy were assessed for two hours following rauwolscine and amphetamine administration the same selective antagonism of amphetamine-induced increases in locomotion was noted (data not shown). At all doses of rauwolscine tested (1–10 mg/kg) there was an inhibition of amphetamine induced increases in locomotor activity. This effect was dose-related in magnitude and duration.

The effects of rauwolscine, clozapine and chlorpromazine on scopolamine and methylphenidate-induced increase in locomotor activity and stereotypy are summarized in Table 2. Briefly, it was observed that although clozapine and rauwolscine preferentially inhibit amphetamine-induced increases in locomotor activity when compared to stereotypies. This was not the cause when scopolamine or methylphenidate were utilized as the simulant.

## DISCUSSION

This report demonstrates that many alpha$_2$-antagonists inhibit amphetamine-induced increases in locomotion. This effect is very likely *not* due to alterations in amphetamine metabolism since stereotypy was often affected only at much higher doses of the alpha$_2$-antagonists. On occasion stereotypies were unaffected even at the highest doses of the alpha$_2$-antagonists tested. Since one amphetamine-induced behavior was preferentially affected this suggests some specificity of action. Two points argue against the decrease in activity being due to the potentiation of a competing behavior (e.g., stereotypy). First, at higher doses the alpha$_2$-antagonists tended to reduce stereotypy. Second, imipramine (10 mg/kg) which potentiates many of the affects of amphetamine produced an *increase* in the number of stereotypies at a dose that decreased locomotor activity (data not shown). Furthermore, the reduction of amphetamine-induced increases in locomotor activity by the alpha$_2$-antagonists is not due to additive effects since larger doses of the alpha$_2$-antagonists were often required to reduce baseline locomotor activity.

Evidence that the effect of the six alpha$_2$-antagonists is mediated via alpha$_2$-adrenergic receptors can be inferred from the observations that corynanthine, an alpha$_1$ selective antagonist, which is an isomer of yohimbine and rauwolscine, was inactive and the relative potencies of the alpha$_2$-antagonists for attenuating amphetamine-induced increases in locomotion correlate with their potencies for antagonizing clonidine-induced antinociception [5].

Some alpha$_2$-antagonists (e.g., yohimbine, rauwolscine and piperoxan) have been reported to stimulate dopamine metabolism in the striatum [11]. However, this effect was not observed with RX781094 [11]. Nor is the effect of yohimbine on dopamine synthesis in the striatum antagonized by the alpha$_2$-agonist clonidine [3]. This apparent inconsistency may be due to some regional selectivity of the behavioral affect. Thus the nucleus accumbens is implicated as the site of action for amphetamine-induced increases in locomotion whereas the striatum seems to be more involved in amphetamine-induced stereotypies [6]. Stereotypies were only weakly affected by many of the alpha$_2$-antagonists tested and only at higher doses which also often had effects on baseline stereotypies. Tolazoline and RX781094, two antagonists which have been reported *not* to effect striatal dopamine utilization [3,11], did not effect amphetamine-induced stereotypy in the dose-range tested. The dose-range was extended to doses that were high enough to decrease baseline stereotypies.

The mechanism by which the alpha$_2$-antagonists attenuate amphetamine-induced increases in locomotor activity is unclear at present. Skolnick *et al.* [12] have reported that clonidine, and the alpha-adrenergic antagonist phentolamine, both inhibit norepinephrine-induced accumulation of cyclic AMP. This may play a role in the effects we have observed, though this awaits further study. Other data which may implicate a mechanism for the effects of alpha$_2$-antagonists on amphetamine-induced increases in locomotor behavior is suggested by the observations of Anden *et al.* [1]. These investigators treated mice with reserpine and observed a concomitant decrease in locomotor activity. When apomorphine was administered motor activity was temporarily restored. This restoration was potentiated by the alpha$_2$-agonist clonidine. These investigators suggested that norepinephrine can modulate the effects of dopamine on motor activity. Our results are consistent with this.

The antagonism of the effects of the various stimulants suggest some differences in mechanism of action. Thus clozapine and rauwolscine only selectively inhibited amphetamine-induced hyperactivity and not scopolamine or methylphenidate-induced hyperactivity. This may be due to the different mechanisms of action of the various stimulants or sites of action.

The profile of activity for the alpha$_2$-antagonists on amphetamine-induced locomotion was similar to that observed with the antipsychotic compounds, chlorpromazine, haloperidol and clozapine. Furthermore the alpha$_2$-antagonist profile was qualitatively more similar to that seen with the atypical antipsychotic compound clozapine (i.e., greater selectivity in antagonizing amphetamine-induced locomotion vs. stereotypy). Caution must be used in interpreting these results since clonidine has been reported to have antipsychotic activity in humans [4]. The alpha$_2$-agonist, clonidine, has also been reported to antagonize the increase in locomotion induced by amphetamine [7,12]. We did not observe this effect on locomotion. Although the reason for this difference is not clear at present, it may be due to differences in species (mice vs. rats) or other methodological considerations.

In summary alpha$_2$-antagonists were observed to preferentially inhibit amphetamine-induced increases in locomotion.

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