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Previous Exposure to Cocaine Enhances Cocaine Self-Administration in an Alpha 1-Adrenergic Receptor Dependent Manner

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Noradrenergic transmission is implicated in the biochemical and behavioral effects of cocaine. Recently, we demonstrated that the alpha 1-adrenergic receptor antagonist prazosin attenuates cocaine-induced reinstatement of drug-seeking behavior. We now assessed whether prazosin could counter the effect of previous exposure to cocaine to enhance subsequent self-administration behavior. Rats were pre-exposed to systemic injections of either saline, prazosin (0.3 mg/kg), saline + cocaine (10 mg/kg), or prazosin + cocaine for 5 days. Starting 15–18 days after the last pre-exposure injection, rats were trained to self-administer cocaine (0.5 mg/kg/infusion) under a fixed ratio 3 (FR3) schedule of reinforcement. Several tests were conducted. First, responding for cocaine under an FR3 schedule was assessed across several doses (0.125–1.0 mg/kg/infusion). Second, responding for cocaine (0.5 mg/kg/infusion) under a progressive-ratio (PR) schedule was examined for 6 consecutive days. Finally, responding for cocaine (0, 0.5, and 1.0 mg/kg/infusion) was determined under the PR schedule of reinforcement. Results showed that cocaine pre-exposed rats self-administer more cocaine compared to saline pre-exposed rats when tested under both the FR and PR schedules. Rats pre-exposed to cocaine plus prazosin did not show enhanced cocaine self-administration. These rats, as well those pre-exposed to prazosin alone, showed levels of cocaine self-administration similar to saline pre-exposed rats. Thus, previous exposure to cocaine enhanced cocaine self-administration, an effect that appears to involve activation of alpha 1-adrenergic receptors. These data, along with several recent studies, show further support for the contribution of noradrenergic transmission in the behavioral effects of cocaine.
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INTRODUCTION

Repeated intermittent exposure to cocaine and other psychostimulants results in a progressive and enduring enhancement of the behavioral and neurochemical responses to subsequent exposures, a phenomenon termed sensitization (Kalivas and Stewart, 1991; Robinson and Becker, 1986). Sensitized responses have been suggested to underlie certain aspects of cocaine and other psychostimulant addiction (Robinson and Berridge, 1993; Shippenberg and Heidbreder, 1995; Vezina *et al*, 2002). Consistent with this view, previous repeated exposure to cocaine or amphetamine enhances the acquisition of cocaine and amphetamine self-administration when rats were tested with low to moderate doses under low fixed ratio (FR) schedules of reinforcement (Horger *et al*, 1990; Piazza *et al*, 1990; Pierre and Vezina, 1997). Similar amphetamine pre-exposure regimens promote self-administration of higher doses when tested under a progressive ratio (PR) schedule of reinforcement as well as the reinstatement of extinguished responding for drug (Lorrain *et al*, 2000; Suto *et al*, 2002). These findings suggest that previous repeated exposure to cocaine or amphetamine enhances the rewarding or incentive motivational effects of cocaine.

The induction of behavioral sensitization to cocaine and amphetamine involves neuroadaptations in the ventral tegmental area (VTA), particularly its dopamine (DA)-containing cell bodies that project to the nucleus accumbens (NAc) (Cornish and Kalivas, 2001; Vanderschuren and Kalivas, 2000; Vezina and Stewart, 1989). Indeed, the cocaine-induced increase in extracellular DA levels in NAc is an effect considered critical to its behavioral and reinforcing effects (DiChiara, 1999; Koob and Bloom, 1988; Robbins and Everitt, 1999). Yet, cocaine not only binds to the DA transporter (DAT), it also binds to norepinephrine (NE) and serotonin transporters (Florin *et al*, 1994; Ritz

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et al, 1987). And, by blocking the reuptake of these monoamines into presynaptic terminals, cocaine increases concentrations of these neurotransmitters at synapses in the NAc (Harris and Baldessarini, 1973; Pettit and Justice, 1989). Although much attention has been paid to alterations in the regulation of DA to understand the neurochemical bases of the behavioral effects of cocaine, DAT knockout (KO) mice self-administer cocaine intravenously (Rocha *et al*, 1998) and show cocaine conditioned place preference (Sora *et al*, 1998). Further, both cocaine and amphetamine increase extracellular levels of DA in the NAc of DAT-KO mice (Carboni *et al*, 2001). Thus, these results suggest that other neurotransmitters also contribute to these long-term behavioral and neurochemical effects of cocaine.

Noradrenergic transmission is implicated in the biochemical and behavioral effects of cocaine. Indeed, a number of studies in mice and rats demonstrate interactions between ascending NE and DA systems (Lategan *et al*, 1990; Shi *et al*, 2000). Increases in locomotor activity and DA levels induced by D-amphetamine are inhibited by either systemic or local injection into the prefrontal cortex with prazosin, an alpha 1-adrenergic antagonist (Blanc *et al*, 1994; Darracq *et al*, 1998; Drouin *et al*, 2002; Wellman *et al*, 2002). Locomotor sensitization induced by cocaine, D-amphetamine, or morphine is decreased in alpha1-b subtype of adrenergic receptors KO mice compared to wild-type littermates (Drouin *et al*, 2002). Further, we have recently demonstrated that prazosin pretreatment attenuated cocaine-induced reinstatement of extinguished drug-seeking behavior in rats (Zhang and Kosten, 2005). These results suggest that NE systems also contribute to the behavioral and neurochemical effects of cocaine.

We hypothesized that pre-exposure to cocaine would lead to a long-lasting enhancement of cocaine self-administration and that this enhancement would involve alpha 1-adrenergic receptor activation. The purpose of the present experiment was to test this hypothesis using both FR and PR schedules of reinforcement. Testing under a FR schedule would provide information on whether cocaine pre-exposed rats would show altered sensitivity to cocaine. Testing under a PR schedule would allow us to demonstrate whether cocaine pre-exposed rats will work more to obtain the drug. The contribution of NE transmission to these effects was examined by pretreating rats with prazosin alone or in combination with cocaine.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats (Charles River, Wilmington, MA, USA) weighing about 300 g at the start of the study were used. They were housed individually in hanging, stainless-steel cages in a temperature- and humidity-regulated vivarium on a 12 h light–dark cycle (lights on at 0700 hours). Food and water were freely available, except during initial lever press training for food. Procedures were approved by the Institutional Animal Care and Use Committee in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Facilities were accredited by the American Association of Laboratory Animal Care.

Drugs

Prazosin (0.3 mg/kg) was obtained from Sigma (St Louis, MO) and cocaine hydrochloride (10 mg/kg) was obtained gratis from the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC). All drugs were mixed in 0.9% NaCl and given intraperitoneally (i.p.) in a volume of 1 ml/kg. Cocaine solutions for self-administration were sterile filtered and prepared fresh daily based on individual body weight that was measured daily.

Groups

Animals were randomly assigned to one of four pre-exposure conditions: saline–saline (S–S; $n = 6$), prazosin + saline (P–S; $n = 6$), saline–cocaine (S–C; $n = 7$), or prazosin + cocaine (P–C; $n = 6$).

Pre-Exposure

Fifteen to eighteen days before catheter implant surgery, rats received a total of five sets of i.p. injections, one per day, corresponding to their pre-exposure condition: saline followed by cocaine (10 mg/kg; S–C), saline followed by saline (S–S), prazosin (0.3 mg/kg) followed by cocaine (P–C), or prazosin followed by saline (P–S). The first injection was administered 30-min before the second injection.

The pretreatment time and doses of cocaine and prazosin were selected based, in part, on our previous findings (Haile *et al*, 2003; Zhang and Kosten, 2005). A 30-min pretreatment time was used in our prior study and also in other studies (Blanc *et al*, 1994; Darracq *et al*, 1998). In addition, the 30-min pretreatment time is within the rise time of peak absorption with oral administration (Cavero and Roach, 1980). The dose of cocaine was selected because it is sufficient to induce sensitization of its locomotor activating effects and reinstate extinguished cocaine self-administration behavior. The choice of prazosin dose (0.3 mg/kg) was based on our finding that higher prazosin doses ($\geqslant$0.5 mg/kg) completely suppressed responding for food and lower doses ($\leqslant$0.3 mg/kg) had minimal effects on food responding. Thus, we selected the maximal prazosin dose that did not disrupt ongoing behavior.

Surgery

Rats were implanted with chronically-indwelling catheters, made of Silastic tubing, into the right jugular vein under ketamine (10 mg/kg) and xylazine HCl (0.2 mg/kg) anesthesia. Catheters passed under the skin and were threaded out of an incision made on the back. The catheter was connected to a single-guide cannula (22 G; Plastics One, Roanoke, VA) attached to a covance infusion harness (Instech Laboratories). Rats were allowed to recover for a minimum of 4 days and were treated with antibiotic during the first 3 days. The catheter was flushed at least 5 days/week to maintain patency.

Apparatus

Standard operant conditioning chambers (Coulbourn Instruments, Allentown, PA) were housed in ventilated, sound-

attenuating cubicles (Coulbourn) with fans to mask outside noise. As described previously (Kosten *et al*, 2004; Zhang and Kosten, 2005), on one wall of each chamber, there were two response levers and above each were three 'cue' lights. Minimal downward pressure (about 25 g) on a lever could result in a programmed response. Between these two levers was a recessed food receptacle into which food pellets (45 mg each; Bio-Serv Inc., Frenchtown, NJ) could be dispensed from a pellet dispenser. A house light was also located within the chamber. These chambers were equipped with syringe pump systems that consisted of an infusion pump (Razel model A, Stamford, CT) with a 20-ml glass syringe connected by a single channel 22 Ga swivel and clamp (Instech Laboratories, Plymouth Meeting, PA) with Teflon tubing. The tubing was connected to the animal's catheter system in order to deliver the cocaine solution intravenously for self-administration. Experimental parameters (schedules of reinforcements, time periods, etc) were programmed using a software package (Graphic State Notation, Coulbourn) installed on a PC computer.

Cocaine Self-Administration

Before implantation of intravenous catheters, rats were food-restricted to 85% of their free-feeding body weight and allowed to lever press for food in the operant conditioning chambers. Rats were trained initially to press a lever for food pellets under a fixed ratio 1 (FR1) schedule of reinforcement overnight. Operant training for food-maintained behavior was continued until a criterion of at least 100 pellets was achieved. The animals were then returned to *ad libitum* food availability and implanted with jugular catheters 2 to 3 days later.

Cocaine self-administration sessions were 3-h in length and were conducted 5–7 days per week. Sessions began with the administration of two noncontingent intravenous drug infusions (0.5 mg/infusion, delivered over 10 s). Rats were initially trained to lever press for cocaine (0.5 mg/kg/infusion) under an FR1 schedule of reinforcement. One depression of the active lever resulted in a 100-μl infusion of cocaine solution delivered through the catheter over 10-s. Lever presses on the inactive lever were tabulated but had no programmed consequences. The house light was illuminated at the session onset and remained on until the active lever was depressed. The cue lights located over the active lever were illuminated during the 10-s cocaine infusion and all lights were then turned off for the subsequent 5-s time-out periods. During the infusion and time-out periods, the active lever was inactivated. Once stable response rates were seen, the schedule was switched to FR3 and continued under this schedule until responding was stable (standard deviation of the mean for 3 consecutive days was less than 20% of the mean). After this time, the experimental tests were conducted.

Procedure Sequence

The overall sequence of procedures is as follows. First, rats received their respective treatments according to their drug pre-exposure group assignment. Second, catheter surgery was performed. Third, training for cocaine self-administration behavior was conducted. Fourth, maintenance of cocaine self-administration tests conducted under the FR schedule was performed in Experiment 1. Finally, in Experiment 2, tests were conducted under the progressive ratio for 6 consecutive days, keeping cocaine dose constant, and then rats were tested under this schedule with different cocaine doses.

Experiment 1: Cocaine dose–response tests under an FR3 schedule. Determination of the dose–response function was conducted in 3-h sessions under an FR3 schedule of reinforcement. Dose–response data were established by allowing the rat to self-administer each of four doses (0.125, 0.25, 0.5 and 1.0 mg/kg/infusion) on separate test sessions. Tests were run one day for each drug dose. To control for order effects, the animals were tested with the four different doses in a nonsystematic sequence. At least 2–3 days of self-administration sessions intervened in which the training dose of cocaine was available to ensure that the animal had returned to its baseline level of responding.

Analyses of responding included tabulating numbers of self-administered infusions and inactive lever presses for each dose. Inactive lever press data served as a control for general activity levels. Data are presented as mean ($\pm$SEM) and significance levels were set at 0.05. These dose–response data were analyzed using a $2 \times 2 \times 4$ analysis variance (ANOVA) representing the between-group factors of cocaine pre-exposure and prazosin pre-exposure with repeated measures on dose. *Post hoc* comparisons between groups were made at each dose using the Newman–Keuls procedure.

Experiment 2: Cocaine responding under a PR schedule. After completion of cocaine self-administration tests under the FR3 schedule, rats were given daily access to the training dose of cocaine (0.5 mg/kg/infusion) until stable baselines of self-administration under this schedule were re-established for at least 3 days. Subsequently, tests were conducted daily in one 6-h session per day under a PR schedule of reinforcement for 6 consecutive days with the training dose of cocaine available. Under this schedule, the response requirement for each successive injection increased by progressive increments according to the following series: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, and 603 (Richardson and Roberts, 1996). Two rats (one in the S–S group and one in the S–C group) were not able to be run in this experiment.

After completion of 6 days of cocaine self-administration under the PR schedule, rats were allowed to self-administer either saline, 0.5 mg/kg/infusion or 1 mg/kg/infusion cocaine on separate 6-h sessions under the same PR schedule. Tests were conducted in a nonsystematic sequence. At least 2–3 days of cocaine self-administration training (FR3; 0.5 mg/kg/infusion) intervened to ensure that the animal had returned to its baseline level of responding.

The main dependent measures were the total numbers of cocaine infusions self-administered as well as final ratios completed. Final ratios completed were analyzed, not break points, because sessions were terminated at the end of 6 h, regardless of whether rats had discontinued self-administration. We also analyzed the numbers of inactive lever

presses emitted as a control for general activity levels. Data obtained under the PR schedule over 6 days were analyzed using a $2 \times 2 \times 6$ ANOVA representing the between-group factors of cocaine pre-exposure and Prazosin pre-exposure with repeated measures on day. Data obtained from the dose-response tests conducted under the PR schedule were analyzed using a $2 \times 2 \times 3$ ANOVA representing the between-group factors of cocaine pre-exposure and prazosin pre-exposure with repeated measures on dose. Analysis of final ratios completed were performed using the nonparametric Mann–Whitney *U*-test because these data were derived from an escalating exponential function and violate the assumption of the homogeneity of variance for ANOVA (Richardson and Roberts, 1996). *Post hoc* comparisons were made at each dose and day using the Newman–Keuls procedure (Winer, 1962).

RESULTS

Experiment 1: Cocaine Dose–Response Tests Under an FR3 Schedule

Responding for various cocaine doses under the fixed ratio 3 (FR3) schedule are shown in Figure 1 for the four groups of rats (S–S, S–C, P–S, and P–C). Rats in all groups exhibited decreasing numbers of self-administered infusions as cocaine dose increased. The effect of dose was significant, $F(3,72) = 71.32$; $P < 0.0001$. Cocaine pre-exposure was associated with greater numbers of self-administered infusions as supported by the significant cocaine pre-exposure effect, $F(1,23) = 29.98$; $P < 0.0001$. Cocaine pre-exposure appears to result in a steeper dose response function as seen in Figure 1 and supported by the significant cocaine pre-exposure × dose interaction, $F(3,72) = 16.28$; $P < 0.0001$. However, rats that were pretreated with prazosin before cocaine injections (P–C) did not self-administer as much cocaine as the rats that were pretreated with saline before cocaine injections (S–C). This statement is supported by the significant interaction of cocaine pre-exposure × prazosin pre-exposure, $F(1,23) = 4.33$; $P < 0.05$. On the other hand, prazosin pre-exposure itself did not have any significant effect on cocaine self-administration as the main effect of prazosin pre-exposure failed to reach significance ($P > 0.10$). *Post hoc* comparisons revealed that the S–C group showed significantly greater cocaine self-administration compared to the other three groups at all four doses ($P < 0.05$). The P–C group shows greater self-administration than the S–S and P–S groups at the 0.125 and 0.25 mg/kg cocaine doses ($P < 0.05$) and the P–C group shows greater self-administration than the S–S group at the 0.5 and 1.0 mg/kg doses ($P < 0.05$). All other group and dose comparisons are not significant.

Numbers of inactive lever presses emitted under the FR3 schedule of reinforcement across cocaine doses are presented in Table 1A. There are no significant main effects or interactions of prazosin or cocaine pre-exposures on numbers of inactive lever presses that were minimal across doses ($P > 0.10$).

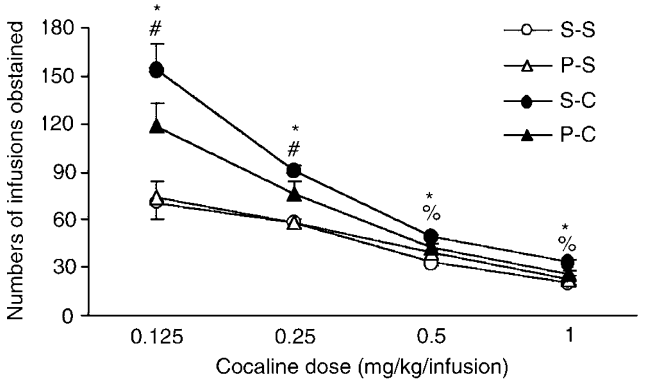

Figure 1 Mean (±SEM) numbers of self-administered cocaine infusions by saline–saline (S–S; open circles; $n = 6$), prazosin (0.3 mg/kg)–saline (P–S; open diamonds; $n = 6$) saline–cocaine (10 mg/kg; S–C; closed circles; $n = 7$), and prazosin–cocaine (P–C; closed diamonds; $n = 6$) groups under the fixed-ratio 3 (FR3) schedule are presented by cocaine dose (mg/kg/infusion). The S–C group (*) shows greater cocaine self-administration compared to the other three groups at all four doses ($P < 0.05$). The P–C group (#) shows greater self-administration than the S–S and P–S groups at the 0.125 and 0.25 mg/kg cocaine doses ($P < 0.05$) and the P–C group (%) shows greater self-administration than the S–S group at the 0.5 and 1.0 mg/kg doses ($P < 0.05$). All other group and dose comparisons are not significant.

Table 1 Mean (±SEM) Inactive Lever Presses for Rats Pre-Exposed to Saline (S–S), Prazosin (0.3 mg/kg) and Saline (P–S), Saline and Cocaine (10 mg/kg; S–C), and Prazosin and Cocaine (P–C)

(a) Inactive lever presses by cocaine dose for FR dose-response tests

	Cocaine dose (mg/kg/infusion)			
Group	**0.125**	**0.25**	**0.5**	**1**
S–S	0.5 (0.3)	0.7 (0.5)	0.3 (0.2)	1.5 (1.0)
P–S	1.0 (0.5)	0.2 (0.2)	0.3 (0.2)	0.2 (0.2)
S–C	1.4 (0.7)	0.3 (0.2)	0.3 (0.3)	1.1 (0.6)
P–C	0.7 (0.3)	0.3 (0.3)	0.7 (0.7)	0.3 (0.2)

(b) Inactive lever presses by day for progressive-ratio tests

	Day					
Group	**1**	**2**	**3**	**4**	**5**	**6**
S–S	9.8 (3.9)	4.0 (1.3)	7.5 (2.7)	9.0 (3.8)	4.0 (1.8)	5.5 (2.3)
P–S	9.0 (2.3)	7.7 (2.4)	7.8 (1.7)	8.5 (2.2)	8.5 (3.9)	7.5 (2.1)
S–C	9.3 (2.7)	9.5 (2.8)	8.3 (2.9)	8.8 (2.9)	8.2 (1.9)	8.3 (2.6)
P–C	5.3 (2.8)	5.8 (1.8)	7.7 (1.7)	6.3 (3.0)	5.0 (1.9)	6.3 (2.4)

(c) Inactive lever presses by cocaine dose for progressive-ratio dose response tests

	Cocaine dose (mg/kg/infusion)		
Group	**0**	**0.5**	**1**
S–S	7.8 (4.7)	3.4 (0.9)	4.4 (2.5)
P–S	10.2 (3.3)	8.5 (3.9)	9.0 (1.8)
S–C	7.3 (1.9)	8.7 (2.8)	7.0 (2.8)
P–C	6.3 (3.0)	5.0 (1.9)	6.3 (2.4)

Experiment 2: Cocaine Responding Under a PR Schedule

Responding for cocaine (0.5 mg/kg/infusion) under a PR schedule across 6 consecutive days are shown in Figure 2 for the four groups of rats (S–S, S–C, P–S, and P–C). Cocaine pre-exposure was associated with greater numbers of self-administered infusions as supported by the significant cocaine pre-exposure effect, $F(1,21) = 17.16$; $P < 0.001$. Prazosin pre-exposure blocked this enhancement in responding for cocaine by cocaine pre-exposure as evidenced by the significant main effect of prazosin pre-exposure, $F(1,21) = 5.35$; $P < 0.05$. Further, the interaction of cocaine and prazosin pre-exposure effects was significant, $F(1,21) = 8.12$; $P < 0.01$. *Post hoc* comparisons indicate that the S–C group self-administered more cocaine on all 6 days compared to the other three groups ($P < 0.05$).

Data on final ratios completed across days are presented in Table 2a for the four groups of rats (S–S, S–C, P–S, and P–C). The S–C group that was pre-exposed to cocaine showed greater cocaine self-administration compared to the other three groups across all 6 days ($P < 0.05$).

Numbers of inactive lever presses emitted under the PR schedule of reinforcement across days are presented in Table 1b. There were no significant main effects or interactions of prazosin or cocaine pre-exposures on numbers of inactive lever presses that were minimal across doses ($P > 0.10$).

Responding for various doses of cocaine under the PR schedule are shown in Figure 3. Minimal self-administered infusions were seen when saline (0 mg/kg) was available for delivery and numbers of self-administered infusions increased when cocaine (0.5 and 1.0 mg/kg) was available. This is supported by the significant dose effect, $F(2,42) = 142.45$; $P < 0.0001$. Again, cocaine pre-exposure was associated with enhanced responding as supported by the significant cocaine pre-exposure effect, $F(1,21) = 12.74$; $P < 0.01$, and by its significant interaction with dose, $F(2,42) = 20.33$; $P < 0.0001$. Prazosin pre-exposure eliminated the enhancement in responding due to cocaine pre-exposure as supported by the significant main effect of prazosin, $F(1,21) = 4.83$; $P < 0.05$, and its interaction with cocaine pre-exposure, $F(1,21) = 13.17$; $P < 0.005$. *Post hoc* comparisons indicate that the S–C group showed significantly greater self-administration than the other three groups at both the 0.5 and 1.0 mg/kg/infusion doses ($P < 0.001$).

Data on final ratios completed across doses are presented in Table 2b for the four groups of rats (S–S, S–C, P–S, and P–C). The S–C group that was pre-exposed to cocaine showed greater cocaine self-administration compared to the other three groups when cocaine was available for delivery ($P < 0.05$).

Table 2 Median Final Ratios Completed Under the PR Schedule are Presented for Rats Pre-Exposed to Saline (S–S), Prazosin (0.3 mg/kg) and Saline (P–S), Saline and Cocaine (10 mg/kg; S–C), and Prazosin and Cocaine (P–C)

(a) Final ratios completed by day for PR tests

	Day					
Group[a]	1	2	3	4	5	6
S–S	62	77	95	95	62	77
P–S	77	95	95	95	95	118
S–C	219	219	328	328	219	328
P–C	77	77	77	77	62	77

(b) Final ratios completed by cocaine dose for PR dose-response tests

	Cocaine dose (mg/kg/infusion)		
Group[a]	0	0.5	1.0
S–S	12	77	62
P–S	9	77	95
S–C	15	219	328
P–C	15	62	77

[a]Significant group effect (S–C > other groups).

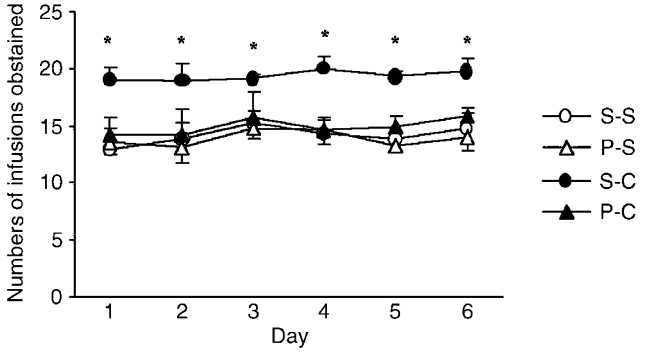

Figure 2 Mean (±SEM) numbers of self-administered cocaine infusions by saline–saline (S–S; open circles; $n = 5$), prazosin (0.3 mg/kg)–saline (P–S; open diamonds; $n = 6$), saline–cocaine (10 mg/kg; S–C; closed circles; $n = 6$), and prazosin–cocaine (P–C; closed diamonds; $n = 6$) groups under the PR schedule for 6 consecutive days are presented. The cocaine dose available for delivery was 0.5 mg/kg/infusion on all 6 days in 6-h sessions. The S–C group (*) shows greater cocaine self-administration compared to the other three groups across all 6 days (P's < 0.05). All other group and dose comparisons are not significant.

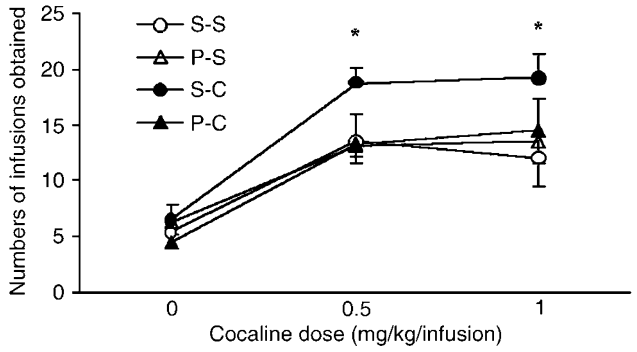

Figure 3 Mean (±SEM) numbers of self-administered cocaine infusions by saline–saline (S–S; open circles; $n = 5$), prazosin (0.3 mg/kg)–saline (P–S; open diamonds; $n = 6$), saline–cocaine (10 mg/kg; S–C; closed circles; $n = 6$), and prazosin–cocaine (P–C; closed diamonds; $n = 6$) groups under the PR schedule are presented by cocaine dose (mg/kg/infusion). Test sessions were 6-h in length. The S–C group (*) shows greater cocaine self-administration compared to the other three groups at the 0.5 and 1.0 mg/kg/infusion doses ($P < 0.001$). All other group and dose comparisons are not significant.

Numbers of inactive lever presses emitted under the PR schedule of reinforcement across cocaine doses are presented in Table 1c. There were no significant main effects or interactions of prazosin or cocaine pre-exposures on numbers of inactive lever presses that were minimal across doses ($P > 0.10$).

DISCUSSION

In the present study, we demonstrate that pre-exposure to cocaine enhances maintenance of cocaine self-administration under both FR and PR schedules. We also show that this facilitation of cocaine self-administration behavior involves activation of alpha 1-adrenergic receptors. Pharmacological blockade of these receptors with the alpha 1-adrenergic antagonist, prazosin, during cocaine pre-exposure attenuates or prevents this facilitation. Attenuation of heightened responding for cocaine by prazosin co-treatment with cocaine is seen under the FR schedule, whereas a blockade of this effect is seen under the PR schedule. Yet, pre-exposure to prazosin alone had no effect on rats' subsequent self-administration of cocaine under either schedule of reinforcement. Hence, our findings suggest that activation of alpha 1-adrenergic receptors plays an important role in the induction of sensitization by cocaine to its subsequent enhancement of cocaine self-administration.

Cocaine pre-exposure facilitated maintenance of cocaine self-administration behavior but did not alter inactive lever press responding, indicating a specific effect on cocaine reinforcement. These findings of the present study are largely consistent with previous studies. Prior studies report that drug pre-exposure enhanced acquisition of self-administration of cocaine (Horger *et al*, 1990) and amphetamine (Piazza *et al*, 1990) under conditions of low to moderate test doses and low FR schedules. The enhancement of cocaine self-administration by cocaine pre-exposure was observed in the present study under maintenance conditions across a wide dose range with a modest FR3 schedule. Further, cocaine self-administration behavior was facilitated when rats were required to work progressively more for each successive cocaine infusion under the PR schedule of reinforcement (Richardson and Roberts, 1996). And, this latter effect was seen with moderate to high cocaine doses. Similarly, previous studies report that responding for amphetamine or cocaine is enhanced under a PR schedule by amphetamine pre-exposure (Lorrain *et al*, 2000; Mendrek *et al*, 1998; Suto *et al*, 2002). However, amphetamine pre-exposure did not facilitate responding under an FR schedule, unlike the results of the present study (Lorrain *et al*, 2000; Suto *et al*, 2002). Differences in pre-exposure drugs and pretreatment regimens, test doses, and schedules of reinforcement between the prior studies and the present study may contribute to the discrepancy. That is, we tested multiple doses of cocaine under an FR3 schedule, whereas the prior studies tested one dose of amphetamine or cocaine under FR1 and FR2 schedules. Nonetheless, the present study adds to the literature showing that drug pre-exposure facilitates subsequent drug self-administration by demonstrating this effect with cocaine pre-exposure and cocaine self-administration under maintenance conditions. Enhanced self-administration observed following pre-exposure may reflect cocaine-induced sensitization that can be presumed to model psychostimulant addiction, as suggested before (Robinson and Berridge, 1993).

The facilitation of self-administration behavior seen in the present and prior studies may reflect augmentation of drug-induced increases in NAc DA levels. Repeated exposure to cocaine augments the NAc DA responses to cocaine (Kalivas and Duffy, 1990; Zapata *et al*, 2003) and NAc DA has been linked to the reinforcing effects of cocaine (Bergman *et al*, 1989; Ritz *et al*, 1987). Increased NAc DA levels induced by prior drug exposure have also been linked to increases in the locomotor activating effects of amphetamine and cocaine (Kalivas and Stewart, 1991; Robinson and Becker, 1986). It is generally thought that the sensitization induced by psychostimulants involves changes in DA transmission from VTA to NAc (Cornish and Kalivas, 2001; Vanderschuren and Kalivas, 2000; Vezina and Stewart, 1989). Similarly, DA transmission in the VTA–NAc pathway contributes to the ability of repeated amphetamine exposure to facilitate self-administration behavior (Suto *et al*, 2002). Data from the present study suggest that, in addition to DA, NE transmission contributes to the facilitation of self-administration by prior drug exposure because pretreatment with the alpha 1-adrenergic antagonist, prazosin, decreased this effect.

The ability of prazosin to attenuate or block the effects of cocaine pre-exposure to enhance self-administration behavior suggests that alpha 1-adrenergic receptors contribute to the development of cocaine-induced sensitization. However, the mechanisms by which this occurs are not known. Nonetheless, numerous studies show that NE and DA systems interact and that this may occur via stimulation of alpha 1-adrenergic receptors. Systemic or local administration of prazosin into the prefrontal cortex blocks increased DA and locomotor responses induced by systemic D-amphetamine (Darracq *et al*, 1998). Prazosin decreased spontaneous bursting of VTA DA cells (Grenhoff and Svensson, 1993), whereas direct application of an alpha 1-adrenergic receptor agonist, phenylephrine, in brain slices, depolarized some cells (Grenhoff *et al*, 1995). Increases burst firing of VTA DA cells was induced by systemic administration of reboxetin, a specific inhibitor of the NE transporter (Linner *et al*, 2001). In fact, Shi *et al* (2000) suggest that D-amphetamine has two effects on DA cells; a DA-mediated inhibition and a non-DA-mediated excitation, with the latter thought to act through alpha 1-adrenergic receptors.

Stimulation of cortical alpha 1-adrenergic receptors by systemic D-amphetamine may facilitate DA transmission in the NAc through a cortico-VTA glutamatergic pathway that would drive VTA dopaminergic cells into a bursting activity (Darracq *et al*, 1998). The inhibitory properties of prazosin on D-amphetamine-induced increases in extracellular DA levels in the NAc and in locomotor activity may reflect inhibition of the activity of glutamatergic excitatory cortical neurons bearing D1 receptors and projecting directly or indirectly through the NAc to the VTA (Karreman and Moghaddam, 1996). Moreover, activation of a glutamatergic projection from the prefrontal cortex to the NAc underlies cocaine-primed reinstatement of drug-seeking behavior (McFarland *et al*, 2004). A recent electrophysiological study

also shows that the NE system could serve to alter the reward value of stimuli that have significant effects on DA neuron firing patterns through mGluRs. Decreased alpha-adrenoceptor regulation of the mGlu-R-mediated current may be a cellular mechanism associated with effects of withdrawal from cocaine after repeated drug administration (Paladini *et al*, 2004). Taken together, these findings suggest that alpha 1-adrenergic transmission may regulate the behavioral effects of psychostimulants by influencing glutamatergic systems or its interaction with DA systems. The results from the present study along with data from our recent investigation showing that prazosin-attenuated cocaine-induced reinstatement of drug-seeking behavior show strong support for the contribution of NE to the motivational effects of cocaine (Zhang and Kosten, 2005).

While the data from the present study along with our prior study suggest that alpha 1-adrenoreceptors contribute to the motivational effects of cocaine, there are some limitations. Prazosin was administered systemically and only one dose was tested. However, we utilized the maximal dose that did not disrupt ongoing behavior based on our prior study. In the present study, pretreatment with this dose resulted in maximal effects under the PR schedule tests and less than maximal, but significant, effects under the FR schedule tests. Second, while the tests under the FR schedule suggest that prazosin attenuates the ability of cocaine pre-exposure to facilitiate self-administration, it is difficult to interpret shifts in dose-response curves under FR schedules of reinforcement (Mello and Negus, 1996). In fact, the increase in responding in the cocaine pre-exposed group could be interpreted as an increase in tolerance to the reinforcing effects of cocaine. Yet, prazosin also blocked the ability of cocaine pre-exposure to enhance responding for cocaine under the PR schedule of reinforcement. Finally, that we did not test responding for saline under an FR3 schedule of reinforcement poses a slight limitation in the study. However, there was no effect of prazosin pretreatment on responding for saline under the PR experiment. Further, we typically find that response levels are minimal when saline is available for delivery and thus would likely not provide a sensitive test of the effects of prazosin pretreatment with cocaine. Nonetheless, it is unlikely that nonspecific effects of prazosin contributed to the effects seen in the present study. There were no effects of prazosin on inactive lever press responding in this study and we previously showed that the prazosin dose chosen did not disrupt ongoing operant responding for food or affect responding for saline (Zhang and Kosten, 2005). Although the mechanisms that underlie the ability of prazosin to attenuate the facilitation of cocaine self-administration by prior cocaine exposure remain to be elucidated, data from our studies point to the role of NE in the motivational effects of cocaine.

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