Published in final edited form as:
Biol Psychiatry. 2011 October 15; 70(8): 712–719. doi:10.1016/j.biopsych.2011.06.010.

Alpha-2 adrenergic and imidazoline receptor agonists prevent cue-induced cocaine-seeking

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Abstract

Background—Drug-associated cues can elicit stress-like responses in addicted individuals, indicating thatcue- and stress-induceddrug relapse may share some neural mechanisms.It is unknown whetherα_2 adrenergic receptor agonists, which are known toattenuate stress-induced reinstatement of drug-seeking in rats,also reduce cue-induced reinstatement.

Methods—Rats were tested for reinstatement of drug-seekingfollowing cocaine self-administration and extinction. We first evaluated the effects ofclonidine, an agonist at α_2 and imidazoline-1 (I_1) receptors, on relapse to cocaine-seeking. To explore possible mechanisms of clonidine's effects, we then tested more specific α_2 or I_1 agonists, post-synaptic adrenergic receptor (α_1 and β) antagonists, andcorticotropin-releasing factor receptor-1 (CRF R1) antagonists.

Results—We found that clonidine,andthe more selective α_2 agonists UK-14,304 and guanfacine, decreased cue-induced reinstatement of cocaine-seeking.The specific I_1 receptor agonist moxonidine reduced cue-induced as well as cocaine-induced reinstatement.Clonidine or moxonidine effects on cue-induced reinstatementwere reversed by the selective α_2 receptor antagonist RS-79948, indicatinga role for α_2 receptors.Prazosin and propranolol, antagonists at the $\alpha_1$and β receptor, respectively, reducedcue-induced reinstatement only when administered in combination. Finally, the CRF R1 antagonist CP-154,526reduced cue-induced reinstatement, as previouslyobservedfor stress-induced reinstatement, indicating possible overlap between stress and cue mechanisms.

Conclusions—These results indicate that α_2 and I_1 receptor agonists are novel therapeutic options for prevention of cue-induced cocaine relapse. Given that α_2 receptor stimulation is associated with sedation in humans, the I_1agonist moxonidineseems to have substantial potential for treating addictive disorders.

Keywords

cocaine; self-administration; relapse; norepinephrine; imidazoline; corticotropin-releasing factor

Introduction

Prevention of relapseisa primary goal of addiction recovery. Understanding the neural mechanisms involved in relapse facilitates rationale development of new therapeutics to

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Financial Disclosures The authors report no biomedical financial interests or potential conflicts of interest.

treat addictive disorders. Animal modelsof relapse revealeda role for the central noradrenergic (NA)and corticotropin-releasing factor (CRF) systemsinstress-induced relapse (reviewed in 1, 2-3). Administration of $\alpha_2$adrenergic agonistsor CRF receptor-1 (R1) antagonists attenuatedstress-induced reinstatement of extinguished drug-seeking for cocaine, heroin, ethanol, and nicotine in rats(4-11). Further, lesioningthe ventral noradrenergic fiber bundle blocked stress-induced reinstatement of heroin-seeking (6). However, it is unknown whether adrenergicsignaling alsoplays a role in relapse triggered by drug-associated cues, and to what degree the neural mechanisms of cue- and stress-induced reinstatement may overlap.

Human studies indicate that cues and stress may share common neural mechanisms for provoking drug craving. Cocaine-dependent individuals exhibited increased drug craving, anxiety, and activation of the hypothalamic-pituitary-adrenal (HPA) axis in response to both drug-related stimuli and stress-related imagery(12-13). Similar HPA axis activationwas seen in rats following cue- or stress-induced reinstatement of cocaine-seeking (14-15). Additionally, stress- and cue-induced craving were reduced in individuals dependent upon opioids or cocaine following treatment with an α_2 agonist, supporting a role of adrenergic signaling in both processes(16-17).

Here, we tested a role for NA signaling in cue-induced reinstatement of cocaine-seekingusingtheα_2 agonist clonidine, as well as α_1 orβreceptor antagonists, to determine the contributions of pre- and post-synaptic adrenergic receptors.Given that clonidine acts at both α_2 and imidazoline-1 (I_1) receptors (18), we also administered agonists with varying affinities for α_2 and I_1receptors. I_1 receptors are implicated in the central regulation of blood pressure and are the primary target of second-generation antihypertensive agents such as moxonidine and rilmenidine, which lack clonidine-like sedation due to their low affinity for α_2 receptors (19-21).In addition to reducing hypertension, I_1 receptor agonists may have the advantage of reducing conditions associated with metabolic syndrome X, including insulin resistance and glucose intolerance(21-25).A potential role for I_1 receptor signaling in addiction is supported by recent findings that I_1 agonists reduced opiate and ethanol withdrawal effects in rats (26-29). However, the possible useof I_1 receptor agonists as anti-relapse therapeutics has been less explored. Studies presented here reveal that stimulation ofα_2 and I_1receptors prevents relapse of cocaine-seeking, indicating new pharmacologic approaches foraddiction treatment.

Methods and Materials

Animals

Male Sprague Dawley rats (initial weight 250-300 g; Charles River, Raleigh, NC) were single- or pair-housed in a temperature- and humidity-controlled, AAALAC-accredited animal facility at MUSC. Rats were housed under a reversed 12-hr light/dark cycle (lights off at 6 a.m.), with ad libitum food and water (except for food self-administration study, described below). All experiments were approved by the Institutional Animal Care and Use Committee at MUSC and conducted according to specifications of the National Institutes of Health as outlined in the Guide for the Care and Use of Laboratory Animals.

Catheter surgery

Following acclimation to the animal facility, rats to be given cocaine self-administration were anesthetized with ketamine/xylazine (and equithesin in some cases), given nonsteroidal anti-inflammatory analgesics, and implanted with intravenous catheters. Silastic tubing was inserted into and secured to the right jugular vein, while the other end passed subcutaneously over the shoulder and exited the back via either a chronic indwelling cannula (30) or an

external infusion harness (31), as previously described. Beginning 3 days after surgery, catheters were flushed once daily with 0.1 ml each of the antibiotic cefazolin (100 mg/ml) and heparin (100 U/ml). Self-administration sessions began after 1 week of recovery from surgery.

Cocaine self-administration

Operant chambers were housed in sound-attenuating cubicles. Stimulus presentations, cocaine infusions, and data recording were controlled via MED-PC IV software (Med-Associates, St. Albans, VT). During 2-hr daily sessions, presses on an active lever resulted in a cocaine infusion (fixed ratio-1; 0.2 mg in 50 μl via motorized pump) paired with tone and light cues (78 dB, 2900 Hz; white stimulus light above the active lever), followed by a 20-sec timeout. Presses on an inactive lever had no consequence. Rats were given 10 self-administration sessions in which they earned at least 10 infusions per session.Rats then were given daily extinction sessions, during which lever presses had no consequence (no drug or cues). Prior to reinstatement testing, rats were required to meet an extinction criterion of ≤ 25 active lever presses for 2 consecutive days (at least 7 extinction sessions prior to the first reinstatement session, and 2 extinction sessions prior to subsequent reinstatement sessions).

For cue-induced reinstatement, active lever presses resulted in presentation of tone and light cues in the same manner as during self-administration, but no drug was delivered. For cocaine-induced reinstatement, rats were injected with a priming injection of cocaine (10 mg/kg, i.p.) immediately prior to the reinstatement test session. Animals undergoing abstinence prior to extinction remained in their home cages and were periodically handled. NA and CRF receptor ligands were administered 30 min prior to reinstatement or extinction sessions (except RS-79948, which was administered 60 min prior).In a within-subject design, each animal received 1 reinstatement session with vehicle pretreatment and 1-2 reinstatement sessions with a NA or CRF receptor ligand (1-2 doses of a single ligand), for which the order of treatments was counterbalanced among animals.

Food self-administration

Rats were food-restricted throughout the study, so that each rat received 10 g of rat chow daily in the home cage at the end of the day; in combination with the food earned during the self-administration session, this was sufficient for each animal to maintain its weight (range among rats was 350-400 g). During 1-hr daily sessions in operant chambers similar to those used for cocaine self-administration, presses on an active lever resulted in the delivery of a single food pellet into a hopper (fixed ratio-1; 45-mg grain-based pellets from Bio-Serv, Frenchtown, NJ) with no cue pairings and no timeout period. Presses on an inactive lever had no consequence. Once rats acquiredfood self-administration, they were given 6 test sessions for which different NA ligands were administered in a counterbalanced order 30 min prior to food self-administration sessions, in a within-subject design. At least one intervening self-administration session was given between test sessions to ensure that rates re-stabilized.

Locomotor testing

Rats were tested for spontaneous locomotion during a 60-min test session in clear acrylic chambers (40 × 40 × 30 cm) equipped with Digiscan monitors (AccuScan Instruments, Inc., Columbus, OH) that monitored horizontal (16 × 16 photobeam array) and vertical activity (16 photobeams). NA receptor ligands were administered 30 min prior to locomotor testing. Each rat was given 2 locomotor tests, for which they were randomly assigned to receive 2 different test ligands, for a between-subjects design.

Drugs

Cocaine HCl (National Institute on Drug Abuse) was dissolved in 0.9% sterile saline. NA and CRF receptor ligands(Table 1) were administered in a volume of 1 ml/kg (i.p.) 30 min prior to reinstatement testing, except RS-79948 (60 min prior to test).Vehicles and receptor affinities are reported on Table 1(18, 20, 32). All NA and CRF receptor ligands were purchased through Tocris Bioscience (Ellisville, MO), except clonidine (Sigma-Aldrich, St. Louis, MO).UK-14,304 tartrate dosing was based on free weight.Moxonidine was dissolved in a few drops of 1M HCl prior to adding saline (and then brought to pH 7).Prazosin was dissolved in its vehicle by heating and vortexing. Vehicles for the CRF antagonists were prepared by heating and stirring (and then adding to the antagonist).

Experimental groups

NA and CRF receptor ligands were administered prior to cue-induced reinstatement (*n*=6-15 per group, within-subject comparisons), extinction (*n*=8 per group, between-subjects comparisons), locomotor testing (*n*=6-7 per group, between-subjects comparisons), or food self-administration (*n*=8, within-subject comparisons). Exact group numbers are presented in the figure legends.

Data analyses

All results are presented as means ± SEM. Animals were completely removed from data analyses if they lacked reinstatement (< 20 active lever presses) on allreinstatement tests, including vehicle sessions. Reinstatement with vehicle pretreatment was not significantly different among groups of rats receiving different doses of a given NA or CRF receptor ligand (using t-test or ANOVA, depending on number of groups), so data were pooled into a single bar for graphs. However, statistical analyses were run separately on each group of rats for within-subject comparisons of reinstatement effects.

Reinstatement data were analyzed using paired t-testsor one-way repeated-measures ANOVAs (with Tukey-Kramer's post-hoc comparisons), depending on whether a given group received 2 or 3 reinstatement sessions (as described in cocaine self-administration methods); Bonferroni correction was employed when more than one statistical test was performed for analysis ofa given NA or CRF receptor ligand. Mixed-model ANOVAs with Bonferroni post-hoc comparisons were used to analyze extinction behavior across sessions (Figs. 1d, 2d) and the effects of RS-79948 alone (Fig. 1c). Locomotor and food self-administration data were analyzed using a one-way ANOVA (repeated measures for food self-administration) with Dunnett's Multiple Comparison Test for post-hoc analyses against saline.

Results

For all rats undergoing cocaine self-administration (*n*=189), the means (± SEM) for the last 2 days of self-administration were 38.5 (±0.9) and 39.6 (±1.0) infusions(appx.20 mg/kg per day), and 54.7 (±3.7) and 53.6 (±3.2) active lever presses. The mean (± SEM) active lever presses for Days 1 and 7 of extinction were 84.3 (±3.2) and 18.2 (±0.8), respectively, for animals that received no treatment on extinction days.Unless noted, there were no significant differences for responding on the inactive lever during testing.

Alpha$_2$ agonists reduced cue-induced reinstatement

We first tested clonidine, an α_2 agonist with comparable affinity for I_1 receptors, to determine whether noradrenergic signaling might be involved in cue-induced reinstatement.Clonidine reduced cue-induced reinstatement of extinguished cocaine-seeking at 20 μg/kg (t_{14}=4.50; p=0.0005), but not 5 μg/kg (t_7=1.57; p=0.16) or10 μg/kg (t_6=2.68;

p=0.037, not sig. after Bonferroni correction), as compared to vehicle(Fig. 1a). Clonidine also reduced inactive lever pressing at 10 μg/kg (t_6=3.33; p=0.016, sig.) and 20 μg/kg (t_{14}=3.81; p=0.0019; not shown).

In a separate group of rats, pretreatment with the selectiveα_2 antagonist RS-79948 (1 mg/kg) reversed the effects of 20 μg/kg clonidine on cue-induced reinstatement($F_{2,9}$=9.42; p=0.0016). Post-hoc analyses revealed that combined treatment with RS-79948/clonidine was different from vehicle/clonidine (p<0.01) but not from vehicle/vehicle (p>0.05), whereas vehicle/clonidine reduced reinstatement as compared to vehicle/vehicle (p<0.05; Fig. 1b).Importantly, administration of RS-79948 alone did not trigger reinstatement during extinction and did not potentiate cue-induced reinstatement ($F_{1,15}$=1.66; p=0.22; Fig. 1c).

Clonidine (20 μg/kg) also reduced cocaine-seeking when given prior to the first day of extinction. Across the first 7 days of extinction, there was a significant effect for clonidine($F_{1,49}$=4.42; p=0.041) and an interaction between clonidine and extinction session ($F_{6,49}$=2.53; p=0.033). Post-hoc analyses revealed that clonidine reduced active lever pressing on the first day of extinction only, when clonidine was administered (p<0.001; Fig. 1d).Clonidine also reduced inactive lever pressing on Day 1 of extinction(p<0.001; not shown).

We then tested ligands with more selective affinity forα_2 receptors on cue-induced reinstatement. The selective α_2 agonist UK-14,304 reduced cue-induced reinstatement at 200 μg/kg (t_5=5.55; p=0.0026, sig. after Bonferroni correction), but not at 50 or 100 μg/kg ($F_{2,11}$=3.10; p=0.065), as compared to vehicle (Fig. 1e). Additionally, the selective α_{2A}receptor agonist guanfacine reduced cue-induced reinstatement ($F_{2,8}$=4.85; p=0.023) when administered at 1 mg/kg (post-hoc, p<0.05), but not 0.2 mg/kg (p>0.05), as compared to vehicle (Fig. 1f).

I_1 receptor agonist reduced reinstatement

We next tested moxonidine on cue-induced reinstatement to evaluate whether actions at I_1 receptors may contributeto clonidine's effects on reinstatement. This compound has 40-fold selectivity for I_1 over α_2 receptors (18);more selective I_1 agonists or antagonists are not readily available. Moxonidine reduced cue-induced reinstatement ($F_{2,9}$=8.66; p=0.0023)at 1 mg/kg (post-hoc, p<0.01), but not 0.2 mg/kg (p>0.05), as compared to vehicle (Fig. 2a).

Pretreatment with the α_2 antagonist RS-79948 (1 mg/kg) reversed the effects of moxonidine (1 mg/kg) on cue-induced reinstatement ($F_{2,11}$=12.19; p=0.0003). Post-hoc analyses revealed that combined treatment with RS-79948/moxonidine was different from vehicle/ moxonidine (p<0.001) but not from vehicle/vehicle (p>0.05), whereas vehicle/moxonidine reduced reinstatement as compared to vehicle/vehicle (p<0.05; Fig. 2b).Inactive lever pressing was also different across the sessions ($F_{2,11}$=3.46; p=0.049), but there were no significant post-hoc effects.

Moxonidine (1 mg/kg) also reduced reinstatement elicited by a cocaine prime (t_{11}=2.72; p=0.020; Fig. 2c). In a separate group of rats, moxonidine reduced first-day extinction responding, as compared to vehicle-treated rats,following 3 weeks of abstinence from cocaine self-administration(post-hoc, p<0.001; Fig. 2d). These rats continued to receive treatment with either moxonidine or vehicle chronically throughout the first 7 days of extinction, followed by2 days of extinction with no treatment. Analysis of these 9 days of extinctionrevealed a significant effect oftreatment ($F_{1,63}$=15.40; p=0.0002) and an interaction between treatment and extinction session ($F_{8,63}$=5.66; p<0.0001), such that moxonidine- and vehicle-treated rats were different on Days 1-3 of extinction (p<0.001, 0.001, 0.05, respectively) and on Day 8, when all rats received no treatment for the first time

($p<0.001$; Fig. 2d).Responding on the inactive lever during these 9 days of extinction was different only on Day 1(post-hoc, $p<0.01$; not shown).In these same rats, we then tested for potential carry-over effects of chronic moxonidine on subsequent reinstatement conductedin the absence of any treatment (i.e., no moxonidine or vehicle injections).There were no differencesin cue- or cocaine-induced reinstatementfor animals that previously received chronic moxonidine during extinction, as compared to vehicle animals ($p>0.05$; not shown).

Combined $\alpha_1$and βadrenoceptorantagonist treatmentreduced cue-inducedreinstatement

Next we determined whether ligandsfor other adrenergic receptors would also decrease relapse to cocaine-seeking. The α_1 antagonist prazosin had no effect on cue-induced reinstatement at 1 or 3 mg/kg ($F_{2,14}=0.64$; $p=0.53$), as compared to vehicle (Fig. 3a). Administration of the β antagonist propranolol also had no effect on cue-induced reinstatement at 5 mg/kg ($t_{10}=1.00$; $p=0.34$) or 10 mg/kg ($t_8=0.61$; $p=0.56$), as compared to vehicle (Fig. 3b). However, a combination of prazosin (1 mg/kg) and propranolol (10 mg/kg) reduced reinstatement as compared to vehicle ($t_8=2.80$; $p=0.023$; Fig. 3c).

Most adrenergic ligands reduced activity in a novel locomotor chamber

Possible sedative effects of adrenergic ligands were tested in a novel locomotor chamber following administration of doses that reduced reinstatement, even if not significantly (20 μg/kg clonidine, 200 μg/kg UK-14,304, 1 mg/kg guanfacine, 1 mg/kg moxonidine, 3 mg/kg prazosin, or 1/10 mg/kg of prazosin/propranolol).There was a significant effect of drug administration on horizontal activity ($F_{6,41}=4.34$; $p=0.002$), such that clonidine, UK-14,304, and guanfacine reduced activity as compared to saline ($p<0.01$; Fig. 4a). There was also a significant effect of drug administration on vertical activity ($F_{6,41}=4.69$; $p=0.001$), such that clonidine, UK-14,304, guanfacine, prazosin, and prazosin/propranolol reduced activity as compared to saline ($p<0.01$ for all, except $p<0.05$ for prazosin/propranolol; Fig. 4a). Notably, moxonidine had no effect on horizontal or vertical activity ($p>0.05$).

Most adrenergic ligands had little effect on food self-administration

When adrenergic ligands were administered prior to food self-administration, the effects were much less pronounced than those on cocaine-seeking. However, analysis of active lever-pressing following administration of adrenergic ligands at doses that reduced reinstatement of cocaine-seeking showed an overall significant effect($F_{5,35}=3.27$; $p=0.016$), and post-hoc analyses revealed that guanfacine and moxonidine were significantly different than saline ($p<0.05$; Fig. 4b). CRF antagonists have previously been shown to have no effect on food self-administration(7, 33).

CRF antagonist reduced cue-induced reinstatement

A role for CRF in cue-induced reinstatement of cocaine-seeking was tested to further evaluate possible common mechanisms underlying cue- and stress-induced reinstatement. We tested two different CRF receptor-1 (R1) antagonists, CP-154,526 and antalarmin.Administration of CP-154,526 at 20 mg/kgsignificantly reduced reinstatement as compared to vehicle ($t_5=6.34$; $p=0.020$; Fig. 5a).Antalarmin had no effect on reinstatement at 10 mg/kg ($t_{11}=0.39$; $p=0.71$), but showed a trend for reducing reinstatement at 20 mg/kg ($t_{10}=1.50$; $p=0.16$), as compared to vehicle(Fig. 5b).

Discussion

Our results indicate that the I_1 receptor-preferring agonist moxonidine is a potential therapeutic option for maintaining cocaine abstinence. Moxonidine reduced cocaine-seeking triggered by either drug-associated cues or cocaine prime, without signs of sedation.These

reinstatement findings highlight the potential functional importance of the relatively-unknown imidazoline signaling system in drug addiction.In addition, the current studies revealed that α_2 adrenergic receptor agonists and CRF R1 antagonists reduced cue-induced reinstatement, as previously observed for stress-induced reinstatement (4-11). These resultsindicate that neural pathways linked to stress-induced reinstatement of drug seeking also may be critically involved in the ability of cues to elicit drug craving and relapse.

Alpha$_2$ and imidazoline receptor actions

We found that ligands with affinities for $\alpha_2$adrenoceptors and I_1 receptors blocked cue-induced reinstatement of cocaine-seeking. The mixed α_2 / I_1 agonist clonidine attenuated cue-induced reinstatement as well as responding on the first day of extinction (Fig. 1), consistent with recent human studies showing that clonidine reduced cue- and stress-induced cocaine craving (17). The selective α_2 agonists UK-14,304 and guanfacinealso reduced cue-induced reinstatement, showing that α_2 receptor activation is sufficient to block reinstatement of cocaine-seeking (Fig. 1).This is in contrast to previous findings that the α_2 agonist lofexidine did not block cue-induced reinstatement of drug-seeking for a cocaine-heroin combination (speedball) (34). Here, we found that the more specific I_1 receptor agonist moxonidinereduced cue-induced reinstatement, cocaine-induced reinstatement, and extinction responding following 3 weeks of abstinence, when drug-seeking appeared to be elevated(Fig. 2D, as compared to Fig. 1D)(35).Taken together with previous findings that clonidine did not block cocaine-induced reinstatement of drug-seeking in self-administration or conditioned place preference paradigms (4, 36), these results indicate thatmoxonidine reduces drug-seeking behaviors more broadly than clonidine, highlighting its potential usefulness in addiction treatment.

The selective α_2 antagonist RS-79948 blocked the reinstatement effects for both clonidine and moxonidine, indicating that at least some stimulation of α_2 receptors is necessary for their actions (Fig. 1 and 2).It is unknown whether I_1stimulation plays an equally important role, and there are no selective I_1 receptor antagonists available to test this. However, we found that moxonidine did not causeclonidine-like reductions in locomotor activity (Fig. 4a)that have been linked to α_2 receptor actions (19, 37-38). This indicates that moxonidine may be acting as a weak or partial agonist at α_2 receptors as compared to clonidine, or that I_1 receptor signaling requires coincident stimulation at α_2 receptors. Previous studies also noted thatmoxonidine actions at α_2 receptors are required for its hypotensive effects (39-40). Coincident stimulation of I_1 and α_2 receptors may play an important role in moxonidine's unique ability to attenuate cue- and cocaine-induced reinstatement.

Reversal of reinstatement blockade by RS-79948 indicates that α_2 receptors play a critical, although not necessarily sufficient, role in cocaine-seeking behavior. The α_2 receptors involved in the reinstatement effect may be located on adrenergic neurons, where they act as autoreceptors, or located on non-adrenergic cells, where several actions have been described for $\alpha_2$receptors(41-43). As an important control, we showed that RS-79948 alone did not drive reinstatement of drug-seeking (Fig. 1), in contrast to previous observations for the non-selective α_2 antagonist yohimbine(5, 44-45). Recent studies similarly found that RS-79948 does not trigger reinstatement, and indicate that yohimbine-induced reinstatement might not reflect adrenergic effects, but instead may be caused by dopamine and serotonin receptor actions (46-49).Notably, although selective α_2 antagonists do not trigger reinstatement, norepinephrine itself does (47). Therefore, the recent finding that cue-induced cocaine-seeking was attenuated by the NA reuptake inhibitor atomoxetine(50) can most likely be attributed to specificstimulation of $\alpha_2$adrenoceptors (paralleling the effects seen here), and not due toenhanced NA signaling overall.

In humans, I_1 agonistssuch as moxonidine and rilmenidineare preferred over α_2 agonists for treatment of hypertension due to their ability to reduce blood pressure without the side effects associated with clonidine, such as dry mouth, decreased alertness, and sedation (51-52).However, moxonidine's effects on reinstatement are most likely not related to its hypotensive properties, as other hypotensive agents tested here (e.g. prazosin and propranolol) did not reduce reinstatement.We found that most NA agents reduced activityin a novel locomotor chamber (Fig. 4a), which may indicate subtle sedative effects. Importantly, we found no profound effects of α_2 / I_1 agonistson lever-pressing during food self-administration (Fig. 4b), indicating that reinstatement blockade cannot be attributed to reductions in motivated behavior or operant ability (all rats showed > 100 presses/hr). However, although we found no reductions infood self-administration following most α_2 agonists, corroborating previous observations for clonidine and UK-14,304 (6-7, 53), there were significant reductions following guanfacine and moxonidine treatment.Reductions in food intake following moxonidine might be related to satiety mechanisms, as moxonidine beneficially affects key metabolic signals involved in the regulation of feeding (21-25). Given that moxonidine appeared to have no clonidine-like sedative effects in the locomotor chamber and that food self-administration was largely intact following its administration, the current results favor moxonidine as a promising anti-relapse therapeutic.

Alpha$_1$ and β receptor antagonists

Although α_2 and I_1 agonists reduced cue-induced reinstatement, the post-synapticα_1 receptor antagonist prazosin or β receptor antagonist propranolol did not reduce cue-induced reinstatement of cocaine-seeking unless administered in combination (Fig. 3).The dosing used for each of these antagonists alone is known to be effectivein other behavioral tasks (53-55). The lack of effect with propranolol is particularly surprising in light of previous studies showing that localmicroinfusions ofβ antagonists into extended amygdala attenuate stress-induced reinstatement of cocaine-seeking(56). This indicates that β receptor signaling may be more involved in reinstatement elicited by stress than bycues. However, this discrepancy may also be due to differences in receptor selectivity with local and systemic dosing of β receptor antagonists.

The finding that reinstatement is only reduced by a prazosin/propranolol combination may indicate that cue-induced reinstatement involves NA, but that signaling through either α_1 or β receptors is sufficient,so dual blockade is necessary to reduce reinstatement. However, reinstatement blockade following the prazosin/propranolol combination may also be explained by general effects onmemoryor cognition. Combined treatment with α_1 and β receptor antagonistshas been shown to affect cognitive processing and memory retrieval (57-58).

Cue and stress commonalities

We found that systemic administration of the CRF R1 antagonist CP-154,526 (20 mg/kg) reduced cue-induced reinstatement (Fig. 5), as previously observed for stress-induced reinstatement(7-11). CP-154,526 has been shown to reduce cue-induced reinstatement of cocaine- and methamphetamine-seeking, as well as extinction responding for cocaine (14, 59-60). We found a trend for reduced reinstatement with another CRF R1 antagonist, antalarmin, at 20 mg/kg (Fig. 5). Antalarmin has been shown previously to reduce reinstatement elicited by yohimbine or footshock stress, as does CP-154,526 (9-10, 61-63).

As described above, we also found that α_2 agonists reduced cue-induced reinstatement of cocaine-seeking (Fig. 1 and 2), as previously observed for stress-induced reinstatement of drug-seeking (4-7). These findings indicate that drug-associated cues and stress may share some neural mechanisms for driving relapse. This is supported by prior studies in rats and

dependent humans, in which cocaine-associated cues increased anxiety and HPA axis activation (12-14, 64-65). Additionally, previous studies have shown that both cue- and stress-induced reinstatement are reduced by the orexin-1 receptor antagonist SB-334867, the corticosterone synthesis inhibitor ketoconazole, environmental enrichment, or manipulation of bed nucleus of striaterminalis(14-15, 30, 56, 66-69). Finally, cues have been shown to potentiate stress-induced reinstatement in rats(70-72).

Conclusions

We found that cue-induced reinstatement of cocaine-seeking is blocked by stimulation of $\alpha_2$adrenoceptors and I_1 receptors, or antagonism of CRF R1s, as previously observed for stress-induced reinstatement of drug-seeking. These findings indicate that drug-associated cues and stress share at least some common mechanisms for driving relapse. Future experiments may further explore this overlap by evaluatingcue-induced reinstatement followingventral noradrenergic fiber bundle lesions or local microinfusions of NA or CRF receptor ligands.The current studies support the therapeutic use of I_1 receptor agonists such as moxonidine as anti-relapse medicationsdue to theirability to reducecocaine-seeking behaviors triggered by a variety of factors without potential sedative side effects.

Acknowledgments

This work was supported by National Institutes of Health grants R37-DA06214, T32-DA007288, and P50-DA015369, and conducted in an animal facility constructed with support from National Institutes of Health grantC06-RR015455 from the Extramural Research Facilities Program of the National Center for Research Resources.We thank Rebecca Fallon, Phong Do, EleniBucuvalas, and Paul Knackstedt for technical assistance.

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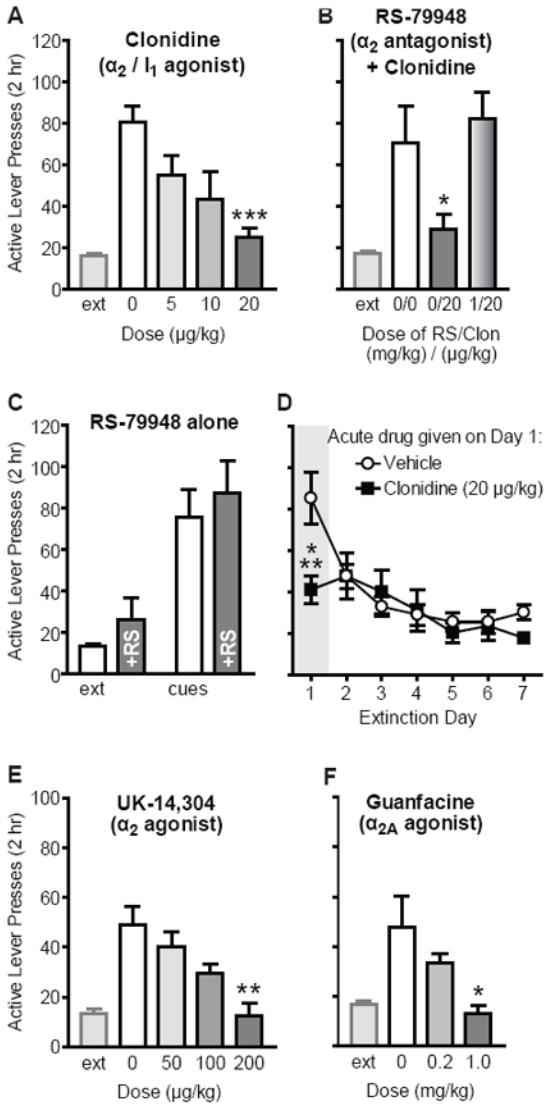

Figure 1.

Stimulation of α_2 adrenergic receptors blocked cue-induced reinstatement of extinguished cocaine-seeking. **(A)** The mixed α_2/ imidazoline-1 (I_1) receptor agonist clonidine significantly attenuated cue-induced reinstatement of active lever pressing at 20 µg/kg (*n*=15), but not 5 µg/kg (*n*=8) or10 µg/kg (*n*=7), as compared to vehicle (***p<0.001). Extinction (ext) levels of responding prior to reinstatement are also shown. **(B)** Pretreatment with the selective α_2 antagonist RS-79948 (1 mg/kg) blocked the effects of 20 µg/kg clonidine on cue-induced reinstatement (*n*=10; *p<0.05, as compared to 0/0 vehicle condition). **(C)** RS-79948 alone did not trigger reinstatement when administered prior to an extinction session (ext; *n*=8), and did not potentiate cue-induced reinstatement (cues; *n*=9). **(D)** Acute clonidine reduced cocaine-seeking on the first day of extinction (*n*=8 and 8; ***p<0.001). Clonidine- and vehicle-treated rats were not significantly different on subsequent extinction days when no pretreatment was given. **(E)** The selective α_2 agonist UK-14,304 significantly attenuated cue-induced reinstatement at 200 µg/kg (*n*=6), but not 50 or 100 µg/kg (*n*=12), as compared to vehicle (**p<0.01). **(F)** The selective α_{2A} agonist guanfacine significantly attenuated cue-induced reinstatement at 1 mg/kg, but not 0.2 mg/kg (*n*=9), as compared to vehicle (*p<0.05).

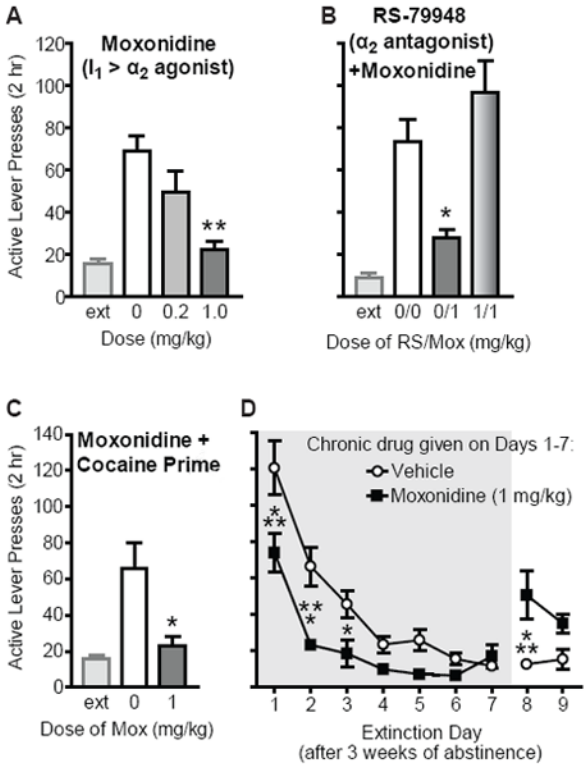

Figure 2.

Moxonidine, an imidazoline-1 (I1) receptor-preferring agonist, reduced cue-induced reinstatement of extinguished cocaine-seeking, as well as cocaine-induced reinstatement and extinction responding. **(A)** Moxonidine significantly attenuated cue-induced reinstatement at 1 mg/kg, but not 0.2 mg/kg (n=10), as compared to vehicle (**p<0.01). Extinction (ext) levels of responding prior to reinstatement are also shown. **(B)** Pretreatment with the selective α_2 antagonist RS-79948 (1 mg/kg) blocked the effects of 1 mg/kg moxonidine on cue-induced reinstatement (n=12; *p<0.05, as compared to 0/0 vehicle condition), indicating that the effects of moxonidine required actions at α_2 receptors. **(C)** Moxonidine significantly attenuated reinstatement elicited by a cocaine prime (n=12), as compared to vehicle (*p<0.05). **(D)** Chronic moxonidine reduced cocaine-seeking on the first days of extinction following 3 weeks of abstinence from self-administration (n=8 and 8). There was a rebound of responding when moxonidine pretreatment was discontinued after 7 days (*p<0.05, ***p<0.001).

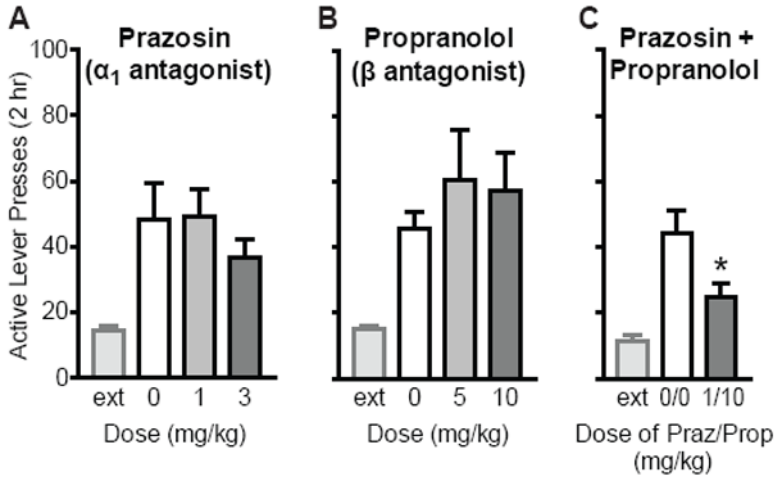

Figure 3.

Prazosin plus propranolol reduced cue-induced reinstatement of extinguished cocaine-seeking. **(A)** The α_1 antagonist prazosin had no significant effect on cue-induced reinstatement at 1 or 3 mg/kg (*n*=15). Extinction (ext) levels of responding prior to reinstatement are also shown. **(B)**The β antagonist propranolol had no effect on cue-induced reinstatement at 5 mg/kg (*n*= 11) or 10 mg/kg (*n*=9). **(C)** Combined pretreatment with prazosin (1 mg/kg) plus propranolol (10 mg/kg) significantly attenuated cue-induced reinstatement (*n*=9), as compared to vehicle (*p<0.05).

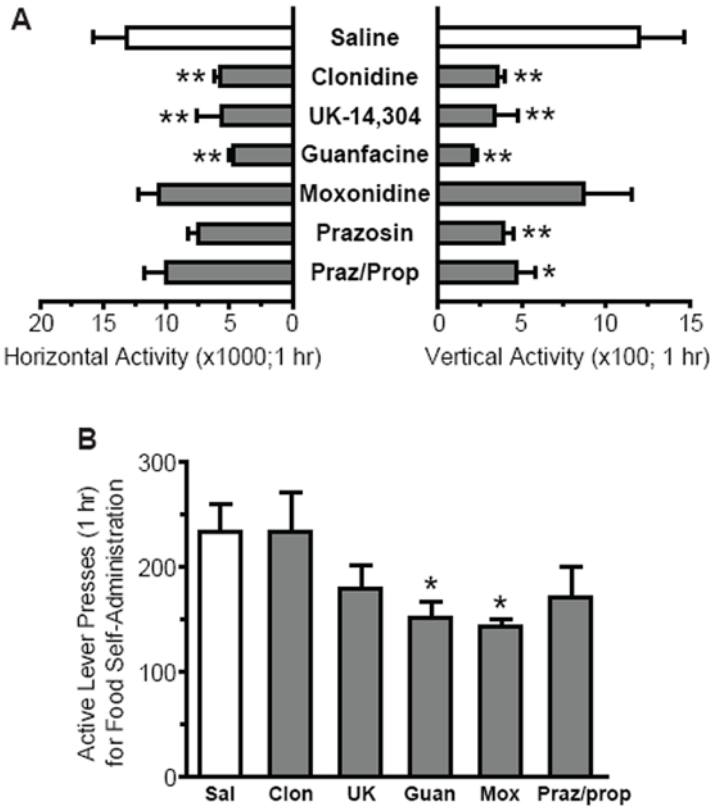

Figure 4.

Most adrenergic ligands reduced activity in a novel locomotor chamberwith little effect on food self-administration.**(A)**Activity (number of beam breaks in 1 hr) was assessed in a locomotor chamber following pretreatment with saline (*n*=6), clonidine (20 μg/kg; *n*=7), UK-14,304 (200 μg/kg; *n*=7), guanfacine (1 mg/kg; *n*=7), moxonidine (1 mg/kg; *n*=7), prazosin (3 mg/kg; *n*=7), or prazosin plus propranolol (1+10 mg/kg; *n*=7). Horizontal activity was significantly reduced by clonidine, UK-14,304, and guanfacine, as compared to saline (**$p<0.01$). Vertical activity was significantly reduced by all ligands except moxonidine (*$p<0.05$, **$p<0.01$).**(B)** Food self-administration was tested following pretreatment with saline,clonidine (20 μg/kg), UK-14,304 (200 μg/kg), guanfacine (1 mg/kg), moxonidine (1 mg/kg), or prazosin plus propranolol (1+10 mg/kg) in a within-subject, counterbalanced design (*n*=8).Guanfacine and moxonidine reduced responding, as compared to saline (*$p<0.05$).

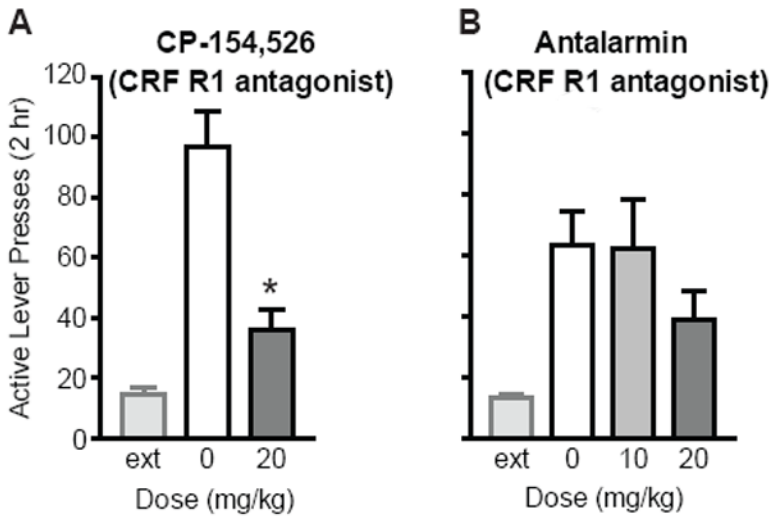

Figure 5.

Corticotropin-releasing factor receptor-1 (CRF R1) antagonists reduced cue-induced reinstatement of extinguished cocaine-seeking. **(A)**CP-154,526 significantly attenuated cue-induced reinstatement at 20 mg/kg (*n*=6), as compared to vehicle (**p*<0.05). Extinction (ext) levels of responding prior to reinstatement are also shown. **(B)**Antalarmin showed a trend for reducing cue-induced reinstatement at 20 mg/kg (*n*=11), but not 10 mg/kg (*n*=12), as compared to vehicle.

Table 1

Noradrenergic (α, β), imidazoline (I_1) and corticotropin-releasing factor (CRF R1) receptor ligands used in the current studies.

Drug	Receptor action	Doses (mg/kg)	Vehicle
Clonidine	I_1> α_2 agonist (4-fold)	0.005, 0.01, 0.02	Saline
RS-79948	α_2 antagonist (selective)	1.0	Saline
UK-14,304	α_2> I_1 agonist (100-fold)	0.05, 0.1, 0.2	Water
Guanfacine	α_{2A} agonist (selective)	0.2, 1.0	Saline
Moxonidine	I_1> α_2 agonist (40-fold)	0.2, 1.0	Saline
Prazosin	α_1 (and α_{2B}) antagonist	1.0, 3.0	10% DMSO in water
(*S*)-(-)-Propranolol	β antagonist	5, 10	Saline; (*R*)-(-)-Prop control
CP-154,526	CRF R1 antagonist	20	5% cremophor in saline
Antalarmin	CRF R1 antagonist	10, 20	10% cremophor in water