Pharmacology Biochemistry and Behavior, Vol. 42, pp. 765–770, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Naloxone Reduces Amphetamine-Induced Stimulation of Locomotor Activity and In Vivo Dopamine Release in the Striatum and Nucleus Accumbens

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Received 10 January 1992

HOOKS, M. S., D. N. C. JONES, J. B. JUSTICE, JR. AND S. G. HOLTZMAN. *Naloxone reduces amphetamine-induced stimulation of locomotor activity and in vivo dopamine release in the striatum and nucleus accumbens.* PHARMACOL BIOCHEM BEHAV **42**(4) 765–770, 1992. – This study tested the possibility that naloxone (NX), an opioid antagonist, reduces the behavioral effects of amphetamine (AMPH) in rats by attenuating the dopaminergic response to AMPH. In the first experiment, adult, male rats were injected SC with either NX (5.0 mg/kg) or saline and 30 min later received doses of AMPH (0.0, 0.1, 0.4, 1.6, and 6.4 mg/kg) cumulatively at 30-min intervals. Gross locomotor counts following AMPH administration were significantly lower for rats pretreated with NX than for rats pretreated with saline. In the second experiment, the same drug treatments were given while performing microdialysis in either the striatum (STR) or nucleus accumbens (NACC). STR rats treated with vehicle showed a larger percentage increase in DA levels following AMPH treatment than did NACC rats treated with vehicle. NX pretreatment did not affect dopamine concentrations in either brain region. However, compared to pretreatment with saline pretreatment with NX significantly decreased the dopaminergic response to AMPH in the STR. There was no difference between the two groups in the peak dopaminergic response to AMPH in the NACC, but there was a significant AMPH × treatment × time interaction due to differences between the groups during the later portion of the response to 6.4 mg/kg AMPH. There was also a difference in locomotor activity following AMPH treatment between NX- and saline-treated subjects during dialysis. These findings suggest that a decrease in the dopaminergic response to AMPH is the mechanism by which NX attenuates behavioral stimulant effects of AMPH. In addition, there is a difference between the STR and NACC in dopaminergic responsiveness to AMPH.

Locomotor activity *d*-Amphetamine Naloxone Dopamine In vivo microdialysis Striatum Nucleus accumbens

NALOXONE and naltrexone are specific and potent opioid receptor antagonists. However, these compounds can modify the effects of nonopioid drugs under some circumstances (28). For example, there have been a number of reports of interactions between naloxone or naltrexone and psychomotor stimulant drugs, such as amphetamine. These opioid antagonists attenuate amphetamine-induced increases in motor activity in a variety of animal species, including mouse, rat, guinea pig, and squirrel monkey (1,2,6,13,14,32,34). In the rat, they reduce amphetamine-induced increases in operant responding maintained by schedules of food reinforcement (3,12) or shock avoidance (14). They also block amphetamine-induced increases in rate of responding for electrical self-stimulation of the brain (10,15), as well as the lowering of the threshold for electrical self-stimulation of the brain produced by amphetamine (9). Naloxone can prevent amphetamine from establishing a conditioned place preference in rats (33).

The psychomotor stimulant properties of amphetamine have been ascribed to the ability of the drug to enhance dopaminergically mediated neurotransmission in the brain, particularly in the striatum (STR) and nucleus accumbens (NACC) (18,23,35). The mechanism(s) by which opioid antagonists modify the behavioral effects of amphetamine is unclear. Opioid receptors have been identified on dopaminergic neurons of the nigrostriatal and mesolimbic tracts (11,20). This raises the possibility that these dopaminergic neurons are under an

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opioidergic influence that can be blocked by appropriate opioid antagonists. If this is indeed the case, opioid antagonists might be expected to attenuate the neuronal release of dopamine that is induced by amphetamine. The present study was designed to examine this possibility using the technique of microdialysis. Microdialysis probes were inserted into the STR or NACC of conscious rats. We measured the release of dopamine from these regions in response to a range of doses of *d*-amphetamine. The drug was administered by a cumulative dosing procedure that enables determination of a complete dose-response function in a single experimental session. The dopamine response to amphetamine was compared in rats that had been pretreated with either saline or naloxone. Locomotor activity was measured concurrently with the microdialysis. The dose of naloxone tested in the microdialysis experiments was selected on the basis of the results of dose-response determinations for combinations of naloxone and amphetamine on the locomotor activity of a separate group of untethered and otherwise normal rats.

METHOD

Subjects

Male rats of Sprague-Dawley descent (Sasco, Omaha, NE) weighing 300–350 g were used in both experiments. Between experiments, rats were housed in group cages in a temperature-controlled room. Food and water were always present in the home cage and a 12 L : 12 D cycle was maintained (lights on from 0700–1900 h). Testing was conducted between 0800–1500 h.

Drugs

Naloxone hydrochloride and *d*-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) were dissolved in 0.9% saline solution and injected SC in a volume of 1.0 ml/kg body weight. Drug doses are expressed as the free base.

Experiment 1

Apparatus. Motor activity was measured with six two-channel Electronic Activity Monitors (No. 31404, Stoelting Co., Chicago, IL). A single rat was placed in a polycarbonate cage (51 × 41 × 21 cm), which was centered on a sensor platform within a sound-attenuating chamber that was ventilated and illuminated by a small fluorescent bulb. The counting thresholds of each sensor were calibrated with a swinging pendulum so that one channel measured gross movements in the horizontal plane corresponding to locomotion and the other channel measured total movements; the difference between the two channels represented fine movements. Activity counts were recorded by a microcomputer.

Behavioral methods. All rats were habituated to the test chambers for 1 h on each of the 5 days prior to the start of the experiment. On the day of testing, subjects were injected SC with either saline or naloxone (0.2, 1.0, or 5.0 mg/kg). Thirty minutes later, all rats were injected with saline, SC, and then placed in the test chamber; activity was recorded for 20 min beginning 10 min later. Rats received additional SC injections at 30-min intervals: 0.1, 0.3, 1.2, and 4.8 mg/kg *d*-amphetamine, resulting in cumulative amphetamine doses of 0, 0.1, 0.4, 1.6, and 6.4 mg/kg. In each instance, activity was recorded for 20 min commencing 10 min after injection.

Data analysis. Gross and fine activity counts for the 20-min periods were subjected to separate analyses of variance (ANOVAs) with repeated measures; posthoc analysis was performed, where appropriate, using Student's *t*-test corrected for multiple pairwise comparisons.

Experiment 2

Apparatus. Locomotor activity was measured in Plexiglas photocell cages (39 × 25 × 34 cm) interfaced to an IBM personal computer (16). Dialysis probes were constructed by a procedure similar to that described previously (17). Briefly, the probe consisted of two sections of fused silica tubing (40 μm i.d.; 105 μm o.d.; Polymicro Technologies, Phoenix, AZ) inserted into an 8-mm section of hollow dialysis fiber (220 μm o.d.; 6000 MW cutoff; Spectro/Por, Houston, TX). The ends of the dialysis membrane were sealed with polyamide resin (Alltech State College, PA). The distance between the inlet and outlet tubes was 4 mm for the STR and 2 mm for the NACC. A single-channel fluid swivel (5) was connected to the inlet flow of the dialysis probe. This allowed the rat to rotate freely during testing. The fluid swivel was connected to a 500-μl Hamilton syringe via PE-10 tubing. Artificial cerebrospinal fluid (CSF) was contained in the perfusion syringe. CSF was composed of 145 mM sodium chloride, 2.8 mM potassium chloride, 1.2 mM magnesium chloride, 1.2 mM calcium chloride, 0.25 mM ascorbic acid, and 5.4 mM glucose, adjusted to a pH of 7.2–7.4 with 0.1 M sodium hydroxide.

The concentration of dopamine was measured with high-performance liquid chromatography using electrochemical detection (HPLC-ED) as described previously (25). A 0.5-μl volume of perfusate was injected on a 0.5-mm i.d. HPLC column (5 μM C-18 ODS-2 stationary phase). Electrochemical detection of dopamine was accomplished with an amperometric detector (model 400, EG&G Princeton Applied Research, Princeton, NJ) with an applied potential of +700 mV vs. an Ag/AgCl reference electrode.

Surgery. Rats were anesthetized with 50 mg/kg IP sodium pentobarbital (Nembutal). Subjects were placed in a stereotaxic frame (David Kopf, Tujunga, CA), and a stainless steel guide cannula (20 g; Plastics One, Roanoke, VA) was lowered to access either the STR or NACC. The stereotaxic coordinates for the STR were AP +2.5 from bregma, lat 2.7 (equal number on each side), DV −2.7 from dura with the incisor bar set at +5 mm (24). The stereotaxic coordinates for the NACC were AP +3.4 from bregma, lat 1.7 (equal number on each side), DV −5.5 from dura with the incisor bar set at +5 mm (24). The guide cannulae were secured in place with the use of skull screws and dental cement. Intramuscular penicillin (60,000 U) was administered immediately following surgery. A recovery period of 7–8 days was allowed following surgery before dialysis was performed.

Behavior and microdialysis. The day before microdialysis was performed, subjects were placed in the photocell cages at 1400 h and allowed to habituate. Subjects had free access to food and water. At 1900 h, the dialysis probe was implanted and flow initiated at 0.1 μl/min. This was to allow subjects to become habituated to the dialysis probe assembly and levels of dopamine to return to baseline after the disturbance caused by probe implantation. Following probe implantation, lights were turned off to maintain the rat's normal circadian rhythm. At 0700 h the following day, lights were turned on, food removed, and the perfusate flow rate increased to 0.60 μl/min. Dialysate samples were subsequently collected every 10 min and injected directly onto the HPLC. Locomotor activity was monitored simultaneously in 10-min bins.

In the test cage, subjects were administered either naloxone

(5.0 mg/kg) or saline after three consecutive dialysis samples varied less than 10% in peak height. Rats received additional SC injections at 30-min intervals: 0.1, 0.3, 1.2, and 4.8 mg/kg *d*-amphetamine, resulting in cumulative amphetamine doses of 0, 0.1, 0.4, 1.6, and 6.4 mg/kg as in Experiment 1. Dialysate dopamine concentration and locomotor activity was monitored until 1 h after administration of the final cumulative dose of amphetamine.

Histology. At the end of the experiment, rats were anesthetized with chloral hydrate (400 mg/kg) and perfused first with 50 ml 0.9% saline and then 50 ml 10% formalin. Coronal sections (75 μm) were cut on a freezing microtome following fixation. Each section through the area of interest and associated structures was mounted on a glass slide and stained with thionine for determination of cannulae placements.

Data analysis. Locomotor activity counts and dialysate dopamine concentrations (expressed as % baseline) were subjected to ANOVA with repeated measures; Newman-Keuls posthoc tests were performed where appropriate. The three time points that preceded drug administration served as the baseline.

RESULTS

Experiment 1

The cumulative administration of amphetamine (0.1-6.4 mg/kg, SC) caused a dose-dependent increase in the locomotor activity of rats pretreated with saline and rats pretreated with 5.0 mg/kg naloxone (Fig. 1). This was demonstrated by significant increases in both the gross, $F(4, 88) = 35.06$, $p < 0.0001$, and fine, $F(4, 88) = 27.45$, $p < 0.0001$, motor activity counts. Pretreatment with naloxone at either 0.2 or 1.0 mg/kg had no significant influence upon the increase in motor activity induced by amphetamine (data not shown). However, administration of a higher dose of naloxone, 5.0 mg/kg, attenuated significantly the amphetamine-induced increase in gross locomotor counts, $F(1, 22) = 11.61$, $p < 0.01$. Posthoc analysis revealed significant differences between rats pretreated with 5.0 mg/kg naloxone and those with saline following 0.4 and 1.6 mg/kg amphetamine; gross counts were reduced from 550 ± 85 and 1,245 ± 104, respectively, in saline-pretreated rats to 223 ± 50 ($p < 0.05$) and 837 ± 119 ($p < 0.01$) in naloxone-pretreated animals (Fig. 1). Naloxone (5.0 mg/kg) did not significantly alter the fine motor activity response to amphetamine, $F(1, 22) = 0.089$, n.s. A separate analysis revealed a naloxone-induced reduction in locomotor activity following saline injection. For example, the gross locomotor counts were reduced from 154 ± 18 for the saline group to 37 ± 8 for animals pretreated with 5.0 mg/kg naloxone ($p < 0.05$, two-tailed *t*-test).

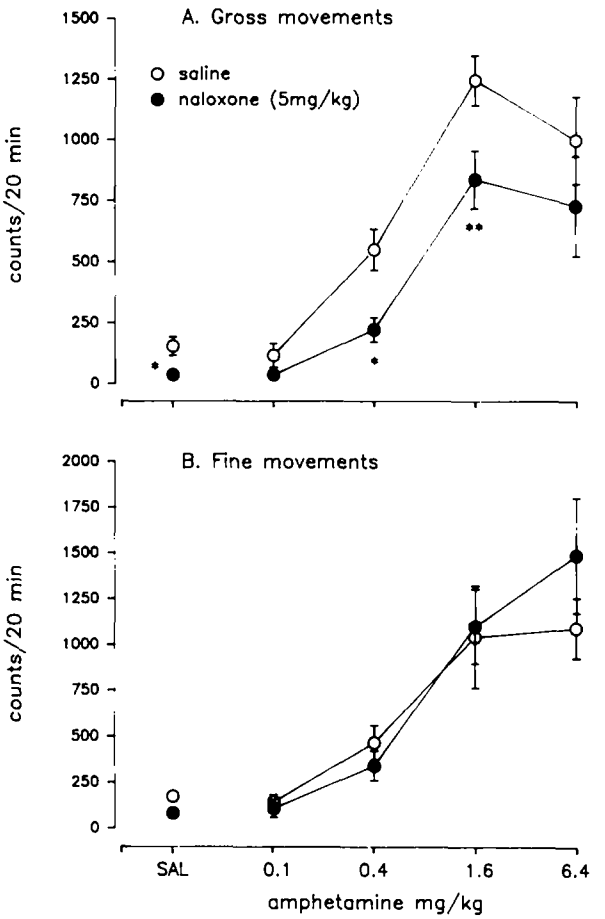

FIG. 1. Naloxone, 5.0 mg/kg, attenuates the locomotor response of rats to amphetamine. Rats were injected SC with either saline or naloxone, then 30 min later received an SC injection of saline (points above SAL). Thereafter, at 30-min intervals rats were injected SC with the indicated cumulative doses of amphetamine. Activity was recorded for 20-min periods, beginning 10 min after each injection. Shown are the mean ± SEM. ($n = 12$) counts per 20 min for: (A) gross movements corresponding to locomotion and (B) fine movements. Significant differences from the corresponding point of rats pretreated with saline are indicated by *($p < 0.05$) and **($p < 0.01$).

Experiment 2

Dialysis overall. When subjects from both the STR ($n = 16$) and NACC ($n = 12$) were analyzed together, there was a lower dialysate dopamine concentration after amphetamine treatment in subjects administered naloxone compared to those receiving saline, $F(16, 27) = 7.02$, $p < 0.02$. The difference between saline- and naloxone-treated subjects was dependent upon the dose of *d*-amphetamine administered, as shown by a naloxone × amphetamine interaction, $F(7, 644) = 7.643$, $p < 0.0001$. This response differed across time, as reflected by a naloxone × amphetamine × time interaction, $F(14, 48) = 3.95$, $p < 0.0001$.

As expected, ANOVA revealed a difference in dopamine dialysate concentration as a function of amphetamine treatment, $F(7, 644) = 62.8$, $p < 0.0001$. Rats responded differently across time following amphetamine, as indicated by an amphetamine × time interaction, $F(14, 48) = 29.00$, $p < 0.0001$. Posthoc analysis showed that the 1.6-mg/kg cumulative amphetamine dose produced increases in dopamine concentration over baseline, naloxone, or saline treatment and 0.0, 0.1, and 0.4 mg/kg amphetamine ($p < 0.01$ for each comparison). The 6.4-mg/kg dose produced increases over baseline, saline, or naloxone and 0.0, 0.1, 0.4, and 1.6 mg/kg amphetamine ($p < 0.01$ for each comparison).

STR dialysis. There was an overall smaller dopaminergic response in STR rats treated with naloxone compared to those treated with saline, $F(1, 15) = 5.97$, $p < 0.05$ (Fig. 2A). No difference existed in dialysate dopamine concentration during the baseline period between rats treated with saline (0.148 ± 0.011 pmol/μl) and rats treated with naloxone (0.149 ± 0.012 pmol/μl), $F(1, 15) = 0.01$, n.s. There was an effect with am-

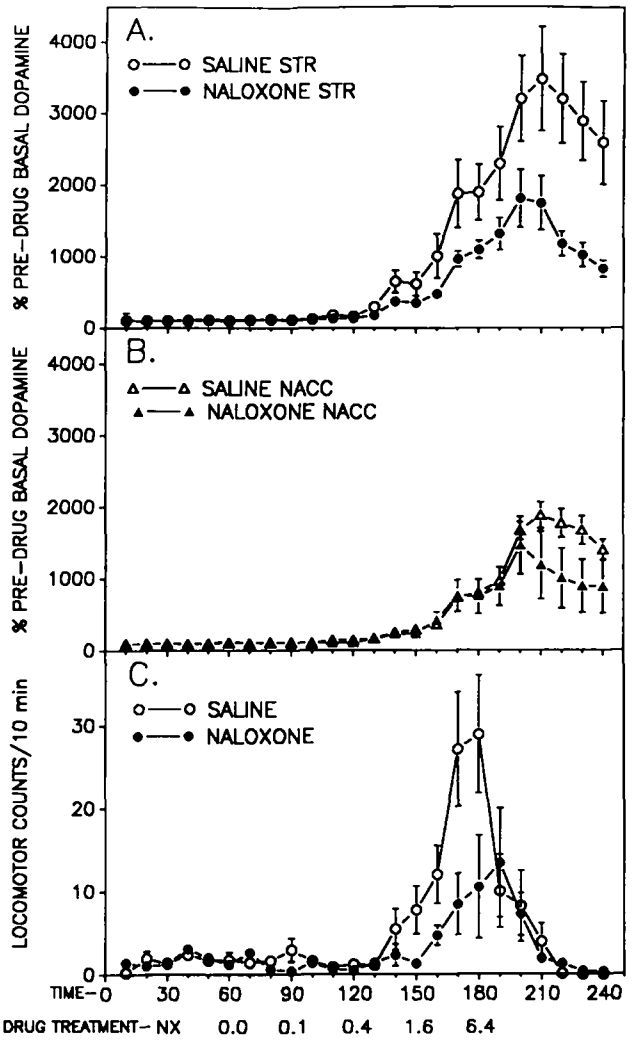

FIG. 2. Naloxone, 5.0 mg/kg, attenuates both the dopaminergic and locomotor responses of rats to amphetamine during microdialysis. Rats were injected SC with either saline or naloxone (NX) 30 min after measurements began, then 30 min later received an SC injection of saline (0.0). Thereafter, at 30-min intervals rats were injected SC with the indicated cumulative doses of amphetamine: 0.1, 0.4, 1.6, and 6.4 mg/kg. Dialysates and locomotor activity counts were collected over 10-min intervals. Each point is a mean ± SEM for: (A) dopamine content of dialysates from striatum (STR; n = 8/point), expressed as a percent of predrug basal concentration (the three points from 0-30 min that preceded the injections of saline or naloxone); (B) dopamine content of dialysates from nucleus accumbens (NACC; n = 6/point), expressed as a percent of predrug basal concentration; (C) locomotor activity counts for rats from both dialysis groups (n = 14/point).

phetamine treatment, as revealed by a main effect of amphetamine, $F(7, 368) = 37.78$, $p < 0.0001$. The response to amphetamine varied across time, as shown by an amphetamine × time interaction in STR rats, $F(14, 28) = 16.76$, $p < 0.0001$. The greater dopaminergic response in subjects treated with saline compared to subjects treated with naloxone was dependent upon the dose of amphetamine, as revealed by a naloxone × amphetamine interaction, $F(7, 368) = 6.18$, $p < 0.0001$. There was no difference between naloxone- and saline-treated subjects during the baseline period, the 30 min following naloxone injection, or following 0.0, 0.1, or 0.4 mg/kg amphetamine. There was less of a dopaminergic response in naloxone-treated subjects following 1.6, $F(1, 15) = 4.52$, $p < 0.05$, and 6.4, $F(1, 15) = 6.93$, $p < 0.02$, mg/kg amphetamine. This is illustrated by the fact that at 30 min following the 6.4 mg/kg dose of amphetamine saline rats were at 3,488 ± 726% baseline dopamine while naloxone-treated subjects were at only 50% of this levels at 1,748 ± 372% baseline dopamine. In addition, there was a naloxone × amphetamine × time interaction, $F(14, 28) = 1.99$, $p < 0.02$.

NACC dialysis. Amphetamine treatment increased dialysate dopamine concentration in a dose-dependent manner in NACC rats, $F(7, 276) = 31.94$, $p < 0.0001$ (Fig. 2B). No difference existed in basal dialysate dopamine concentration between rats treated with saline (0.081 ± 0.007 pmol/μl) and those treated with naloxone (0.079 ± 0.008 pmol/μl) during the baseline period, $F(1, 15) = 0.01$, n.s. The effect of naloxone treatment was not as great on NACC dopamine response, as shown by the fact there was not an overall difference between subjects treated with naloxone and those treated with saline, $F(1, 11) = 1.15$, n.s., or a naloxone × amphetamine interaction, $F(7, 276) = 1.80$, n.s. There was, however, a difference across time following amphetamine between naloxone- and saline-treated rats, as shown by a naloxone × amphetamine × time interaction, $F(14, 20) = 3.41$, $p < 0.0001$.

Differences between regions in dopamine dialysate response. The STR showed a larger dopaminergic response compared to the NACC, as revealed by a main effect of region, $F(1, 27) = 5.09$, $p < 0.05$. This difference was dependent upon amphetamine treatment, as shown by a region × amphetamine interaction, $F(7, 644) = 4.10$, $p < 0.0005$. The two regions responded differently following amphetamine across time, as indicated by a region × amphetamine × time interaction, $F(14, 48) = 1.86$, $p < 0.05$. In subjects pretreated with saline, no differences between regions were observed in the time following 0.0 mg/kg amphetamine, $F(1, 13) = 0.01$, n.s. The greater dopaminergic responses in STR rats compared to NACC rats was dependent upon amphetamine dose, as revealed by a region × amphetamine interaction, $F(7, 322) = 3.91$, $p < 0.001$. This difference was most pronounced 30 min following the 6.4-mg/kg cumulative dose, as STR rats showed almost twice the increase in basal dopamine (3,488 ± 726% of baseline) compared to NACC rats (1,883 ± 184% of baseline). If subjects were treated with naloxone, no differences in % baseline increases in dopamine were observed between STR and NACC following administration of amphetamine, as revealed by the lack of a region × amphetamine interaction in naloxone-treated rats (compare naloxone STR in Fig. 2A with naloxone NACC in Fig. 2B), $F(1, 14) = 0.81$, n.s.

Locomotor activity in dialyzed rats. There was no overall difference in locomotor response between rats with STR probes and those with NACC probes $F(1, 27) = 3.28$, n.s. Therefore, data from the two groups were combined and are displayed in Fig. 2C. There was no overall difference in locomotor activity between naloxone- and saline-treated rats, $F(1, 27) = 2.87$, n.s. Locomotor activity changed with differing amphetamine doses, $F(7, 644) = 15.95$, $p < 0.0001$. There was a difference between naloxone- and saline-treated rats that was dependent upon dose of amphetamine administered, as shown by a naloxone × amphetamine interaction, $F(7, 644)$, $p < 0.0005$. This is evident by the fact that peak locomotor activity was more than twice as high in saline-treated

rats (29 ± 8) compared to naloxone-treated rats (13 ± 7). In addition, there was a shift to the right of locomotor activity, as revealed by both a naloxone × time interaction, $F(2, 168) = 11.73$, $p < 0.0001$, and a naloxone × amphetamine × time interaction, $F(14, 48) = 1.94$, $p < 0.05$.

DISCUSSION

The results of these experiments show that naloxone attenuates not only the locomotor response of rats to amphetamine but the dopaminergic response as well. The latter effect of naloxone was more prominent in the STR than in the NACC. This first in vivo confirmation of the suppression by naloxone of a drug-elicited dopaminergic response provides further evidence for the hypothesis that the dopaminergic response to amphetamine is regulated by endogenous opioid systems. In addition, these results indicate that in rats not treated with naloxone the STR has a larger dopaminergic response to amphetamine than does the NACC.

It is well established that systemic administration of amphetamine increases extracellular dopamine in a dose-dependent manner in both the STR and NACC (7,26,29). The present experiment demonstrated a dose-dependent increase in extracellular dopamine in both the STR and NACC using a cumulative dosing protocol. There were no differences in the extracellular dopamine levels in the STR or NACC of saline-treated rats following saline administration. However, the increase in extracellular dopamine subsequent to amphetamine treatment was much greater in the STR than in the NACC. This is in contrast with previous reports of either a greater increase in dopamine levels in the NACC than in the STR (7) or no difference between the regions in dopaminergic responsiveness (27).

The differences in reports of the responsiveness of these two structures to amphetamine treatment might be explained by the differences in the methodologies employed. The *trans*-striatal probes utilized by Di Chiara and Imperato (7) sample from different portions of the STR and NACC than the vertical probes used in the present study and that of Robinson et al. (27); the apparent discrepancies between studies might represent differences in responsiveness within the different parts of the STR. Perhaps more importantly, in the present study rats underwent surgery for placement of the guide cannulae at least 1 week prior to the experiment and the dialysis probe was simply inserted into the guide cannulae 12 h before testing without the need for anesthesia or further surgery. This contrasts with the methods employed by these other investigators (7,27), who surgically implanted the dialysis probes into anesthetized rats directly into the brain structure being monitored less than 24 h prior to testing. The trauma from such surgery might well affect the pharmacological responsiveness of the tissue after only 24 h. Alternatively, the differences in results among studies may be a consequence of differences in the methods of drug administration. In the present study, amphetamine was administered using a cumulative dosing regimen whereas single doses of amphetamine were administered in the other studies (7,27).

However, the major finding of this study was that a dose of the opioid antagonist, naloxone, which attenuated the locomotor response of untethered rats to amphetamine, also attenuated both the amphetamine-induced increases in locomotor activity and in extracellular dopamine in the dialysate of tethered rats. This provides the first in vivo neurochemical correlate to the well-documented ability of naloxone and naltrexone to attenuate behavioral effects of amphetamine (see the introductory section).

Opioid agonists have been shown to affect levels of extracellular dopamine in the STR and NACC, presumably by acting on the dense populations of opioid receptors that have been identified in the nigrostriatal and mesolimbic regions of the brain (11,20,36). For example, μ- and δ-opioid agonists increase the levels of extracellular dopamine in these two regions following systemic or intracerebral administration (7, 8,19,30,31), effects that are blocked by opioid antagonists (7,30). In contrast, κ-opioid agonists decrease the neuronal release of dopamine (7,21,36). Naloxone could attenuate amphetamine-stimulated release of dopamine by blocking μ- or δ-opioid receptors, thereby eliminating direct and/or indirect opioidergic modulation of dopaminergic neurons. The relatively high doses of naloxone usually necessary to attenuate behavioral effects of amphetamine suggests that naloxone is interacting with opioid receptors other than μ.

The use of a relatively high dose of naloxone in this study, 5.0 mg/kg, may have been necessitated by the length of the cumulative dosing protocol, 3.0–3.5 h, and the short plasma half-life of naloxone in the rat, approximately 30 min (4,22). In shorter procedures, the minimum dose of naloxone for attenuating behavioral effects of amphetamine in the rat has ranged from 0.02 mg/kg (33) to 4.0 mg/kg (9), possibly reflecting, in part, the different sensitivities of the different dependent behavioral measures.

The 5.0-mg/kg dose of naloxone significantly reduced the baseline locomotor activity of rats in Experiment 1. This result raises the possibility that the attenuation of effects of amphetamine by naloxone is merely the consequence of a nonspecific behavioral depression. However, several points argue against this possibility. First, although naloxone reduced the amphetamine-stimulated increases in extracellular dopamine it had no effect on baseline dopamine levels prior to the administration of amphetamine. Second, naloxone did not reduce the baseline locomotor activity of tethered rats in Experiment 2. Third, in the same animals in which gross movements were reduced by naloxone fine movements were unaffected. Fourth, under conditions similar to those of the present study naloxone decreased baseline locomotor activity but did not modify the stimulant effect of cocaine (Jones and Holtzman, in preparation). Therefore, it appears that the ability of naloxone to attenuate behavioral and neurochemical responses to amphetamine is the consequence of a specific interaction between naloxone and opioid systems that modulate the neuronal substrates mediating those responses. The results of this study provide the first direct evidence of a neurochemical basis for the ability of opioid antagonists to attenuate behavioral effects of amphetamine-type psychomotor stimulant drugs.

ACKNOWLEDGEMENTS

This research was supported in part by Grants DA00541 (S.G.H.) and DA07532 (J.B.J.) and by Research Scientist Award DA00008 (S.G.H.), all from the National Institute on Drug Abuse.

REFERENCES

1. Adams, P. M.; Beauchamp, R.; Alston, C. Potentiation of apomorphine and *d*-amphetamine effects by naloxone. Life Sci. 28: 629–634; 1981.

2. Andrews, J. S.; Holtzman, S. G. Effects of naloxone and diprenorphine on amphetamine-stimulated behavior in guinea pigs and rats. Neuropharmacology 26:1115–1120; 1987.

3. Andrews, J. S.; Holtzman, S. G. The interaction of *d*-amphetamine and naloxone differs for rats trained on separate fixed-interval or fixed-ratio schedules of reinforcement. Pharmacol. Biochem. Behav. 26:167-171; 1987.
4. Berkowitz, B. A.; Ngai, S. H.; Mempstead, J.; Spector, S. Disposition of naloxone: Use of a new radioimmunoassay. J. Pharmacol. Exp. Ther. 195:499-504; 1975.
5. Brown, Z. W.; Amit, Z.; Weeks, J. R. Simple flow-thru swivel for infusions into unrestrained animals. Pharmacol. Biochem. Behav. 5:363-365; 1976.
6. Dettmar, P. W.; Cowan, A.; Walter, D. S. Naloxone antagonizes behavioural effects of *d*-amphetamine in mice and rats. Neuropharmacology 17:1041-1044; 1978.
7. Di Chiara, G.; Imperato, A. Opposite effects of mu and kappa opiate agonist on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J. Pharmacol. Exp. Ther. 244:1067-1080; 1988.
8. Di Chiara, G.; Imperato, A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA 85: 5274-5278; 1988.
9. Esposito, R. U.; Perry, W.; Kornetsky, C. Effects of *d*-amphetamine and naloxone on brain stimulation reward. Psychopharmacology (Berl.) 69:187-191; 1991.
10. Franklin, K. B. J.; Robertson, A. Effects and interactions of naloxone and amphetamine on self-stimulation of the prefrontal cortex and dorsal tegmentum. Pharmacol. Biochem. Behav. 16: 433-436; 1982.
11. Goodman, R. R.; Alder, B. A.; Pasternak, G. W. Regional distribution of opioid receptors. In: Pasternak, G. W., ed. The opiate receptors. Clifton, NJ: The Humana Press; 1988:197-228.
12. Harris, R. A.; Snell, D. Interactions between naltrexone and nonopiate drugs evaluated by schedule-controlled behavior. Neuropharmacology 19:1087-1093; 1980.
13. Hitzemann, R.; Curell, J.; Hom, D.; Loh, H. Effects of naloxone on *d*-amphetamine- and apomorphine-induced behavior. Neuropharmacology 21:1005-1011; 1982.
14. Holtzman, S. G. Behavioral effects of separate and combined administration of naloxone and *d*-amphetamine. J. Pharmacol. Exp. Ther. 189:51-60; 1974.
15. Holtzman, S. G. Comparison of the effects of morphine, pentazocine, cyclazocine and amphetamine on intracranial self-stimulation in the rat. Psychopharmacologia 46:223-227; 1976.
16. Hooks, M. S.; Jones, G. H.; Smith, A. D.; Neill, D. B.; Justice, J. B., Jr. Individual differences in locomotor activity and sensitization. Pharmacol. Biochem. Behav. 38:467-470; 1991.
17. Hooks, M. S.; Jones, G. H.; Smith, A. D.; Neill, D. B.; Justice, J. B., Jr. Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 9:121-128; 1991.
18. Kuczenski, R. Biochemical actions of amphetamine and other stimulants. In: Creese, I., ed. Stimulants: Neurochemical, behavioral, and clinical perspectives. New York: Raven Press; 1983: 31-61.
19. Leone, P.; Pocock, D.; Wise, R. A. Morphine-dopamine interaction: Ventral tegmental morphine increases nucleus accumbens dopamine release. Pharmacol. Biochem. Behav. 39:469-472; 1991.
20. Mansour, A.; Khachaturian, H.; Lewis, M. E.; Akil, H.; Watson, S. J. Anatomy of CNS opioid receptors. Trends Neurosci. 7:308-314; 1988.
21. Manzanares, J.; Lookingland, K. J.; LaVigne, S. D.; Moore, K. E. Activation of tuberohypophysial dopamine neurons following intracerebroventricular administration of the selective kappa opioid receptor antagonist norbinaltorphimine. Life Sci. 48:1143-1149; 1991.
22. Misra, A. L.; Pontani, R. B.; Vadlamani, N. L.; Mule, S. J. Physiological disposition and biotransformation of [allyl-1′,3′-^{14}C]naloxone in the rat and some comparative observations on nalorphine. J. Pharmacol. Exp. Ther. 196:257-268; 1976.
23. Moore, K. E. Amphetamines: Biochemical and behavioral actions in animals. In: Iversen, L. L.; Snyder, S. H., ed. Handbook of psychopharmacology. vol. 2. New York: Plenum Press; 1978:41-98.
24. Pellegrino, K. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.
25. Pettit, H. O.; Pan, H.; Parsons, L. H.; Justice, J. B., Jr. Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. J. Neurochem. 55:798-804; 1990.
26. Robinson, T. E.; Jurson, P. A.; Bennett, J. A.; Bentegen, K. M. Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: A microdialysis study in freely moving rats. Brain Res. 462:211-222; 1988.
27. Robinson, T. E.; Camp, D. M. Does amphetamine preferentially increase the extracellular concentration of dopamine in the mesolimbic system of freely moving rats? Neuropsychopharmacology 3:163-173; 1990.
28. Sawynok, J.; Pinsky, C.; LaBella, F. S. On the specificity of naloxone as an opiate antagonist. Life Sci. 25:1621-1632; 1979.
29. Sharp, T.; Zetterstrom, T.; Ljungberg, T.; Ungerstedt, U. A direct comparison of amphetamine-induced behaviours and regional brain dopamine release in the rat using intracerebral dialysis. Brain Res. 401:322-330; 1987.
30. Spanagel, R.; Herz, A.; Shippenberg, T. S. The effects of opioid peptides on dopamine release in the nucleus accumbens: An in vivo microdialysis study. J. Neurochem. 55:1734-1740; 1990.
31. Spanagel, R.; Herz, A.; Shippenberg, T. S. Identification of the opioid receptor types mediating beta-endorphin-induced alterations in dopamine release in the nucleus accumbens. Eur. J. Pharmacol. 190:177-184; 1990.
32. Swerdlow, N. R.; Amalric, M.; Koob, G. F. Nucleus accumbens opiate-dopamine interactions and locomotor activation in the rat: Evidence for a pre-synaptic locus. Pharmacol. Biochem. Behav. 26:765-769; 1987.
33. Trujillo, K. A.; Belluzzi, J. D.; Stein, L. Naloxone blockade of amphetamine place preference conditioning. Psychopharmacology (Berl.) 104:265-274; 1991.
34. Winslow, J. T.; Miczek, K. A. Naltrexone blocks amphetamine-induced hyperactivity, but not disruption of social and agonistic behavior in mice and squirrel monkeys. Psychopharmacology (Berl.) 96:493-499; 1988.
35. Wise, R. A.; Bozarth, M. A. A psychomotor theory of addiction. Psychol. Rev. 94:469-492; 1987.
36. Wood, P. L.; Iyengar, S. Central actions of opiates and opioid peptides: In vivo evidence for opioid receptor multiplicity. In: Pasternak, G. W., ed. The opiate receptors. Clifton, NJ: The Humana Press; 1988:307-356.