Published in final edited form as:

Eur Neuropsychopharmacol. 2008 April ; 18(4): 303–311.

α_1 -Noradrenergic System Role in Increased Motivation for Cocaine Intake in Rats with Prolonged Access

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Abstract

In rodents, extended access to cocaine produces an escalation in cocaine self-administration that has face and construct validity for human compulsive drug intake. Here we report that rats with six-hour access (long access, LgA) to cocaine self-administration produced a higher breakpoint for cocaine using a progressive-ratio schedule than rats with one-hour access (short access, ShA), and prazosin (α_1 receptor antagonist) reduced the higher breakpoint for cocaine in LgA rats. Additionally, the number of neurons with α_1 -adrenergic receptor-like immunoreactivity in the bed nucleus of stria terminalis (BNST) was found to be much lower in LgA rats than in ShA and drug-naïve rats. In contrast, UK14304 (α_2 receptor agonist) and betaxolol (β_1 receptor antagonist) had no effect on cocaine self-administration in either group. The data suggest that activation of the α_1 -noradrenergic system, perhaps in the BNST, is associated with increased motivation for cocaine in rats with extended access.

Keywords

cocaine; self-administration; escalation; alpha-1 noradrenergic receptor; rats; bed nucleus of stria terminalis

1. Introduction

Cocaine dependence is associated with compulsive use and loss of control over intake (American Psychiatric Association, 2000). In rodents, extended access to cocaine and methamphetamine produced an escalation in self-administration of the drugs (Ahmed and Koob, 1998; Kitamura et al., 2006), which has face validity for compulsive drug intake in humans. Therefore, the investigation of neuropharmacological changes during escalation in cocaine self-administration in rodents may provide key information about the neural mechanisms underlying the development of drug dependence in humans.

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Conflict of Interest

All the authors (S.W., C.D.M., D.M.L., G.F.K.) have no conflict of interest to declare.

Author Disclosures

Author S. Wee performed the self-administration experiment and wrote the first draft of the manuscript. Author C.D. Mandyam performed immunohistochemistry experiment. Authors S. Wee, C.D. Mandyam and G.F. Koob contributed to the design of the study, analysis of the data and the final manuscript. D.M. Lekic performed part of the self-administration study. All the authors approved the final manuscript.

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Cocaine increases extracellular norepinephrine (NE), dopamine, and serotonin by blocking monoamine reuptake transporters (Reith et al., 1997; Rothman et al., 2001). These findings prompted researchers to investigate the role of monoamines in cocaine self-administration. Focusing on norepinephrine, early studies using a self-administration paradigm found no convincing evidence of the involvement of NE in the acute reinforcing effects of psychostimulants. For example, direct and indirect noradrenergic receptor agonists did not function as positive reinforcers in monkeys or dogs (Risner and Jones, 1976; Wee and Woolverton, 2004; Woolverton, 1987) although the reinforcing effect of clonidine, an α_2 -adrenergic receptor agonist, has been noted (Davis and Smith, 1977; Woolverton et al., 1982, respectively). Moreover, pretreatment with noradrenergic receptor ligands did not affect amphetamine or cocaine self-administration under a fixed-ratio (FR) schedule in rats or monkeys (Wilson and Schuster, 1974; Woolverton, 1987; Yokel and Wise, 1976; Tella, 1995; Yokel and Wise, 1978). Depleting central nervous system NE did not alter cocaine self-administration under an FR schedule in rats (Roberts et al., 1977).

However, some studies using paradigms other than self-administration seem to support the involvement of NE in the acute rewarding effects of psychostimulants. In conditioned place preference, NE depletion in the medial prefrontal cortex blocked the development of cocaine-associated place preference in mice (Ventura et al., 2007). α_1 noradrenergic receptor knockout mice also showed reduced cocaine preference compared to wild type mice under a two-bottle choice paradigm (Drouin et al., 2002). In contrast, dopamine β -hydroxylase knockout mice (NE depletion) showed enhanced sensitivity to cocaine-induced place preference, suggesting the development of compensatory neural responses in knockout mice (Weinshenker et al., 2002). Taken together, the role of noradrenergic activity in the acute reinforcing/rewarding effects of psychostimulants remains speculative.

One possibility is that extended access to cocaine self-administration may alter the noradrenergic system by repeatedly stimulating the system, which may in turn contribute to the development of drug dependence. It was shown that repeated administration of amphetamine produced a sensitized response of NE release in the prefrontal cortex (Salomon et al., 2006). An increased number of norepinephrine transporters in the brain were also found in rhesus monkeys after 100 days of cocaine self-administration compared to 5 days of cocaine self-administration (Macey et al., 2003; Beveridge et al., 2005). Moreover, previous cocaine exposure by repeated passive administration increased a breakpoint for cocaine self-administration on a progressive-ratio (PR) schedule, which was blocked by administration of prazosin in rats (Zhang and Kosten, 2007).

The purpose of the present study was to test the hypothesis that neuroadaptation in the central noradrenergic system has a role in the increased cocaine self-administration in rats with extended access. Our hypothesis was that increased cocaine self-administration under a PR schedule in rats with extended access may be mediated by an altered noradrenergic system. To test this hypothesis, cocaine self-administration in rats with one-hour and six-hour access was tested with pretreatment by prazosin (a selective α_1 -noradrenergic receptor antagonist; Boyajian and Leslie, 1987), UK14304 (a selective α_2 -noradrenergic receptor agonist; Andorn et al., 1988; Ernsberger et al., 1997) and betaxolol (a β_1 -noradrenergic receptor antagonist; Smith and Teitler, 1999). Additionally, the density of α_1 -noradrenergic receptors in brain regions that are associated with drug dependence (Koob, 2003) was compared between groups.

2. Experimental procedures

All animal use procedures were approved by The Scripps Research Institute Animal Care and Use Committee and were in accordance with the National Institutes of Health guidelines.

Self-administration

Animals and apparatus—Twenty-seven male Wistar rats (Charles River, Hollister, CA), each weighing between 250 and 275 g at the beginning of the study, served as subjects. They were housed in groups of three in plastic cages with a 12 h:12 h light/dark cycle with lights off at 8:00AM. Food and water were available *ad libitum*. During experimental sessions, each rat was placed in a standard operant chamber, which was placed in a light- and sound-attenuating cubicle (28 × 26 × 20 cm; Med Associates Inc., St Albans, VT). A drug injection was delivered by a syringe pump (Razel Scientific Instruments, Georgia, VT) located on top of the cubicle. Experimental sessions were controlled and recorded by a PC computer with custom interface and software in the experimental room.

Procedure—Rats were implanted with a silastic catheter (0.3 mm i.d. × 0.64 mm o.d.; Dow Corning, Midland, MI) into the right external jugular vein under aseptic conditions. The catheter was flushed daily with heparinized saline (30 units/ml). The patency of catheters in the rats was tested using an ultra short-acting barbiturate, Brevital® (methohexital sodium, 10 mg/ml, 2 mg/rat) whenever a catheter failure was suspected during the study. Generally, a total loss of muscle tone within 3 s after a Brevital injection indicated the patency of a catheter. Detailed procedures of animal care and surgery have been previously described (Wee et al., 2007).

Experimental sessions were conducted once a day during the dark (active) cycle. The start of a session was signaled by the presentation of two response levers into the chamber. Responding on the right lever resulted in the delivery of 0.1 ml of a drug injection over 4 s. During an injection, stimulus lights above both levers were illuminated and lasted throughout a time-out period (20 s) that followed each injection. Pressing the left lever was counted but had no other programmed consequences. Self-administration procedure

After surgery, rats were trained to self-administer cocaine at a high dose of 1 mg/kg/injection for three days under an FR1 schedule of reinforcement, where one lever press resulted in the delivery of drug injection. The dose of cocaine was then reduced to 0.5 mg/kg/injection, and the rats were allowed to self-administer 0.5 mg/kg/injection of cocaine for six more sessions under an FR1 schedule.

After the baseline sessions, the rats were divided into two groups balanced by the number of injections/session in the last baseline session. During an escalation period, one group of rats (long access, LgA) self-administered 0.5 mg/kg/injection of cocaine in 6 h sessions, whereas the other group (short access, ShA) did so in 1 h sessions. Escalation sessions lasted for a minimum of 14 sessions and until there was no trend of increased or decreased responding for three consecutive sessions. After escalation sessions, the dose-response function of cocaine was determined under a PR schedule of reinforcement. For the PR schedule, the response requirement began at 1 response/injection and increased according to the following equation: $\text{responses/injection} = [5 \times e^{(\text{injection number} \times 0.2)}] - 5$ (Richardson and Roberts, 1996). When a rat failed to achieve the response requirement within 1 h, the session ended. A session length under a PR schedule was set at 12 h, and PR sessions lasted an average of 3 h across rats. Test PR sessions were held on Tuesdays and Fridays. Escalation sessions (LgA, 6 h session; ShA, 1 h session under an FR1 schedule) separated two test sessions. After the determination of the cocaine dose-response function, the effect of pretreatment with UK14304 (a selective α_2 -noradrenergic receptor agonist, 0.03–0.3 mg/kg, i.p.), prazosin (a selective α_1 -noradrenergic receptor antagonist, 1–3 mg/kg, i.p.) or betaxolol (a β_1 -noradrenergic receptor antagonist, 0.3–10 mg/kg, s.c.) on cocaine self-administration was examined under a PR schedule. Eleven of the 27 rats were examined with all three drugs. The rest of the rats were tested with either one or two drugs. Prazosin was tested first in 11 rats and last in the other 11 rats. UK14304 was tested first in 16 rats. Betaxolol was tested between prazosin and UK14304. After testing each

noradrenergic drug, the rats were removed from cocaine self-administration for 7–8 days, then 12 days of successive escalation sessions were reestablished (ShA, 1 h session; LgA, 6 h session). Consequently, a total of 19–20 days separated the pretreatment periods of two noradrenergic drugs in all rats. Doses of each drug were tested in a counterbalanced manner across rats. Ten mg/kg of betaxolol was tested only in four rats of each group because of the high cost of the drug. The pretreatment time for UK14304, prazosin, and betaxolol was, respectively, 10, 10, and 30 min before a test session. The injection volume was always 1 ml/kg.

Data analysis—The data were expressed as the mean number of injections/session and the mean number of injections in the first hour of the session for each group of rats. Cocaine self-administration was compared across daily sessions over the initial 14 escalation sessions using a two-way repeated-measures analysis of variance (ANOVA) with the Bonferroni *post hoc* tests (access $\times$ daily session; Prism 4.0, GraphPad, San Diego, CA). Responding for doses of cocaine under a PR schedule was expressed as the number of injections/session and compared between LgA and ShA rats using a two-way repeated-measures ANOVA (access $\times$ cocaine dose). With a significant interaction and main effects of access and cocaine dose, Student's *t*-test was performed on the number of injections/session between LgA and ShA groups at each cocaine dose under the PR schedule. The effect of noradrenergic drugs on cocaine self-administration was compared using a two-way repeated-measures ANOVA with the Bonferroni *post hoc* test (access $\times$ doses of a test drug; Prism 4.0, GraphPad, San Diego, CA). All data under a PR schedule followed the Gaussian distribution when tested using the D'Agostine and Pearson omnibus normality test (Prism 4.0, GraphPad, San Diego, CA).

Immunohistochemistry

After the self-administration study, ShA, LgA, and age-matched drug-naïve rats were anesthetized with chloral hydrate (35%, 3 ml/450 g body weight, i.p.) and perfused transcardially with cold 0.1 M phosphate buffered saline (PBS), and then with 4% paraformaldehyde in 0.1 M PBS (pH 7.4) for 20 min at a rate of 15 ml/min. Brains were cryoprotected in 30% sucrose and sectioned coronally on a freezing microtome (American Optical Corporation, Buffalo, NY) at 40 μ m, and sections were stored in 0.1% NaN₃ in 0.1 M PBS at 4°C before processing for immunohistochemistry.

Immunostaining of α_1 noradrenergic receptors was measured in the medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), bed nucleus of stria terminalis (BNST), and ventral tegmental area (VTA). Rabbit polyclonal anti- α_1 noradrenergic receptor antibody (cat# PA1-047; Affinity BioReagents, Golden, CO; 1:1000, raised against the peptide sequence 339–349 of the human α_1 adrenergic receptor) was used for labeling α_1 noradrenergic receptors. This antibody was non-selective for subtypes of α_1 receptors as was prazosin (see Lomasney et al., 1991; Wang et al., 2007). The left and right hemispheres of sections (bregma 4.20 for OFC, 3.7 and 3.2 for mPFC [including the anterior cingulate, prelimbic and infralimbic cortices], –0.26 and –0.30 mm for BNST, –5.30 and –5.60 mm for VTA; Paxinos and Watson, 1997) through the rat brain were slide-mounted and dried overnight prior to immunohistochemistry. All incubations were performed at room temperature unless otherwise indicated. Slide-mounted sections were subjected to an antigen unmasking pretreatment step as described previously (Mandyam et al., 2004). Slides were incubated with 0.3% H₂O₂ for 30 min to remove any endogenous peroxidase activity. Nonspecific binding was blocked with 5% serum and 0.5% Tween-20 in 0.1 M PBS for 60 min and incubated with the primary antibody (in 5% serum and 0.5% Tween-20) for 48 h at 4°C. After washing with 0.1 M PBS, the sections were exposed to biotinylated secondary immunoglobulin-G for 1 h (Vector Laboratories, Burlingame, CA; 1:200). After secondary antibody incubation, slides were incubated in avidin-biotin complex for 1 h (cat# PK-6100; Vector Laboratories, Burlingame,

CA), and staining was visualized with 3,3-diaminobenzidine (DAB, cat# 34065; Pierce Laboratories, Rockford, IL). Sections were counterstained with Fast Red (Vector Laboratories, Burlingame, CA). Omission or dilution of the primary antibody resulted in lack of specific staining, thus serving as a negative control for antibody experiments.

Data analysis—Analysis was performed with the experimenter blinded to the group analyzed, to localize and quantify immunoreactivity (IR). DAB staining of the coded slides was visualized and quantified with a Zeiss Axiophot photomicroscope. Staining was examined, and X-IR neurons from the left and right hemispheres that were localized in the counting frame (mPFC, OFC and BNST: 0.078 mm²; VTA: 0.09 mm²) were visually quantified (Paxinos and Watson, 1997) (Figure 6). The total number of IR neurons both in the left and right hemispheres was counted and expressed as the total number of IR neurons/mm² in each rat and averaged across rats within a group. A difference in the total number of IR neurons/mm² across groups was found using one-way ANOVA with the Student-Neuman-Keul's *post hoc* test (Prism 4.0, GraphPad, San Diego, CA).

Drugs—The National Institute on Drug Abuse (Rockville MD) provided (–)-cocaine hydrochloride. Prazosin hydrochloride, UK14304, and betaxolol hydrochloride were purchased from Sigma-Aldrich Co. (St Louis, MO). Cocaine was dissolved in sterile 0.9% saline for self-administration. Prazosin, UK14304, and betaxolol were dissolved in 20% dimethylsulfoxide (DMSO) in water, 10% DMSO in water, and sterile water, respectively. Doses of cocaine for self-administration were prepared by varying the concentration of cocaine in drug solutions while the injection volume was kept constant at 0.1 ml/injection. Each drug solution was prepared for each rat based on its body weight and was updated every two or three days. Doses of drugs were expressed as salt. Rabbit polyclonal anti- α_1 adrenergic receptor antibody was purchased from Affinity BioReagents (Golden, CO). Biotinylated secondary IgG was purchased from Vector Laboratories (Burlingame, CA).

3. Results

Cocaine self-administration in LgA rats significantly increased over 14 sessions starting in session 2 and 4 compared to session 1, respectively, within a session and during the first hour of each session (Figure 1). A two-way ANOVA found a significant interaction between access and daily session in escalation of cocaine self-administration within a session as well as during the first hour of each session [session, $F(13,325)=14.74$, $p<0.001$; first hour, $F(13,325)=5.60$, $p<0.001$]. Cocaine self-administration in ShA rats was stable over 14 sessions.

Under a PR schedule, a significant interaction between access and cocaine dose was found in responding under the PR schedule [two-way ANOVA: $F(2,40)=3.72$, $p<0.05$, Figure 2]. Additionally, there were main effects of cocaine dose and access on responding under the PR schedule [cocaine dose, $F(2,40)=59.65$, $p<0.001$; access, $F(1,40)=6.16$, $p<0.05$]. Responding for saline did not differ between LgA and ShA rats (Student *t*-test, $p>0.05$). However, LgA rats produced a significantly higher break -point for cocaine than ShA rats at doses of 0.5 mg/kg and 1 mg/kg/injection (Student *t*-test, $p<0.05$).

Prazosin significantly decreased the breakpoint for 0.5 mg/kg/injection of cocaine in LgA rats at 3 mg/kg (Figure 3). However, the drug had no effect on cocaine self-administration in ShA rats. A two-way ANOVA between prazosin pretreatment and access showed a significant interaction between these two factors and a main effect of prazosin on cocaine self-administration [$F(2,40)=3.29$, $p<0.05$; $F(2,40)=4.59$, $p<0.05$, respectively].

UK14,304 and betaxolol did not produce any effect on cocaine self-administration of 0.5 mg/kg/injection in either LgA or ShA rats [two-way ANOVA; UK14304, $F(3,42)=0.84$, $p>0.05$,

Figure 4; betaxolol, $F(3,42)=1.07$, $p>0.05$, Figure 5]. When the dose of UK14304 was increased to 1 mg/kg, all rats lost their muscle tone immediately after the injection.

The distribution of α_1 noradrenergic receptor staining was similar to the pattern of mRNA expression previously reported in the striatum and thalamus (Domyancic and Morilak, 1997). α_1 noradrenergic receptor staining was mostly cytoplasmic, and, in a few neurons, staining also was visible in the processes [Figure 6B and E (drug-naïve), C and F (LgA)]. Quantitative analysis in the drug-naïve control rats indicated the highest number of neurons with α_1 noradrenergic receptor-like IR neurons in the dorsal BNST compared with the ventral BNST, the VTA, and the FC [$F(6,38)=68.07$, $p<0.001$; Figure 6G, gray striped bars]. Both the dorsal and ventral BNST of LgA rats showed a significantly decreased number of IR neurons to 46% and 68% the level of drug-naïve rats, respectively [dorsal BNST, $F(2,14)=15.3$, $p<0.001$; ventral BNST, $F(2,14)=10.4$, $p<0.01$; Figure 6 G]. However, no significant change in the number of IR neurons was found in the VTA and in subregions of the FC across groups.

4. Discussion

In agreement with previous studies (Ahmed and Koob, 1998, 1999), cocaine self-administration increased with 6 h access in LgA rats compared to ShA rats. Additionally, responding for doses of cocaine was higher in LgA than in ShA rats under a PR schedule. An upward shift of the cocaine dose-response function was previously found in LgA rats under an FR and a PR schedule (Ahmed and Koob, 1998; Paterson and Markou, 2003). Additionally, it was reported that LgA rats exhibited tolerance to the locomotor-stimulating effect of cocaine challenge (Ben-Shahar et al., 2004, 2005). In humans, compulsive drug use is accompanied by increased drug intake and tolerance (American Psychiatric Association, 2000), lending some face validity to the rodent model of self-administration with extended access.

Acute administration of cocaine is well documented to enhance noradrenergic neurotransmission by inhibiting NE reuptake (Iversen, 1973; Rothman et al., 2001; Wee et al., 2006). Additionally, increased levels of norepinephrine transporters was observed in rhesus monkeys after 100 days of cocaine self-administration compared with 5 days of cocaine self-administration (Macey et al., 2003; Beveridge et al., 2005). Therefore, we hypothesized that cocaine self-administration with extended access would alter noradrenergic activity in LgA rats to a greater extent than in ShA rats, and that these changes may in turn play a role in the increased cocaine self-administration in LgA rats. Under the present conditions, prazosin pretreatment reduced cocaine self-administration under a PR schedule in LgA rats, but not in ShA rats. Moreover, prazosin decreased self-administration of cocaine in LgA rats to the level of ShA rats. This suggests that α_1 noradrenergic receptor activation may be related to increased motivation for cocaine in LgA rats with extended access. Results with experimenter-administered cocaine have shown that passive administration of cocaine (10 mg/kg/day) for five days produced the increased breakpoint for cocaine self-administration in rats under a PR schedule, and co-pretreatment of prazosin (0.3 mg/kg) with cocaine (10 mg/kg/day) inhibited the increase of the breakpoint for cocaine (Zhang and Kosten, 2007). Moreover, similar to the present results, prazosin had no effect on the breakpoint for cocaine in non-cocaine-pretreated rats. No effect of prazosin on non-escalated cocaine self-administration was previously reported in monkeys under FR and fixed-interval schedules (Woolverton, 1987; Howell and Byrd, 1991). Thus, the present and previous findings suggest a relationship between changes in α_1 noradrenergic neurotransmission and the neuroplasticity associated with the increased cocaine intake.

The number of neurons with α_1 noradrenergic receptor-like IR neurons was significantly higher in the BNST than in the VTA and cortices in drug-naïve rats, consistent with the literature that the density of noradrenergic terminals is very high in the BNST (Brownstein and Palkovits,

1984). In addition, the number of IR neurons in the BNST was found to be lower by 40–60% in LgA rats than in ShA and drug-naïve rats, suggesting a decrease in the number of α_1 noradrenergic receptors during extended access to cocaine self-administration. We hypothesize that extended access to cocaine self-administration causes a downregulation of α_1 noradrenergic receptors as a compensatory response to a prolonged overflow of extracellular NE by cocaine. A decreased number of α_1 noradrenergic receptors, perhaps reduced spare receptors, in LgA rats may make noradrenergic signal transmission easily affected by the blockade of the receptors by prazosin, a hypothesis supported by the self-administration data showing that cocaine self-administration in LgA rats was sensitive to the inhibition of α_1 noradrenergic receptors by prazosin. However, this conclusion should be taken with a caveat, and the direct causal relationship between decreased α_1 receptors in the BNST and the effect of prazosin on increased cocaine self-administration in LgA needs further research. The increased norepinephrine transporters in the BNST after 100 days of cocaine self-administration in monkeys (Macey et al., 2003) is consistent with the present results because the increased norepinephrine transporters implies a compensatory response of increased clearance of extracellular NE.

An alternative explanation of the present finding may be that there is an intake-dependent emergence of α_1 noradrenergic regulation of cocaine self-administration down stream from the BNST that makes activity in ascending noradrenergic neurotransmission relevant in LgA rats. The potential recruitment of α_1 noradrenergic pathways in an intake-dependent manner may have resulted in prazosin effects on increased cocaine self-administration in LgA rats regardless of the level of α_1 noradrenergic receptor expression.

It should be noted that we did not observe any significant changes in the number of IR neurons in the VTA and FC that receive noradrenergic projections from the locus coeruleus comprising the dorsal noradrenergic bundle (Moore and Bloom, 1979; Weinshenker and Schroeder, 2006). In contrast, the ventral noradrenergic bundle projects from the pons and medulla to the hypothalamus and the extended amygdala (for review, see Moore and Bloom, 1979), and the BNST is a key component of the extended amygdala. Thus, the present results may suggest that the noradrenergic changes in the ventral noradrenergic projection rather than in the dorsal noradrenergic projection are related to increased cocaine self-administration, thereby increased motivation for cocaine in LgA rats.

One question would be the nonspecific effect of the doses of prazosin (1, 3 mg/kg) on operant behavior. However, the doses of prazosin did not reduce the rate of self-administration in ShA rats under the present conditions. Zhang and Kosten (2005) reported that 0.5 mg/kg of prazosin disrupted the rate of responding for food in Sprague-Dawley rats. In contrast, in Long Evans rats, 3.2 mg/kg of prazosin produced only “small decreases” in the rate of responding under a drug discrimination paradigm (Berthold et al., 1992). Similarly, 2 mg/kg of prazosin (i.p.) did not alter locomotor activity in rats (Wellman et al., 2002). Thus, prazosin in the present study is likely to have decreased cocaine self-administration in LgA rats via a more specific motivational interaction.

In contrast to prazosin, betaxolol pretreatment did not affect cocaine self-administration in any groups under the present conditions. It was shown that pretreatment with propranolol, a nonselective β -noradrenergic receptor antagonist, decreased cocaine self-administration under an FR schedule in rats and squirrel monkeys (Harris et al., 1996; Goldberg and Gonzalez, 1976). However, in a drug discrimination paradigm, Kleven and Koek (1997) implicated the involvement of the β_2 rather than β_1 noradrenergic system in the discriminative stimulus effects of cocaine in rats. Additionally, Leri et al. (2002) found that the mixture of β_1 and β_2 noradrenergic receptor antagonists attenuated stress-induced, but not cocaine-induced,

reinstatement of responding for cocaine. Therefore, we conclude that the β_1 -noradrenergic system is not involved in cocaine self-administration.

With respect to the α_2 -noradrenergic system, clonidine has been shown to have positive reinforcing properties in rats and rhesus monkeys (Davis and Smith, 1977; Woolverton et al., 1982). However, clonidine is a mixed α_2 -noradrenergic and imidazoline-1 receptor agonist, whereas UK14304 is a selective α_2 receptor agonist (Andorn et al., 1988; Ernsberger et al., 1997; Georges et al., 2005; Georges and Aston-Jones, 2003). Therefore, the action of clonidine may be mediated by imidazoline receptors. On the other hand, the α_2 noradrenergic receptor antagonist, yohimbine, was shown to reinstate responding for psychostimulants in monkeys and rats (Lee et al., 2004; Shepard et al., 2004) presumably through disinhibition of ascending noradrenergic neurotransmission. NE reuptake inhibitors have also been shown to reinstate cocaine seeking (Platt et al., 2007). Whether stress-induced reinstatement produced by noradrenergic agents is related to the noradrenergic role in cocaine intake escalation remains to be explored.

An important question is why the effects of α_1 receptor blockade and α_2 receptor stimulation differed in the present study. Forray and colleagues (1999) demonstrated that the local perfusion of UK14304 and prazosin into the BNST produced asymmetric effects on the basal and K⁺ evoked release of the extracellular NE and glutamate in the BNST in rats. Although both drugs increased basal and K⁺ evoked NE release in the BNST, UK14304 also increased basal glutamate release whereas prazosin did not. Additionally, UK14304 and prazosin, respectively, increased and decreased K⁺ evoked glutamate release. Recently, Egli and colleagues (2005) reported that NE modulates glutamate system in the BNST. Therefore, it appears that the stimulation of α_2 receptors may not simply be considered to be equal to the blockade of α_1 receptors.

One might speculate that pretreatment time and/or drug doses contributed to the lack of effects of betaxolol and UK14304 on cocaine self-administration. However, betaxolol (0.01–10 mg/kg, i.p.) was effective on water-maintained responding and responding under a drug discrimination paradigm with 30 or 35 min pretreatment in rats (Zhang et al., 2001; O'Donnell, 1997). Likewise, we observed that 1 mg/kg of UK14304 produced a loss of muscle tone and sedation, indicating that behaviorally active doses were tested. Moreover, UK14304 (0.1 mg/kg) decreased firing rates of rat locus coeruleus neurons immediately after an intraperitoneal injection in *in vivo* cell recordings, suggesting that the drug easily crossed the blood-brain barrier (Georges and Aston-Jones, 2003). UK14304 pretreatment (0.05 mg/kg, i.p.) also increased immediate-early gene expression in the rat cerebral cortex when sacrificed 90 min after injection, indicating that UK14304 was long-acting (Shen and Gundlach, 2000).

In summary, the present data demonstrated that increased cocaine self-administration in LgA rats under a PR schedule was sensitive to α_1 noradrenergic receptor inhibition. Additionally, the number of neurons with α_1 -adrenergic receptor-like IR was significantly lower in the BNST of LgA rats than in ShA and drug-naïve rats. Therefore, the data suggest that altered α_1 -noradrenergic neurotransmission may be related to increased motivation for cocaine self-administration in rats with extended access to the drug, and changes in the noradrenergic projections to the extended amygdala, such as the BNST, may underlie altered motivation for cocaine during cocaine self-administration with extended access. In contrast, the present study did not find any relationship between the α_2 - and β_1 -noradrenergic systems and cocaine self-administration in any groups under the present conditions.

Acknowledgements

We gratefully acknowledge the technical assistance of Mike Pham, JoAnn Lee, Stephanie Chan, and Hanan Jammal, who were undergraduate students of the University of California, San Diego, in the self-administration and the

immunohistochemistry studies. We also thank Mike Arends for editorial assistance. This is publication number 18632 from The Scripps Research Institute.

Role of the funding source

National Institute on Drug Abuse grant DA004398 (G.F.K) supported the study; the NIDA had no further role in study design, in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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Figure 1.

Self-administration of cocaine by rats under a fixed-ratio schedule during the escalation period. Data are expressed as the number of injections on the left axis and mg/kg on the right axis. The upper panel represents data from entire sessions, and the lower panel represents data from the first hour of sessions. Error bars are SEM values. Filled circles indicate self-administration by rats in 6 h sessions (long access, LgA), and open circles indicate self-administration by rats in 1 h sessions (short access, ShA). * $p < 0.05$ compared to session 1.

Figure 2.

Dose-response function of cocaine by rats responding under a PR schedule. Test sessions under a PR schedule ended when rats did not achieve reinforcement within 1 h. Data are expressed as the number of injections/session on the left axis and the ratio per injection on the right axis. Error bars are SEM values. Open bars indicate responding by short access (ShA) rats, and filled bars indicate responding by long access (LgA) rats. * $p < 0.05$ compared to ShA rats at each dose of cocaine.

Figure 3.

Effect of prazosin, an α_1 -noradrenergic receptor antagonist, on the break-point for 0.5 mg/kg/injection of cocaine under a PR schedule. Prazosin was intraperitoneally injected 10 min before a session. Data are expressed as the number of injections/session on the left axis and the ratio per injection on the right axis. Error bars are SEM values. The upper panel represents data from long access (LgA) rats, and the lower panel represents data from short access (ShA) rats.

* $p < 0.05$ compared to vehicle treatment. # $p < 0.05$ compared to ShA rats.

Figure 4.

Effect of UK14304, an α_2 -noradrenergic receptor agonist, on the break-point for 0.5 mg/kg/injection of cocaine under a PR schedule. UK14304 was intraperitoneally injected 10 min before a session. Data are expressed as the number of injections/session on the left axis and the ratio per injection on the right axis. Error bars are SEM values. The upper panel is the data from long access (LgA) rats, and the lower panel is the data from short access (ShA) rats.

Figure 5.

Effect of betaxolol, a β_1 -noradrenergic receptor antagonist, on the break-point for 0.5 mg/kg/injection of cocaine under a PR schedule. Betaxolol was subcutaneously injected 30 min before a session. Data are expressed as the number of injections/session on the left axis and the ratio per injection on the right axis. Error bars are SEM values. The upper panel is the data from long access (LgA) rats, and the lower panel is the data from short access (ShA) rats. The number in parentheses above 10 mg/kg of betaxolol indicates the number of rats that were tested with the dose of the drug.

Figure 6.

Quantification of α_1 -noradrenergic receptors in the frontal cortex (FC), bed nucleus of the stria terminalis (BNST), and ventral tegmental area (VTA) across drug-naive, short access (ShA), and long access (LgA) rats ($n=5$ in each group). A–F: Schematic of a coronal section through the striatum (A) indicating the areas examined for quantitative analysis (area 1 is dorsal-mediolateral BNST; area 2 is ventral-mediolateral BNST), α_1 -adrenergic neurons in the dorsal-mediolateral BNST from a drug naive (B) and LgA rat (C). Schematic of a coronal section through the hippocampus (D) used for quantitative analysis (area 3 is the VTA), α_1 -noradrenergic neurons in the VTA from a drug naive (E) and LgA rat (F). Quantitative neuron counts are expressed as mean $\pm$ SEM of the total number of immunoreactive neurons/mm².

#p<0.01 compared to the dorsal BNST of the drug-naive group in (G) and #p<0.01 compared to the mPFC of the drug naive group in (H). *p<0.001 compared to drug-naive and ShA groups within each brain region. Scale bar in C is 10 μ m (applies to B–C, E–F).