Published in final edited form as:

Neuroreport. 2012 March 28; 23(5): 325–330. doi:10.1097/WNR.0b013e3283517628.

Cocaine self-administration is not dependent upon mesocortical $\alpha 1$ noradrenergic signaling

Laurel E. Ecke,

Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, NIH/DHHS, Baltimore, MD 21224, USA

Greg I. Elmer, and

Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, P.O. Box 21247, Baltimore, MD 21228

Nobuyoshi Suto

Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, NIH/DHHS, Baltimore, MD 21224, USA, TEL: 858-784-7031, FAX: 858-784-7146

Nobuyoshi Suto: nsuto@scripps.edu

Abstract

The rewarding properties of psychomotor stimulants are traditionally thought to be independent of norepinephrine. Recent findings, however, suggest that local noradrenergic signaling via $\alpha 1$ receptors in the medial prefrontal cortex and the ventral tegmental area – brain regions critically important in natural and drug reward – is in a position to influence stimulant reward. Despite this controversy, the contribution of this targeted signaling to stimulant self-administration has not been directly assessed. We have thus examined whether pharmacological blockade of $\alpha 1$ receptors in the medial prefrontal cortex and ventral tegmental area alters cocaine self-administration. Rats were trained to lever-press for cocaine (1.0 mg/kg/infusion) under a fixed ratio 1 schedule of reinforcement for 10 days. Following training, the rats received a bilateral microinjection of an $\alpha 1$ noradrenergic antagonist (terazosin: 1.0, 5.0, or 10 mM/side), a D1 dopaminergic antagonist (SCH23390: 12.3 mM/side), or saline into either the medial prefrontal cortex or ventral tegmental area immediately prior to a cocaine self-administration session. While SCH23390 significantly increased cocaine self-administration when injected into either brain region, terazosin, at all doses and sites tested, failed to alter this behavior. Thus, the maintenance of cocaine self-administration appears to be under the influence of D1 dopaminergic, rather than $\alpha 1$ noradrenergic, signaling at these mesocortical sites.

Keywords

cocaine self-administration; ventral tegmental area; medial prefrontal cortex; dopamine; norepinephrine; D1 receptors; $\alpha 1$ receptors

Correspondence to: Laurel E. Ecke; Nobuyoshi Suto, nsuto@scripps.edu.

Present address: N. Suto, Molecular and Integrative Neurosciences Department, The Scripps Research Institute, 10550 North Torrey Pines Road, SP30-2120, La Jolla, CA 92037

Present address: L.E. Ecke, Department of Pharmacology, University of Pennsylvania, 125 S 31st St, Philadelphia, PA 19104

INTRODUCTION

While psychomotor stimulants, such as amphetamine and cocaine, increase brain concentrations of both dopamine and norepinephrine, studies utilizing systemic administration of dopaminergic and noradrenergic antagonists originally established a dominant role for dopamine in stimulant self-administration [1–4]. Recent findings, however, suggest that regionally specific noradrenergic neurotransmission within the medial prefrontal cortex and ventral tegmental area is in a position to influence stimulant reward by modulating dopamine in the nucleus accumbens, an area critical for mediating both natural and drug rewards. For example, noradrenergic lesions of the medial prefrontal cortex prevent conditioned place preference for cocaine [5] and amphetamine [6]. This blunted preference may be due to impaired signaling at $\alpha 1$ receptors because blockade of these receptors in the medial prefrontal cortex reduces cocaine- [5] and amphetamine-induced [7] elevations in nucleus accumbens dopamine as well as amphetamine-induced hyperlocomotion [7,8]. Furthermore, preference for cocaine, cocaine and amphetamine-induced hyperlocomotion, and amphetamine-induced elevations in nucleus accumbens dopamine are all blunted in mice lacking $\alpha 1b$ receptors [9,10]. Regarding noradrenergic functioning in the ventral tegmental area, amphetamine-induced burst firing of dopamine neurons in this brain region is dependent on activation of local $\alpha 1$ noradrenergic, but not $\alpha 2$ noradrenergic or dopaminergic, receptors [11]. Taken together, these findings suggest that $\alpha 1$ receptor-mediated neurotransmission in the medial prefrontal cortex and ventral tegmental area contributes to the rewarding properties of psychomotor stimulants and their ability to maintain self-administration behavior.

We have thus examined whether local administration of the $\alpha 1$ receptor antagonist terazosin into either the medial prefrontal cortex or ventral tegmental area alters cocaine self-administration at a high dose in well-trained rats. As a positive control, we also tested the D1 dopamine antagonist SCH23390, which is known to increase self-administration at a high dose when microinjected into the medial prefrontal cortex [12] or ventral tegmental area [13].

MATERIALS AND METHODS

Animals

Sixty male Long-Evans rats (Charles River, Raleigh, NC), weighing 350–400 grams at the time of surgery, were used. The guidelines of the National Institutes of Health (NIH publication 86–23, 1996) were followed and all procedures were approved by the local ACUC. Rats were individually housed under a reverse light-dark cycle and were tested during the dark phase.

Surgery

Rats were surgically implanted with bilateral guide cannulae (Plastics One, Roanoke, VA) into either the medial prefrontal cortex or ventral tegmental area and an intravenous catheter made of microrenathane tubing (Braintree Scientific, Braintree, MA) into the right external jugular vein. Guide cannulae were inserted at an angle toward the midline for the medial prefrontal cortex (13o; A/P = +3.0 mm, M/L = ± 0.7 mm, D/V = –3.6 mm) and ventral tegmental area (16o; A/P = –5.7 mm, M/L = ± 0.5 mm, D/V = –8.4 mm) and were positioned 1.0 mm above the injection site. The intravenous catheters were flushed daily with 0.3 mL saline with gentamicin (4 mg/mL) and heparin (30 U/mL). Six rats were excluded from the experiment because their catheters developed leaks.

Experimental design

The current study consisted of two experimental phases: cocaine self-administration training (10–14 days) and cocaine self-administration testing with intracranial microinjection (1 day). Rats were randomly assigned to one of 10 groups defined by the brain region targeted (medial prefrontal cortex or ventral tegmental area) and the drug type used (saline, terazosin [1.0, 5.0 or 10 mM], or SCH23390 [12.3 mM]) for intracranial microinjection prior to cocaine self-administration testing.

Cocaine self-administration training—Following recovery from surgery (3–5 days), rats were trained to self-administer cocaine in daily 4-hr sessions. Ten operant chambers (Med Associates, Georgia, VT), each measuring 25 × 27 × 30 cm and equipped with two levers, were used for all self-administration procedures. Reinforced lever-presses on a retractable “active lever” delivered an infusion of cocaine (1.0 mg/kg/infusion, i.v.). This high dose of cocaine is on the descending limb of the dose response curve [e.g., 14] and has been verified to be sensitive to intra-ventral tegmental area administration of SCH23390 [13] – one of the designated positive controls in the current study. A reinforced lever-press on the active lever also caused a stimulus light above the lever to turn on for 20 seconds during which further active lever-presses were recorded but did not lead to additional infusions. Presses on a stationary “inactive lever” were counted but had no scheduled consequence. Rats were trained for at least 10 days and were required to satisfy predetermined training criteria within 14 days. The training criteria was two consecutive days of no less than 20 infusions and no more than 5 presses on the inactive lever during the 4-hr session. Four rats failed to satisfy these criteria and were excluded.

Cocaine self-administration testing with intracranial microinjection—The day following the last training session, each animal received bilateral microinjections (0.5μl/ side) of terazosin, SCH23390 or saline into either the medial prefrontal cortex or ventral tegmental area immediately prior to a normal cocaine self-administration session. Terazosin hydrochloride and R(+)-SCH23390 hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Terazosin was used instead of more the commonly used α1 antagonist, prazosin, because of its higher solubility in isotonic saline. Three doses of terazosin (1.0, 5.0, and 10 mM) and one dose of SCH23390 (12.3 mM) were tested. The doses of terazosin were determined based on the findings by Darracq *et al.* [7]. In this study, bilateral microinjection of prazosin into the medial prefrontal cortex at 1.0 mM reduced amphetamine-induced elevations in locomotor activity as well as extracellular dopamine in the nucleus accumbens. The receptor binding affinity of terazosin for α1 receptors is approximately 10-fold lower than that of prazosin [15], thus 10 mM of terazosin was chosen as the highest dose. The dose of SCH23390 was based on two previous findings: bilateral microinjections of SCH23390 at 12.3mM into the medial prefrontal cortex [12] or ventral tegmental area [13] increased the rate of cocaine self-administration. The total numbers of infusions, active and inactive lever-presses were recorded and used for statistical analysis.

Cannula placement verification

Upon completion of all experimental procedures, rats were euthanized with pentobarbital (30 mg/kg, i.p.) supplemented with chloral hydrate (140 mg/kg, i.p.). The brains were removed, postfixed in 10% formalin, and sliced into coronal sections (40 μm). The sections were then stained on gelatin-coated slides with cresyl violet for verification of cannula placements. Only data obtained from animals with both cannula tips placed within the medial prefrontal cortex or ventral tegmental area were retained for statistical analysis (Figures 1A and 1B). Eight animals were excluded because one or two of the bilateral cannula were located outside these brain regions. Figures 1A (medial prefrontal cortex) and 1B (ventral tegmental area) depict representative cannula placements.

Statistical analysis

The data collected from the medial prefrontal cortex and ventral tegmental area groups were analyzed separately. The total number of cocaine infusions, active lever-presses, and inactive lever-presses were recorded and separately analyzed using a one-way between ANOVA with the type of microinjection (5 levels) as the between-subjects factor. In addition, in order to assess the effects of terazosin on the pattern of cocaine-seeking behavior throughout the test session, the cumulative number of active lever-presses was divided into 10-minute bins, which were analyzed using a two-way repeated measures ANOVA with the type of microinjection (5 levels: saline, terazosin [1.0, 5.0 or 10 mM], or SCH23390 [12.3 mM]) as the between-subjects factor and time (24 levels) as the within-subjects factor. Post hoc LSD comparisons were conducted when appropriate following ANOVA. All analyses were conducted with Statistica (1999 edition) statistical software.

RESULTS

The bilateral microinjection of terazosin into the medial prefrontal cortex failed to affect cocaine self-administration at all doses tested, while that of SCH23390 significantly increased cocaine intake and cocaine-seeking behavior (Figure 2A and 2B). The ANOVA conducted on the total number of cocaine infusions obtained revealed a significant effect of microinjection ($F_{4,26} = 18.16$, $p < 0.01$). The ANOVA conducted on the cumulative number of active lever-presses per 10-minute bin revealed a significant effect of time ($F_{23,598} = 127.03$, $p < 0.001$) and a significant microinjection $\times$ time interaction ($F_{92,598} = 2.61$, $p < 0.001$). Post hoc LSD comparisons revealed that only rats receiving intra-medial prefrontal cortex SCH23390 obtained significantly more cocaine infusions ($p < 0.05$) and made significantly more active lever-presses than did the saline-treated animals ($p < 0.01$). The ANOVA conducted on the total number of inactive lever-presses revealed no significant effect. Four rats with guide cannula tips located outside the medial prefrontal cortex showed no change in cocaine self-administration following microinjection of terazosin (data not shown).

The bilateral microinjection of terazosin into the ventral tegmental area also failed to affect cocaine self-administration at all doses tested, while that of SCH23390 significantly increased cocaine intake and cocaine-seeking behavior (Figure 2C and 2D). The ANOVA conducted on the total number of cocaine infusions obtained revealed a significant effect of drug ($F_{4,24} = 3.83$, $p < 0.05$). The ANOVA conducted on the cumulative number of active lever-presses per 10-minute bin revealed a significant effect of time ($F_{23,552} = 191.77$, $p < 0.001$) and a significant drug $\times$ time interaction ($F_{92,552} = 2.16$, $p < 0.001$). Post hoc LSD comparisons revealed that only rats receiving intra-ventral tegmental area SCH23390 obtained significantly more cocaine infusions ($p < 0.05$) and made significantly more active lever-presses than saline-treated animals ($p < 0.05$). The ANOVA conducted on the total number of inactive lever-presses revealed no significant effect. Four rats with guide cannula tips located outside the ventral tegmental area showed no change in cocaine self-administration following microinjection of terazosin (data not shown).

DISCUSSION

In the current study, bilateral microinjections of the $\alpha 1$ noradrenergic antagonist, terazosin, into either the medial prefrontal cortex or ventral tegmental area failed to alter cocaine self-administration maintained under a fixed ratio 1 schedule at all doses tested. It is important to note that while $\alpha 1$ manipulation failed to produce even a trend in altering cocaine self-administration (see Figure 2), bilateral microinjections of the D1 dopaminergic antagonist, SCH23390, into either the medial prefrontal cortex or ventral tegmental area significantly increased this behavior, consistent with previous reports [12,13]. As always with negative

findings, it is hard to conclude whether an effective dose of terazosin was used to alter self-administration, however, terazosin's lack of effect is consistent with previous studies demonstrating that systemic injection of another $\alpha 1$ selective antagonist, prazosin, fails to alter cocaine self-administration maintained under fixed ratio schedules [16,17]. Similarly, cocaine self-administration is unaffected following systemic injection of broad-spectrum noradrenergic antagonists [4,18] or lesions of the locus coeruleus [19] – the major source of noradrenergic input to the medial prefrontal cortex and ventral tegmental area. Therefore, unlike D1 dopaminergic signaling in the medial prefrontal cortex and ventral tegmental area [12,13, current study], $\alpha 1$ receptor-mediated noradrenergic neurotransmission at these mesocortical sites does not appear to be critical for cocaine to maintain the steady rate of self-administration behavior that is characteristically observed in well-trained animals under fixed ratio schedules (e.g., Figure 2B and 2D).

Noradrenergic signaling via $\alpha 1$ receptors has been suggested to contribute to cocaine self-administration under certain circumstances. Extended daily access to cocaine is known to lead to an escalated level of cocaine self-administration and is thought to model the excessive cocaine intake observed in addicts [20]. Systemic administration of prazosin (1.0 and 3.0 mg/kg, i.p.) effectively decreases escalated levels of responding for cocaine under a progressive ratio schedule in rats trained with six-hour daily access, while the same noradrenergic manipulation fails to alter the progressive ratio responding in rats trained with limited one-hour daily access to cocaine [20]. Therefore, it is possible that the current noradrenergic manipulation failed to alter cocaine self-administration because the rats were trained with four-hour, instead of six-hour, daily access. It is also possible that $\alpha 1$ signals in brain regions other than the medial prefrontal cortex or the ventral tegmental area may be important in cocaine self-administration.

While these possibilities need to be addressed in future research, the prazosin-induced reduction in the escalated level of cocaine self-administration [20] could reflect not only motivational but also motor deficits that are revealed only when lever-pressing requirements are greatly increased under the progressive ratio schedule. In this regard, a lower dose of prazosin (0.5 mg/kg, i.p.) than the doses effective at reducing the escalated levels of progressive ratio responding for cocaine [20] has been reported to reduce responding for food maintained under a fixed ratio 1 schedule [21] – a finding that is traditionally interpreted to reflect a performance deficit. Indeed, a predominant effect of $\alpha 1$ receptor antagonists, whether administered systemically or intracranially, is locomotor depression. For instance, systemic administration of prazosin decreases both cocaine- and amphetamine-induced hyperlocomotion [22–24], and microinjection of prazosin into the medial prefrontal cortex [7,8] decreases amphetamine-induced hyperlocomotion. Furthermore, microinjection of terazosin into the locus coeruleus depresses spontaneous locomotor activity in the home cage as well as the locomotor activating effects of a novel environment [25]. Mice lacking $\alpha 1b$ receptors also exhibit reduced locomotor activation following cocaine and amphetamine [9,10]. These data suggest that $\alpha 1$ receptor signaling may be involved in the locomotor activation, but not the self-administration, of cocaine.

CONCLUSION

Noradrenergic signaling via $\alpha 1$ receptors in the medial prefrontal cortex and ventral tegmental area does not appear critical for cocaine self-administration in well-trained animals. The maintenance of this behavior appears to be modulated by D1 dopaminergic, rather than $\alpha 1$ noradrenergic, signaling at these mesocorticolimbic sites.

Acknowledgments

Supported by funding from the Intramural Research Program, National Institute on Drug Abuse (NIDA), National Institutes of Health, Department of Health and Human Services as well as NIDA Residual Research Services (N01DA-5-9909). We thank Dr. Roy A. Wise for critical input to the study. We also thank Eric Thorndike for technical assistance with acquisition of self-administration data.

References

1. Yokel RA, Wise RA. Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. *Science* (New York, NY. 1975; 187:547–549.
2. Yokel RA, Wise RA. Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Psychopharmacology*. 1976; 48:311–318. [PubMed: 823588]
3. De Wit H, Wise RA. Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not with the noradrenergic blockers phentolamine or phenoxybenzamine. *Can J Psychol*. 1977; 31:195–203. [PubMed: 608135]
4. Wilson MC, Schuster CR. Aminergic influences on intravenous cocaine self-administration by Rhesus monkeys. *Pharmacol Biochem Behav*. 1974; 2:563–571. [PubMed: 4431820]
5. Ventura R, Morrone C, Puglisi-Allegra S. Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward- and aversion-related stimuli. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:5181–5186. [PubMed: 17360372]
6. Ventura R, Cabib S, Alcaro A, Orsini C, Puglisi-Allegra S. Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and mesoaccumbens dopamine release. *J Neurosci*. 2003; 23:1879–1885. [PubMed: 12629192]
7. Darracq L, Blanc G, Glowinski J, Tassin JP. Importance of the noradrenaline-dopamine coupling in the locomotor activating effects of D-amphetamine. *J Neurosci*. 1998; 18:2729–2739. [PubMed: 9502830]
8. Blanc G, Trovero F, Vezina P, Herve D, Godeheu AM, Glowinski J, et al. Blockade of prefronto-cortical alpha 1-adrenergic receptors prevents locomotor hyperactivity induced by subcortical D-amphetamine injection. *The European journal of neuroscience*. 1994; 6:293–298. [PubMed: 7912614]
9. Auclair A, Cotecchia S, Glowinski J, Tassin JP. D-amphetamine fails to increase extracellular dopamine levels in mice lacking alpha 1b-adrenergic receptors: relationship between functional and nonfunctional dopamine release. *J Neurosci*. 2002; 22:9150–9154. [PubMed: 12417637]
10. Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S, et al. Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. *J Neurosci*. 2002; 22:2873–2884. [PubMed: 11923452]
11. Shi WX, Pun CL, Zhang XX, Jones MD, Bunney BS. Dual effects of D-amphetamine on dopamine neurons mediated by dopamine and nondopamine receptors. *J Neurosci*. 2000; 20:3504–3511. [PubMed: 10777813]
12. McGregor A, Roberts DC. Effect of medial prefrontal cortex injections of SCH 23390 on intravenous cocaine self-administration under both a fixed and progressive ratio schedule of reinforcement. *Behav Brain Res*. 1995; 67:75–80. [PubMed: 7748503]
13. Ranaldi R, Wise RA. Blockade of D1 dopamine receptors in the ventral tegmental area decreases cocaine reward: possible role for dendritically released dopamine. *J Neurosci*. 2001; 21:5841–5846. [PubMed: 11466456]
14. Oleson EB, Roberts DC. Behavioral economic assessment of price and cocaine consumption following self-administration histories that produce escalation of either final ratios or intake. *Neuropsychopharmacology*. 2009; 34:796–804. [PubMed: 18971927]
15. Foglar R, Shibata K, Horie K, Hirasawa A, Tsujimoto G. Use of recombinant alpha 1-adrenoceptors to characterize subtype selectivity of drugs for the treatment of prostatic hypertrophy. *Eur J Pharmacol*. 1995; 288:201–207. [PubMed: 7536677]

16. Howell LL, Byrd LD. Characterization of the effects of cocaine and GBR 12909, a dopamine uptake inhibitor, on behavior in the squirrel monkey. *J Pharmacol Exp Ther*. 1991; 258:178–185. [PubMed: 1677037]
17. Woolverton WL. Evaluation of the role of norepinephrine in the reinforcing effects of psychomotor stimulants in rhesus monkeys. *Pharmacol Biochem Behav*. 1987; 26:835–839. [PubMed: 3496618]
18. Tella SR. Effects of monoamine reuptake inhibitors on cocaine self-administration in rats. *Pharmacol Biochem Behav*. 1995; 51:687–692. [PubMed: 7675844]
19. Roberts DC, Corcoran ME, Fibiger HC. On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav*. 1977; 6:615–620. [PubMed: 122445]
20. Wee S, Mandyam CD, Lekic DM, Koob GF. Alpha 1-noradrenergic system role in increased motivation for cocaine intake in rats with prolonged access. *Eur Neuropsychopharmacol*. 2008; 18:303–311. [PubMed: 17920248]
21. Zhang XY, Kosten TA. Prazosin, an alpha-1 adrenergic antagonist, reduces cocaine-induced reinstatement of drug-seeking. *Biological psychiatry*. 2005; 57:1202–1204. [PubMed: 15866561]
22. Drouin C, Blanc G, Villegier AS, Glowinski J, Tassin JP. Critical role of alpha1-adrenergic receptors in acute and sensitized locomotor effects of D-amphetamine, cocaine, and GBR 12783: influence of preexposure conditions and pharmacological characteristics. *Synapse (New York, NY)*. 2002; 43:51–61.
23. Dickinson SL, Gadie B, Tulloch IF. Alpha 1- and alpha 2-adrenoreceptor antagonists differentially influence locomotor and stereotyped behaviour induced by d-amphetamine and apomorphine in the rat. *Psychopharmacology (Berl)*. 1988; 96:521–527. [PubMed: 2907662]
24. Wellman P, Ho D, Cepeda-Benito A, Bellinger L, Nation J. Cocaine-induced hypophagia and hyperlocomotion in rats are attenuated by prazosin. *Eur J Pharmacol*. 2002; 455:117–126. [PubMed: 12445577]
25. Stone EA, Lin Y, Ahsan R, Quartermain D. Role of locus coeruleus alpha1-adrenoceptors in motor activity in rats. *Synapse*. 2004; 54:164–172. [PubMed: 15452865]

Figure 1. Representative locations of the injection cannula tip placements in the medial prefrontal cortex (A) and ventral tegmental area (B). Symbols refer to drug types for microinjection: saline (○), 10 mM terazosin (▲), and 12.3 mM SCH23390 (●). Line drawings are from Paxinos and Watson (1997). Numbers to the right indicate mm from bregma.

Figure 2.

Effects on cocaine self-administration of bilateral microinjections of terazosin, SCH23390 or saline into the medial prefrontal cortex (A and B) and ventral tegmental area (C and D). While the $\alpha 1$ antagonist terazosin, at all sites (medial prefrontal cortex or ventral tegmental area) and doses (1.0, 5.0, and 10.0 mM) tested, failed to significantly alter cocaine self-administration, local administration of the D1 antagonist SCH23390 into either the medial prefrontal cortex or ventral tegmental area significantly increased cocaine-directed behavior. Figures 2A and 2C depict total number of cocaine infusions as well as active and inactive lever-presses. Data are shown as the mean ($\pm$ SEM) number of infusions and lever-presses. Figures 2B and 2D depict the cumulative number of active lever-presses per 10-minute bins over the 4-hr self-administration testing. Data are shown as the mean ($\pm$ SEM) number of active lever-presses. (b), Total number of active versus inactive lever-presses. * $p < 0.01$ –0.05 vs. saline microinjected rats as revealed by post-hoc LSD comparisons following ANOVA.