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The Interaction of d-Amphetamine and Naloxone Differs for Rats Trained on Separate Fixed-Interval or Fixed-Ratio Schedules of Reinforcement

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ANDREWS, J. S. AND S. G. HOLTZMAN. *The interaction of d-amphetamine and naloxone differs for rats trained on separate fixed-interval or fixed-ratio schedules of reinforcement.* PHARMACOL BIOCHEM BEHAV **26**(1) 167–171, 1987.—The effects of d-amphetamine and naloxone were investigated using two groups of rats trained on either an FR30 or FI2 schedule of reinforcement. Amphetamine (0.1–1.0 mg/kg), and naloxone (1.0 and 10 mg/kg) administered separately reduced responding on the FR procedure in a dose-dependent manner. The combined administration of naloxone with amphetamine had an additive suppressive effect on responding. The same doses of amphetamine and naloxone, when given separately, did not significantly depress responding in the FI procedures. However, naloxone/amphetamine combinations produced a marked inhibition of lever-pressing. Naloxone did not alter the characteristic pattern of responding engendered by amphetamine in this schedule, as measured by the quarter-life and Index of Curvature. It appears that the type of procedure used is a critical factor in demonstrating the effects of naloxone on behavior, and the nature of naloxone/amphetamine interactions.

Naloxone Amphetamine Rats Lever-pressing

AN increasing body of evidence suggests that opiate antagonists exert substantive behavioral effects of their own, and can modulate the actions of non-opiate drugs. For example, opiate antagonists depress locomotor behavior [2,3] when given alone, and reduce amphetamine-stimulated locomotor behavior [14,24]. There are several conflicting reports as to whether opiate antagonists administered alone, such as naloxone, depress responding reinforced by food or brain stimulation [6, 8, 9, 12, 13, 22, 23, 25, 26]. However, naloxone does modify the action of stimulant drugs in numerous operant procedures, e.g., shock avoidance [15], food-reinforced [8,12] and brain-stimulation reinforced paradigms [6, 9, 16, 23].

The interaction between stimulants, such as amphetamine and naloxone, is not a simple dose-related effect, suggesting an indirect mechanism. For example, naloxone might block endogenous opioid systems thought to modulate the central dopaminergic pathways that mediate actions of amphetamine [14,18]. It is presently unclear as to whether opiate antagonists enhance or antagonize the behavioral effects of stimulant drugs. For example, naloxone could depress amphetamine-stimulated locomotor activity or schedule-controlled behavior either by potentiating the ability of amphetamine to induce stereotyped behavior, or by antagonizing its behavioral stimulant properties. There is evidence in support of both possibilities, particularly in procedures examining unconditioned behavior [1, 4, 7, 14, 20, 21].

The purpose of this experiment was to investigate the effects of amphetamine and naloxone co-administration on different food-reinforced operant procedures. This will help to clarify whether the reported interaction of amphetamine and naloxone is dependent on the rate and pattern of responding, or is a more general effect, independent of the specific characteristics of ongoing behavior. To this end rats were trained on either a fixed-ratio (FR) or fixed-interval (FI) schedule of food reinforcement. If naloxone potentiates the stereotypic action of amphetamine, the high rates of responding seen in FR procedures should be especially sensitive to disruption by the combination of drugs due to an increasing interference with lever-pressing from inappropriate (perseverative) behavior. In an FI procedure increasing doses of amphetamine modify the typical scalloped response pattern in each interval by potentiating responding at the beginning of an interval, and depressing responding towards the end. If naloxone enhances the perseverative action of amphetamine then we might expect these effects to be more pronounced at lower doses of amphetamine when co-administered with naloxone.

METHOD

Subjects

The subjects were sixteen male Sprague-Dawley derived

rats (Sasco Inc., Omaha, NE), weighing 275–300 g at the beginning of the experiment. Each animal was housed individually under diurnal conditions (lights on between 0700 and 1900 hr) with water available ad lib, but on a 23-hr food deprivation schedule.

Apparatus

Four rodent operant chambers (Coulbourn Instruments Inc., Lehigh Valley, PA) fitted with a houselight, food-magazine and food-dispenser, stimulus lights and response levers, were connected to, and controlled by, a NorthStar Horizon microcomputer (North Star Computers Inc., Berkeley, CA).

Procedure

All rats were trained initially to lever press for continuous food-reinforcement (45 mg food-pellets, Bio-Serv, Frenchtown, NJ). The rats were then divided into 2 groups of 8. For one group the number of presses required to obtain reinforcement was gradually increased from 1 to 30 (fixed-ratio 30, FR30) in a 60-min session. The second group was trained on a fixed-interval 2 (FI2) schedule. The first response emitted after 2 min resulted in the delivery of 2 food pellets. However, if a rat did not respond within 10 sec after the interval finished, a new 2-min period began. Each session consisted of 30 intervals. Thus, the session length could vary from approximately 60 min for a rat that responded immediately after each interval ended, to 65 min for one that did not respond at all. Animals were tested 5 days per week; drug testing began when daily performance varied within a range of less than 10% on the measures taken.

Drugs

Drugs were administered on Tuesday and Friday of each week. Injections were given SC in a volume of 1.0 ml/kg of body weight, 15 min prior to testing. Each rat received saline, 0.1, 0.3, 0.56, 1.0 and 1.75 mg/kg of d-amphetamine sulfate (Sigma Chemical Company, St. Louis, MO), in combination with saline or with 1.0 or 10 mg/kg of naloxone hydrochloride (National Institute on Drug Abuse, Rockville, MD). All doses are expressed as base dissolved in 0.9% saline, and were administered in a different random order for each subject.

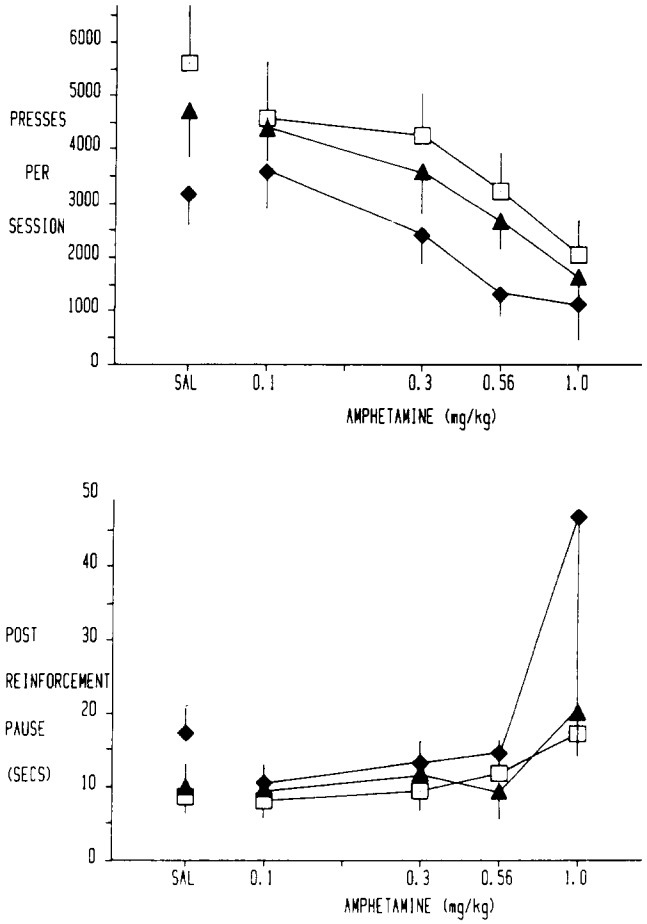

FIG. 1. Top: The effects of naloxone and amphetamine on the mean total responses per session, of rats trained in a food-reinforced FR30 procedure. Bottom: The effects of naloxone and amphetamine on the mean post-reinforcement pause per session of the same group of rats. Key: Open squares—amphetamine/saline injections; triangles—1.0 mg/kg of naloxone in combination with saline and amphetamine; diamonds—10 mg/kg of naloxone in combination with saline and amphetamine. Each point represents the mean and SEM of 6 rats. See text for further details.

Data Analysis

The number of responses emitted in each session was recorded for both the FI and FR procedures. In addition, the mean post-reinforcement pause was calculated for the FR group. This index is calculated as the total time (in sec) between the delivery of a food-pellet and the next press, divided by the number of times this occurs per session. Each 2-min FI was divided into 10 equal subdivisions and responses collected cumulatively in each of the subdivisions throughout the session. The quarter-life (the point when one quarter of the total responses within an interval had been made), and the Index of Curvature (an index which quantifies the extent and direction responding within an interval deviates from a straight line observed after constant rate of responding), were then calculated as previously described [10,11]. The number of reinforcements earned during each session was also recorded.

Values obtained for each of the indices were subjected to a 2 factor repeated measures ANOVA: one factor being dose of naloxone, and the other dose of amphetamine. Where overall significance was indicated, *a posteriori* comparisons were made using the multiple comparisons procedure according to Tukey.

RESULTS

Several animals failed to respond at higher doses of amphetamine (1.0 and 1.75 mg/kg). Only animals that responded at doses up to 1.0 mg/kg of amphetamine were included in the analysis. This allowed a complete analysis of each of the calculated indices for 6 out of 8 rats in the FR group, and 7 out of 8 rats in the FI group. The 3 remaining animals showed trends similar to those of other animals in their respective groups.

FR30

Both naloxone and amphetamine, when administered

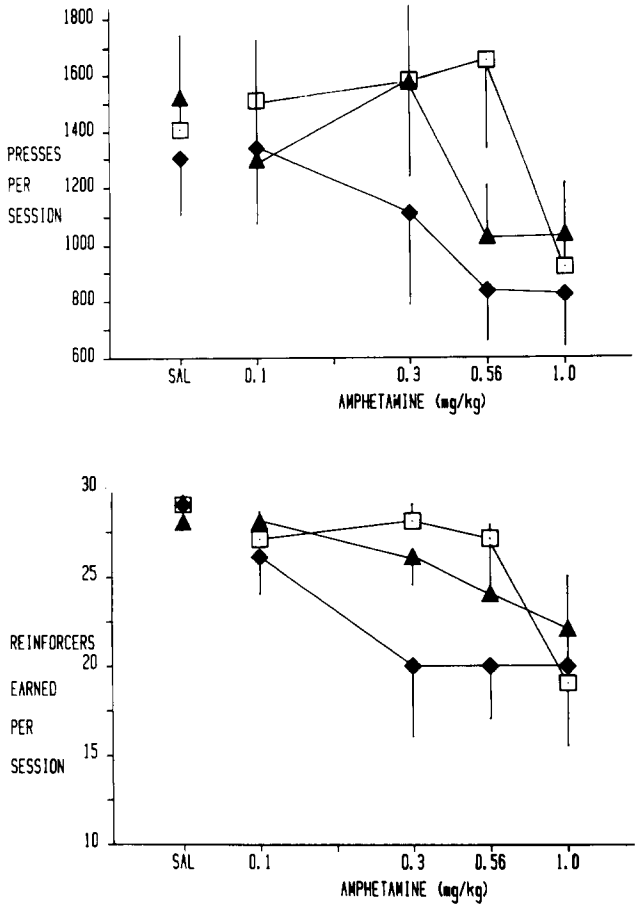

FIG. 2. Top: The effects of naloxone and amphetamine on the mean total responses per session, of rats trained in a food-reinforced FI2 procedure. Bottom: The effects of naloxone and amphetamine on the mean number of reinforcements earned per session by the same rats. Key: Open squares—amphetamine/saline injections; triangles—1.0 mg/kg of naloxone in combination with saline and amphetamine; diamonds—10 mg/kg of naloxone in combination with saline and amphetamine. Each point represents the mean and SEM of 7 rats. See text for further details.

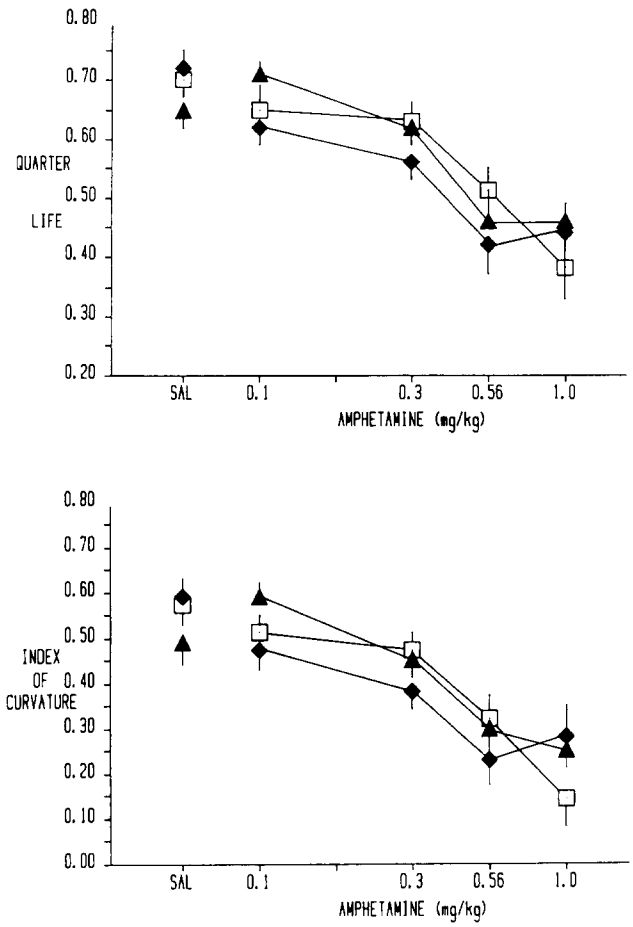

FIG. 3. Top: The effects of naloxone and amphetamine on the average quarter-life per session, of rats trained in a food-reinforced FI2 procedure. Bottom: The effects of naloxone and amphetamine on the average Index of Curvature per session for the same rats. Key: Open squares—amphetamine/saline injections; triangles—1.0 mg/kg of naloxone in combination with saline and amphetamine; diamonds—10 mg/kg of naloxone in combination with saline and amphetamine. Each point represents the mean and SEM of 7 rats. See text for further details.

alone, caused a dose-dependent decrease in responding. Naloxone (10 mg/kg), in combination with amphetamine, significantly reduced responding compared to that found with amphetamine administered alone, $F(2,10)=4.76$, $p<0.05$. However, there was no significant interaction between amphetamine and naloxone: the two suppressive effects appear additive in nature (see Fig. 1). Amphetamine caused a slight but nonsignificant dose-dependent increase in post-reinforcement pause; naloxone had no effect on this measure (Fig. 1).

FI2

The total number of responses per session tended to increase slightly with low doses of amphetamine, and then decrease with higher doses; however, there was no overall amphetamine dose effect. Although naloxone alone had no effect on response rate, overall analysis showed that naloxone (10 mg/kg) in combination with amphetamine significantly reduced responding compared to that observed after amphetamine administered alone, $F(2,12)=4.67$, $p<0.05$. There was no significant effect of naloxone or amphetamine on food-pellets obtained per session, although naloxone tended to reduce the number of pellets obtained at 0.3 and 0.56 mg/kg of amphetamine by 25–30%. These results are illustrated in Fig. 2.

As expected, increasing doses of amphetamine caused a significant dose-dependent reduction in the quarter-life and Index of Curvature. However, the presence of naloxone did not significantly alter these effects (see Fig. 3).

DISCUSSION

The results of the FR30 experiment indicate that naloxone depresses responding in a dose-dependent manner, and that this effect is additive with the action of amphetamine in the same animals. There is no interaction to suggest naloxone potentiates disruptive effects of amphetamine on behavior,

as has been reported previously [8, 12, 15, 16]. This was in contrast to the results observed for rats trained on an FI schedule. Naloxone alone had no obvious effect on responding at either dose (1.0 or 10 mg/kg). However, the effect of naloxone in combination with amphetamine was clearly to suppress responding.

It has been reported that naloxone enhances amphetamine-induced stereotypy [1,7]. Such an effect cannot explain the results presented here. Increasing doses of amphetamine lower the quarter-life: that is, there is an increased tendency for rats to press throughout the interval as opposed to just towards the end of the interval. Because progressively higher doses of amphetamine also enhance the tendency to perseverate, these indices may be viewed as a measure of amphetamine-induced perseveration. If naloxone enhanced the perseverative effect of amphetamine then combinations of these drugs should have depressed these measures further. This is clearly not the case: naloxone, despite lowering response rates when combined with amphetamine, has no effect on the pattern of responding. There is even a suggestion that performance is improved at higher doses of amphetamine and naloxone (see Fig. 3).

The effect of naloxone on lever-pressing may depend on the baseline rate of responding. In this study naloxone had no effect on responding in the FI, but had an obvious dose-dependent effect in the FR procedure, where baseline responding was much higher. Sanger and McCarthy [22], using a food-reinforced FR20, which produced a baseline rate comparable to that observed here, demonstrated effects of 1.0 and 10 mg/kg of naloxone on responding similar to those observed here. Moreover, naloxone had no effect on a food-reinforced FI2 procedure that engendered baseline response rates only slightly higher than observed in this study [12].

However, naloxone does not appear to have a "true" rate-dependent effect. Naloxone does not enhance low rates of responding, nor does it appear to enhance submaximal increases in responding by amphetamine ([8,9], this study). Although the effect of naloxone on amphetamine-maintained behavior is generally to suppress responding, the interpretation depends on the test situation. Thus, naloxone can accentuate rate-decreasing effects, or antagonize rate-increasing effects of amphetamine on operant responding [8, 9, 12, 16].

Naloxone has been reported to exert inconsistent effects on both response rates and thresholds for brain-stimulation reward [6, 9, 16, 23]. The site of the stimulating electrode appears to be important [9,23]. However, it has also been suggested that the effectiveness of naloxone in modulating the effect of amphetamine on self-stimulation depends on the extent to which amphetamine enhances lever-pressing [9]. Naloxone appears to exert a greater effect on responding that has been markedly increased by amphetamine [9,16].

One study has shown that naloxone depresses the amphetamine-stimulated release of dopamine from both accumbens and caudal tissue [14]. Both of these structures have important, but possibly distinct roles in motor behavior [19]. This would suggest a mechanism by which naloxone attenuates the effects of amphetamine, but cannot adequately explain reports of naloxone both increasing or decreasing amphetamine- and apomorphine-induced stereotypy [1, 4, 7, 20, 21]. It may be that naloxone exerts both presynaptic and postsynaptic effects on dopamine-mediated behaviors, or acts on other related systems. It is possible that a reduction in locomotor activity may make stereotypic behavior more obvious, but not necessarily more frequent in occurrence. Multiple sites of action in opioid stimulated behavior has been suggested by one study showing that lesions of the mesolimbic dopamine system do not completely block this behavior [18]. In addition, there are many reports showing naloxone to enhance disruptive effects of amphetamine on operant behavior ([8, 9, 12, 16], this study). In all, these studies demonstrate that the effect of naloxone is not a simple antagonistic effect on dopaminergic systems.

The accumulated evidence suggests that the type and magnitude of the effect of naloxone depends heavily on the test situation, the response rate, and how performance is measured. Although it is clear that naloxone, alone and in combination with other drugs, will depress response rates, this does not necessarily mean that overall performance is disrupted. For example, naloxone had no effect on the FI quarter-life under any condition; nor did it significantly reduce the number of reinforcements obtained in the FI. Because of the anatomical link between opiate and mesolimbic and striatal dopamine systems, many studies have concentrated on motor behavior. However, the interaction between naloxone and amphetamine suggests something more than a general affect on locomotor behavior, or even dopamine-mediated reward mechanisms. Identifying the exact nature of this effect has proven difficult. The differential effect of naloxone on performance in several different procedures makes pharmacokinetic factors seem unlikely. However, these factors may be important in interpreting naloxone interactions across species (see [5,17]).

One possible explanation is that naloxone disrupts performance through effects on motivation. In this case it could be argued that the motivation of the two groups of rats differs markedly, and this accounts for the different effects of naloxone alone and in combination with amphetamine. Although changes in motivational states may be important they are unlikely to be sufficient in explaining these results. The FI group of rats received approximately one third of the number of pellets obtained by the FR group. It could be argued that since naloxone depressed responding and food intake only in the FR group that the FI rats were more motivated to acquire reinforcement. However, it then becomes difficult to explain why naloxone should have a much more dramatic effect on FI response rates when co-administered with amphetamine (which also did not generally depress responding and food intake in the FI group). Differences in motivation may be an important reason for the results obtained here, however, it is difficult to determine exactly how important because of the many differences between the two procedures. In fact a case could also be made for there being no difference in the motivational states of the two groups. Both groups maintained similar weights, and both groups made approximately similar number of responses per pellet. In the FR group responding was approximately 3.5 times higher than in the FI group and the number of pellets obtained 3.3 times higher. Further studies using complex procedures such as signal detection analysis may be appropriate in defining the extent of the effects of naloxone on motivation.

In conclusion, the effect of naloxone, alone and in combination with amphetamine, on food-reinforced responding depends on the procedure used. These effects may be mediated by indirect actions on dopaminergic neurons regulated by an endogenous opioid system. However, further research, possibly involving local injection of opiate antagonists and non-opiate drugs, is required to further characterize the nature and location of this interaction.

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