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Pharmacological Blockade of α_2-Adrenoceptors Induces Reinstatement of Cocaine-Seeking Behavior in Squirrel Monkeys

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Converging evidence suggests a role for noradrenergic mechanisms in stress-induced reinstatement of cocaine seeking in animals. Yohimbine, an α_2-adrenoceptor antagonist, is known to be anxiogenic and induce stress-related responses in humans and animals. Here, we tested the ability of yohimbine to reinstate cocaine-seeking behavior and induce behavioral and physiological signs characteristic of stress in squirrel monkeys. Monkeys were trained to self-administer cocaine under a second-order schedule of i.v. drug injection. Drug seeking subsequently was extinguished by substituting saline for cocaine injections and omitting the cocaine-paired stimulus. The ability of yohimbine and the structurally distinct α_2-adrenoceptor antagonist RS-79948 to reinstate cocaine-seeking behavior was assessed by administering priming injections immediately before test sessions in which the cocaine-paired stimulus was either present or absent. Priming injections of yohimbine (0.1–0.56 mg/kg, i.m.) or RS-79948 (0.01–0.1 mg/kg, i.m.) induced dose-related reinstatement of cocaine-seeking behavior. The magnitude of yohimbine-induced reinstatement was similar regardless of the presence or absence of the cocaine-paired stimulus. Yohimbine also significantly increased salivary cortisol levels, a physiological marker of stress, as well as scratching and self-grooming, behavioral markers of stress in nonhuman primates. In drug interaction experiments, pretreatment with the α_2-adrenoceptor agonist clonidine (0.1–0.3 mg/kg, i.m.) dose-dependently inhibited yohimbine-induced reinstatement of cocaine seeking. In contrast, pretreatment with the dopamine receptor antagonist flupenthixol failed to inhibit yohimbine-induced reinstatement of cocaine seeking. The results show that pharmacological blockade of α_2-adrenoceptors can induce reinstatement of cocaine-seeking behavior and characteristic stress responses in squirrel monkeys, providing a potentially useful model of stress-induced relapse to drug seeking.
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INTRODUCTION

Although stress has been implicated as a risk factor in drug addiction and relapse (Kreek and Koob, 1998; Sinha, 2001), the mechanisms by which stress triggers persistent drug seeking are still poorly understood (Sinha, 2001). There is, however, a growing body of literature linking activation of noradrenergic transmission to the behavioral and physiological consequences of stress (Stanford, 1995; Bremner *et al*, 1996). Recent findings using rodent models of reinstated drug seeking suggest a potentially important role for the noradrenergic system in stress-induced relapse (Stewart, 2000; Shaham *et al*, 2003). In these models, animals typically are trained to self-administer a drug of abuse such as cocaine and, subsequently, drug seeking is extinguished by substituting vehicle for the drug administration. Once drug-seeking behavior is extinguished, pharmacological and nonpharmacological stimuli, including stressors, can be tested for their ability to reinstate the behavior. Using procedures such as these, the α_2-adrenoceptor agonist clonidine has been shown to inhibit footshock-induced reinstatement of cocaine seeking in rats (Erb *et al*, 2000). In addition, a mixture of β_1- and β_2-adrenoceptor antagonists betaxolol and ICI-118,551, when injected into the bed nucleus of the stria terminalis or the central nucleus of the amygdala, can inhibit footshock-induced reinstatement of cocaine-seeking behavior in this species (Leri *et al*, 2002). These results suggest that modulation of central noradrenergic systems may play a role in stress-induced reinstatement of cocaine-seeking behavior. Consistent with this possibility, the selective norepinephrine (NE) reuptake inhibitors such as nisoxetine and talsupram recently have been shown to induce partial reinstatement of cocaine-seeking behavior in monkeys (Platt *et al*, 2001). Further support for a role of the noradrenergic system in stress-induced relapse comes from human laboratory studies

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showing dysregulation of brain NE activity accompanied by anxiety and dysphoria during cocaine withdrawal (McDougle *et al*, 1994).

Yohimbine increases NE release and the firing rate of noradrenergic neurons by blocking α_2-adrenoceptors (Cooper *et al*, 1991). Yohimbine also has been shown to be anxiogenic and to induce stress-related responses in both humans and nonhuman primates (Charney *et al*, 1983; Albus *et al*, 1992). Yohimbine is used frequently as a pharmacological challenge to investigate noradrenergic functions in patients with stress and anxiety disorders (Charney *et al*, 1989; Southwick *et al*, 1993). Stine *et al* (2002) recently reported that yohimbine elevated drug craving and elicited opioid withdrawal symptoms in methadone-maintained patients. Based on these findings, we hypothesized that yohimbine might serve as a pharmacological stressor to induce reinstatement of drug seeking in monkeys. The present experiments investigated the ability of yohimbine to reinstate cocaine-seeking behavior and induce stress-related physiological and behavioral responses in squirrel monkeys. The pharmacological specificity of yohimbine-induced reinstatement was investigated in two ways. First, we tested whether the structurally distinct α_2-adrenoceptor antagonist RS-79948 (Milligan *et al*, 1997) would reinstate cocaine-seeking behavior in a manner similar to yohimbine. Second, we examined the ability of the selective α_2-adrenoceptor agonist clonidine and the selective dopamine receptor antagonist flupenthixol to attenuate yohimbine-induced reinstatement of cocaine seeking.

MATERIALS AND METHODS

Subjects

Adult squirrel monkeys (*Saimiri sciureus*) weighing 0.7–1.2 kg were housed individually in a climate-controlled vivarium, where they had unlimited access to water and received a nutritionally balanced diet of monkey chow (Teklad®, Madison, WI) supplemented with fresh fruit. A total of 14 monkeys were studied, with groups of four to seven monkeys serving as subjects in each experiment (see below). All animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and the 'Guide for the Care and Use of Laboratory Animals' of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH) 85-23, revised 1996. Research protocols were approved by the Harvard Medical School Institutional Animal Care and Use Committee.

Surgery

In experiments involving drug self-administration, indwelling venous catheters were implanted in eight monkeys using aseptic surgical procedures as described by Carey and Spealman (1998). Briefly, monkeys were anesthetized with isoflurane, and the catheter was passed by way of either a femoral or jugular vein to the level of the right atrium. The distal end of the catheter was then passed subcutaneously to the mid-scapular exit point. Catheters were flushed daily with 0.9% saline solution containing 200 U of heparin/ml and were sealed with stainless-steel obturators when not in use. Monkeys wore nylon-mesh jackets (Lomir Biomedical, Canada) at all times to protect the catheter.

Apparatus

Experiments on reinstatement of drug-seeking behavior were conducted in ventilated sound-attenuated chambers, which were equipped with white noise to mask external sounds. Within the chamber, monkeys were seated in Lexan chairs (Med Associates, St Albans, VT) facing a panel that was equipped with a response lever and colored stimulus lights above the lever. Catheters were connected to motor-driven syringe pumps located outside the chamber. Each operation of the pump delivered a 1-s infusion of 0.18 ml saline or cocaine solution into the catheter.

Observational studies were conducted in a ventilated, transparent Plexiglas arena (114 cm long $\times$ 122 cm wide $\times$ 213 cm high) located in a lighted room isolated from other animals (Platt *et al*, 2000). The arena was equipped with perches, suspended plastic chains, and a wood-chip foraging substrate to allow for the expression of a range of species-typical behaviors. A video camera and a videocassette recorder operated continuously during the observation session.

Cocaine Self-Administration and Extinction

Monkeys were trained to self-administer cocaine by pressing a lever under a second-order fixed interval (FI) fixed ratio (FR) schedule of i.v. drug injection identical to the schedule described by Khroyan *et al* (2000). Briefly, in the presence of a white light, completion of every 10th response during a 10-min FI resulted in a 2-s change in illumination from white to red. Completion of the first FR after expiration of the FI resulted in an i.v. injection of cocaine simultaneous with the onset of the 2-s red light (the cocaine-paired stimulus). A 60-s time out (TO) period, during which all lights were off and responses had no scheduled consequences, followed each cocaine injection. If the FR requirement was not completed within 8 min following the expiration of the FI, the component ended automatically without injection. Daily sessions ended after completion of five cycles of the second-order schedule (maximum of 90 min). Initially, the dose of cocaine was varied over a 3–10-fold range to determine the dose that maintained maximum rates of responding for each monkey. For six monkeys, 0.3 mg/kg/injection of cocaine maintained maximum response rates, whereas for two monkeys, 0.18 mg/kg/injection of cocaine maintained maximum response rates. These doses of cocaine were kept constant for the remainder of the study.

Once stable responding under the second-order schedule was maintained consistently, cocaine-seeking behavior was extinguished by substituting saline for cocaine and omitting all presentations of the 2-s stimulus. Extinction sessions were otherwise identical to those described above for cocaine self-administration. Extinction sessions were conducted daily until response rate declined and stabilized at $\leqslant$10% of the rate maintained by active cocaine self-administration (4–15 sessions depending on the subject).

Saline (0.2 ml/kg, i.m.) was injected before each self-administration and extinction session to habituate subjects to the i.m. injection procedures described below.

Reinstatement of Drug Seeking

Tests for priming-induced reinstatement of extinguished cocaine seeking were then begun once the criteria for extinction (see above) were satisfied. Reinstatement test sessions used procedures identical to those during cocaine self-administration except that only saline was available for self-administration. Except when noted below, response-contingent presentations of the cocaine-paired stimulus were restored during reinstatement test sessions because earlier studies showed that reinstatement of drug seeking induced by cocaine priming was greatest when priming injections were accompanied by restoration of the cocaine-paired stimulus (Spealman *et al*, 1999, 2004). Between reinstatement test sessions with different doses of a particular drug, extinction sessions were conducted for 3 or more days to ensure that monkeys maintained low rates of responding in the absence of cocaine self-administration. In addition, between experiments with different drugs, 10 or more sessions of i.v. cocaine self-administration were conducted so that high response rates could be re-established during active self-administration. Subsequently, four or more extinction sessions, in which response rates fell to $\leqslant$10% of the response rates maintained by cocaine self-administration, were conducted before testing another drug was begun. Our previous studies have shown that by periodically re-establishing and then extinguishing drug-seeking behavior between experiments, reinstatement of drug seeking can be reliably induced by cocaine priming over more than 2 years of testing (Khroyan *et al*, 2000).

Reinstatement tests with yohimbine (0.1–0.56 mg/kg), RS-79948 (0.01–0.1 mg/kg), and their respective vehicles were conducted by administering drugs i.m. immediately before the reinstatement test sessions. The doses of yohimbine used in these experiments were selected on the basis of previous studies demonstrating yohimbine-induced behavioral and physiological responses characteristic of stress and anxiety in humans and nonhuman primates (Charney *et al*, 1983; Harris and Newman, 1987). The doses of RS-79948 were selected on the basis of its relative potency compared to yohimbine as determined by *in vitro* receptor binding assays and anxiogenic effects in rats (Milligan *et al*, 1997; White and Birkle, 2001). To investigate the possible interaction of the cocaine-paired stimulus and yohimbine on reinstatement of cocaine-seeking behavior, reinstatement tests were conducted with yohimbine (0.3 mg/kg, i.m.) in four monkeys both in the presence and absence of response-contingent presentations of the cocaine-paired stimulus. The order of doses tested within each drug was varied across monkeys.

Additional experiments investigated the effects of the α_2-adrenoceptor agonist clonidine (0.1 and 0.3 mg/kg) and the dopamine receptor antagonist flupenthixol (0.03 mg/kg) on yohimbine-induced reinstatement of cocaine-seeking behavior. The doses of clonidine and its pretreatment time were selected on the basis of previous studies showing that clonidine antagonized the effects of yohimbine on operant behavior in squirrel monkeys (McKearney, 1983; Khroyan *et al*, 2000). The dose of flupenthixol and its pretreatment time were selected on the basis of previous study showing that 0.03 mg/kg flupenthixol produced maximal inhibition of cocaine-induced reinstatement of drug seeking in squirrel monkeys (Khroyan *et al*, 2000). Clonidine or its vehicle was administered i.m. 10 min before priming with yohimbine (0.3–1.0 mg/kg, i.m.), and flupenthixol or its vehicle was administered i.m. 1 h before priming with yohimbine (0.3–1.0 mg/kg, i.m.). The order of drug testing was varied across monkeys.

Observation Study

After habituation to the observational arena, handling, and injection procedures, 30-min observational sessions were conducted daily, during which the animal's behavior was videotaped continuously. Drug test sessions were conducted once or twice per week, with saline control sessions on intervening days. Yohimbine (0.1–0.56 mg/kg, i.m.) was administered 5 min prior to placing the subject in the observation arena.

Scoring of videotapes was conducted by two observers, who were trained in the use of the behavioral scoring system described by Platt *et al* (2000), but were not informed about the drugs under investigation. Before beginning the study, each observer underwent at least 20 h of training until they reached an interobserver reliability criterion of 90% or more based on percent agreement scores. The behavioral scoring system included 10 categories (Table 1), which were scored by recording the presence or absence of each behavior in 15-s intervals during three 5-min observation periods across the session (0–5, 7–12, 24–29 min). Frequency scores were calculated from these data as the proportion of 15-s intervals in which a particular behavior was observed.

Salivary Cortisol Collection and Assay

The effects of yohimbine on circulating levels of cortisol, a physiological stress marker, were measured in saliva. Saliva was collected noninvasively in the monkey's homecage

Table 1 Behavioral Categories

Locomotion	Any two or more directed steps in the horizontal and/or vertical plane; also active
Object exploration	Any tactile or oral manipulation of features of the observational arena
Foraging	Sweeping and/or picking through wood-chip substrate
Self-grooming	Picking, scraping, spreading, or licking of fur
Scratching	Rapid movement of digits through fur in a rhythmic motion
Visual scanning	Directed high and/or head movements, usually from a sitting position
Rest posture	Species-typical posture: crouched on hind legs, hunched back, upper body
Vocalization	Any utterance including chirps, twitters, peeps, etc
Other	Any notable behavior not defined above (eg yawn, sneeze)

Adapted from Platt *et al* (2000).

between 0830 and noon using the techniques described by Tiefenbacher *et al* (2003). Briefly, a device consisting of a PVC pole with a dried cotton dental rope (3/8 in diameter, 5 cm long, Richmond Dental) flavored with concentrated Kool-Aid® solution was given to a monkey. After the monkey chewed and dampened the dental rope with saliva, the device was retrieved, and the dental rope was placed in a salivette tube (Sarstedt, Germany). The saliva was extracted by centrifugation at 1000 rpm for 12 min and frozen at −40°C for later cortisol assay. Cortisol levels in saliva were determined in duplicate using a commercially available radioimmunoassay kit (Diagnostic Products Corporation, CA). The intra-assay coefficient of variation for cortisol was 3.9%.

On the first day of testing, half of the monkeys received an injection of yohimbine (0.3 mg/kg, i.m.), and the remainder received an equivalent volume of vehicle. On the second day, the monkeys that previously received yohimbine were tested with vehicle, and the monkeys that previously received vehicle were tested with yohimbine. During each test day, saliva samples were collected 30 min before yohimbine or vehicle injection as well as every 30 min after the injection for the next 2 h.

Data Analyses

Drug-seeking behavior was measured by determining the total number of lever presses during each test session. Lever pressing during extinction (before reinstatement test sessions) was averaged across all sessions in each experiment and used in statistical analysis. Data from reinstatement tests were analyzed by Friedman repeated measures analysis of variance (ANOVA) followed by a *post hoc* comparison using either Dunnett's or Student–Newman–Keuls test based on rank, as appropriate. In observational experiments, individual scores for each behavior were averaged over the three 5-min observation periods because no systematic trends were observed across the three observation periods of the test session (as determined by separate repeated measures ANOVAs). Individual scores were then averaged across subjects to provide group means. Dunnett's test was used to assess statistical significance of treatment effects on each behavior. The effect of yohimbine on salivary cortisol levels was assessed by a one-way repeated measures ANOVA followed by a *post hoc* comparison using Dunnett's test.

Drugs

Cocaine hydrochloride and cis-(z)-flupenthixol dihydrochloride were dissolved in 0.9% saline solution. Yohimbine hydrochloride and RS-79948 hydrochloride were dissolved in sterile water. RS-79948 was purchased from Tocris (Ellisville, MO). Other drugs were purchased from Sigma (St Louis, MO).

RESULTS

Cocaine Self-Administration and Extinction

Self-administration of cocaine (0.18 or 0.3 mg/kg/injection; see Materials and methods) maintained high levels of responding in all eight subjects (averaging 1944 ± 350 (SEM) responses per session) under the second-order schedule of i.v. drug injection. During extinction, in which saline was substituted for cocaine and the cocaine-paired stimulus was omitted, responding declined and stabilized at low levels (averaging 64 ± 16 (SEM) responses per session).

Reinstatement of Cocaine-Seeking Behavior by Yohimbine and RS-79948

Priming injections of yohimbine (0.1–0.56 mg/kg, i.m.) resulted in dose-related reinstatement of cocaine-seeking behavior (Figure 1a). ANOVA revealed a significant main effect of treatment ($\chi^2(3) = 11.057$, $P = 0.011$). Subsequent pairwise comparison showed that the number of drug-seeking responses induced by both 0.3 and 0.56 mg/kg yohimbine were significantly different from the number of responses after injection of vehicle ($P < 0.05$; Dunnett's test). The dose of yohimbine that induced maximum reinstatement of cocaine seeking was 0.3 mg/kg in two subjects and 0.56 mg/kg in four subjects. A final subject showed significant reinstatement of responding (257 responses) only when the dose of yohimbine was increased further to 1.0 mg/kg (not shown).

Like yohimbine, RS-79948 (0.01–0.1 mg/kg, i.m.) induced a dose-related reinstatement of cocaine-seeking behavior (Figure 1b). ANOVA revealed a significant main effect of treatment ($\chi^2(3) = 9.686$, $P = 0.021$). Pairwise comparison revealed that priming injections of 0.03 and 0.1 mg/kg RS-79948 induced significant reinstatement of cocaine-seeking behavior compared to vehicle injection ($P < 0.05$; Dunnett's test). As illustrated in Figure 1, the maximum reinstatement of cocaine seeking induced by RS-79948 was similar to the maximum reinstatement induced by yohimbine (compare Figure 1a with Figure 1b). The dose of RS-79948 that induced maximum reinstatement of cocaine-seeking behavior was 0.01 mg/kg for one subject, 0.03 mg/kg for four subjects, and 0.1 mg/kg for two subjects. Averaged across the six monkeys that received both yohimbine and RS-

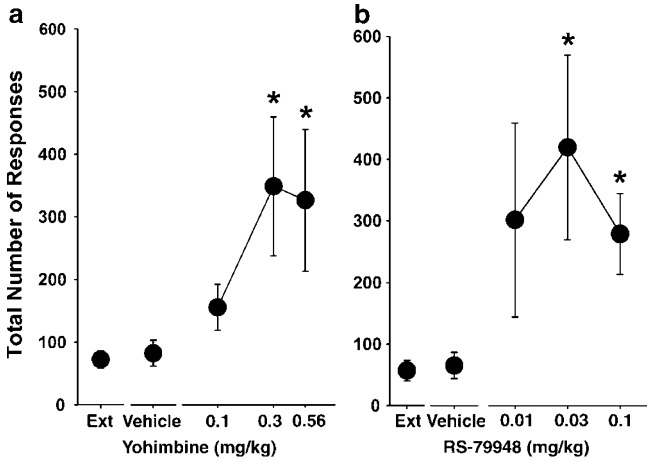

Figure 1 Reinstatement of cocaine-seeking behavior induced by yohimbine (a) and RS-79948 (b). Data are means ± SEM ($n = 7$). Ext: extinction. Average session length (± SEM) was 74 ± 3 min (a) and 75 ± 3 min (b). *$P < 0.05$ as compared with the vehicle treatment.

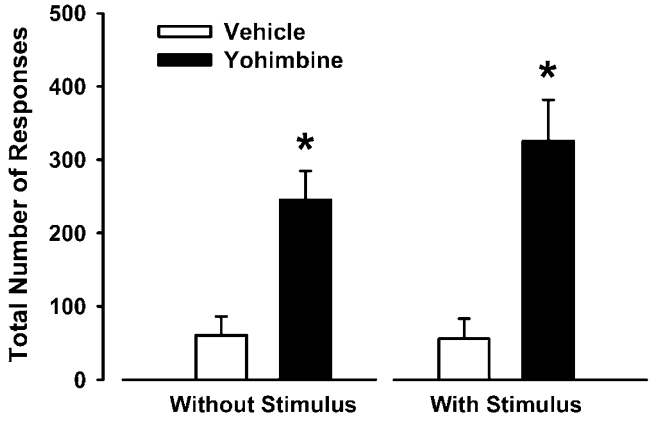

Figure 2 Reinstatement of cocaine-seeking behavior induced by yohimbine in the presence and absence of the cocaine-paired stimulus. Data are means ± SEM ($n = 4$). Average session length (± SEM) was 76 ± 1 min. *$P < 0.05$ as compared with vehicle treatment in each condition.

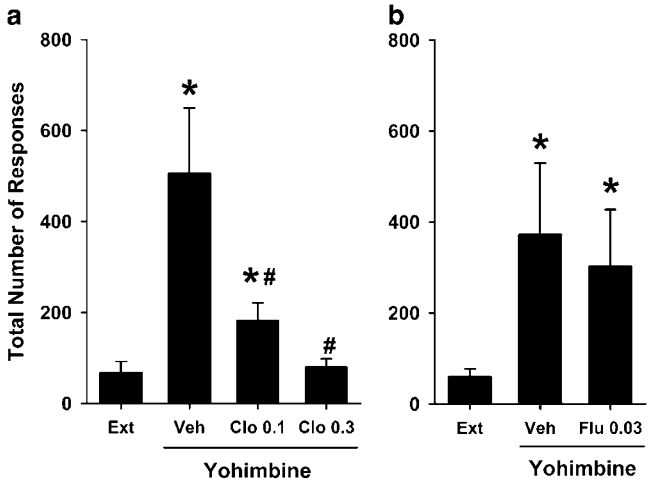

Figure 3 Attenuation of yohimbine-induced reinstatement of cocaine-seeking behavior by clonidine (a) but not flupenthixol (b). Data are means ± SEM ($n = 4$–5). Ext: extinction; Clo: clonidine; Flu: flupenthixol. Average session length (± SEM) was 74 ± 2 min for panel (a) and 74 ± 3 min for panel (b). *$P < 0.05$ as compared with extinction. #$P < 0.05$ as compared with vehicle treatment.

79948, RS-79948 was 18 times more potent than yohimbine in inducing maximum reinstatement of cocaine seeking.

Priming with yohimbine (0.3 mg/kg, i.m.) induced reinstatement of cocaine seeking both in the presence and absence of response-contingent presentations of the cocaine-paired stimulus (Figure 2). ANOVA revealed a significant main effect of drug treatment ($\chi^2(3) = 10.231$, $P = 0.017$). Pairwise comparison revealed that priming injections of 0.3 mg/kg yohimbine induced significant reinstatement of cocaine seeking compared to vehicle injection regardless of whether or not the cocaine-paired stimulus was present ($P < 0.05$; Student–Newman–Keuls test). Although the magnitude of reinstatement of cocaine seeking induced by yohimbine was slightly higher when response-contingent presentations of the cocaine-paired stimulus were restored, this difference did not approach statistical significance ($P > 0.05$; Student–Newman–Keuls test).

Effect of Clonidine and Flupenthixol on Yohimbine-Induced Reinstatement of Cocaine Seeking

Clonidine dose-dependently attenuated the reinstatement of cocaine-seeking behavior induced by maximally effective doses of yohimbine (Figure 3a). Before the reinstatement test sessions, monkeys received vehicle or either 0.1 or 0.3 mg/kg clonidine i.m. 10 min prior to priming injection of yohimbine (0.3 mg/kg in three monkeys, 0.56 and 1.0 mg/kg in one monkey each). Analysis of cocaine-seeking behavior induced by yohimbine with or without clonidine pretreatment revealed a significant main effect of treatment ($\chi^2(3) = 13.560$, $P = 0.004$). Subsequent pairwise comparisons (Student–Newman–Keuls test) showed that the number of responses induced by yohimbine after vehicle pretreatment was significantly greater than the number of responses during extinction. Furthermore, pretreatment with 0.1 mg/kg clonidine significantly attenuated yohimbine-induced reinstatement of cocaine-seeking behavior ($P < 0.05$), but the number of responses was still significantly greater than the number of responses during extinction ($P < 0.05$). Pretreatment with a three-fold higher dose of clonidine, however, blocked yohimbine-induced reinstatement of cocaine seeking nearly completely, and the number of responses was not significantly different from the number of responses during extinction ($P > 0.05$).

The dopamine receptor antagonist flupenthixol (0.03 mg/kg, i.m.) did not significantly alter the reinstatement of cocaine-seeking behavior induced by maximally effective doses of yohimbine (Figure 3b). Before the reinstatement test sessions, monkeys received vehicle or 0.03 mg/kg flupenthixol i.m. 1 h prior to priming injection of yohimbine (0.3 mg/kg in two monkeys, 0.56 and 1.0 mg/kg in one monkey each). ANOVA revealed a significant main effect of treatment ($\chi^2(2) = 6.500$, $P = 0.042$), and subsequent pairwise comparisons (Student–Newman–Keuls test) showed that the number of responses induced by yohimbine was significantly greater than the number of responses during extinction regardless of pretreatment ($P < 0.05$). In addition, the number of responses induced by yohimbine after flupenthixol pretreatment did not differ significantly from the number of responses induced by yohimbine after vehicle pretreatment ($P > 0.05$). Higher doses of flupenthixol were not tested because motoric impairments and ataxia, which sometimes were observed at the end of reinstatement test. Flupenthixol previously has been shown to produce nonselective suppression of operant behavior at doses ⩾ 0.03 mg/kg in squirrel monkeys (Spealman, 1990).

Effects of Yohimbine on Observed Behavior

Yohimbine (0.3 and 0.56 mg/kg) induced significant increases in self-grooming and scratching. As shown in Figure 4, self-grooming behavior increased maximally from an average control score of 1.1 ± 0.6 to 3.3 ± 0.8 after administration of yohimbine (Dunnett's test: $q = 3.02$, $P < 0.05$). Scratching increased maximally from an average control score of 0.5 ± 0.2 to 3.4 ± 1.0 after administration of yohimbine (Dunnett's test: $q = 3.95$, $P < 0.05$). In contrast,

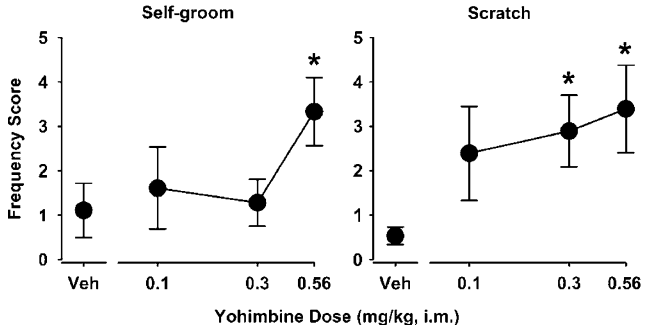

Figure 4 Frequency scores for self-groom and scratch engendered by yohimbine. Data are means ± SEM ($n = 6$). *$P < 0.05$ as compared with vehicle control values.

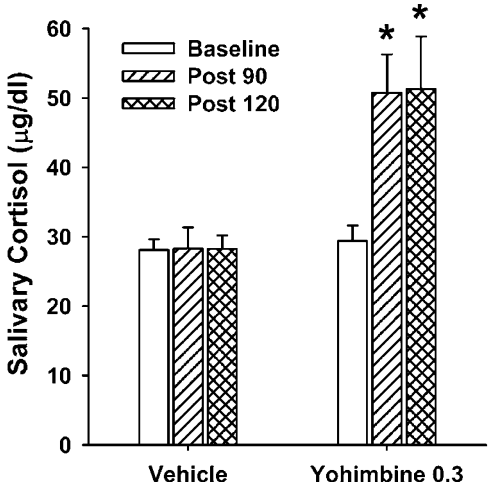

Figure 5 Salivary cortisol levels 30 min before and 90 and 120 min after yohimbine and vehicle administration. Data are means ± SEM ($n = 6$). *$P < 0.05$ as compared with baseline levels.

yohimbine did not significantly alter the frequency of any other observable behaviors including locomotion, object exploration, foraging, visual scanning, vocalization, or resting (data not shown).

Effects of Yohimbine on Salivary Cortisol Levels

Salivary cortisol levels were used as a physiological marker for stress induced by yohimbine treatment. Due to behavioral agitation after yohimbine (0.3 mg/kg, i.m.), we were not able to collect saliva from three of the six subjects at 30 and/or 60 min after yohimbine treatment. Therefore, statistical comparisons were conducted using salivary cortisol levels collected at 30 min before (baseline level) and 90 and 120 min after yohimbine or its vehicle treatment (Figure 5). ANOVA revealed a significant effect of treatment ($F_{(2,10)} = 11.383$, $P = 0.003$) after yohimbine injection, and subsequent pairwise comparisons (Dunnett's test) showed that salivary cortisol levels were significantly higher at 90 and 120 min after yohimbine treatment compared to baseline levels ($P < 0.05$). Salivary cortisol levels did not significantly change from baseline levels after vehicle injection ($P > 0.05$).

DISCUSSION

The present study demonstrates that yohimbine can induce significant reinstatement of cocaine-seeking behavior as well as physiological and behavioral indices of stress in squirrel monkeys. Pharmacological analysis suggests that blockade of α_2-adrenoceptors plays a pertinent role in the ability of yohimbine to reinstate cocaine seeking and that dopaminergic mechanisms do not contribute critically to yohimbine-induced reinstatement of cocaine-seeking behavior.

Stress has been implicated frequently as a factor underlying relapse to drug use among human drug abusers (Kreek and Koob, 1998; Sinha, 2001). Most clinical studies of the relationship between stress and relapse rely on either retrospective or prospective interviews, which are subject to the failures and distortion of recall (Hall *et al*, 1991; McKay *et al*, 1995). In rodent models of drug relapse, inescapable electric footshock has been used as a prototype stressor to reinstate drug-seeking behavior (Shaham *et al*, 2000). The experimental utility of electric footshock not withstanding, efforts to use other stressors that might be more analogous to the types of stress experienced by people have been limited (Shalev *et al*, 2002; Spealman *et al*, 2004). Although food deprivation in rats has been shown to reinstate drug-seeking behavior under some conditions (Shalev *et al*, 2000, 2001), it does not do so consistently under others (Carroll, 1985).

Given that yohimbine induces effects characteristic of stress and anxiety in humans (Grunhaus *et al*, 1989) as well as nonhuman primates (Carey *et al*, 1992; Coplan *et al*, 1992), we explored the ability of yohimbine to reinstate extinguished cocaine-seeking behavior at doses that also induce stress-associated physiological and behavioral changes in monkeys. Our results show that yohimbine reinstates cocaine-seeking behavior. Yohimbine also produced pronounced increases in salivary cortisol levels and displacement behaviors typically precipitated by stress and anxiety in monkeys (Schino *et al*, 1996; Castles *et al*, 1999). These data, to the least of our knowledge, are the first to show that a pharmacological stressor can reinstate cocaine-seeking behavior in a nonhuman primate. A recent human study showed that doses of yohimbine comparable to those used here induced drug craving and exacerbated opioid withdrawal symptoms in methadone-maintained patients (Stine *et al*, 2002).

Although yohimbine has a high affinity and selectivity at α_2-adrenoceptors, it also interacts with other receptors such as α_1-adrenoceptors, 5-HT_{1A} receptors, D_2 receptors, and imidazoline receptors at high concentrations (Scatton *et al*, 1980; Doxey *et al*, 1984; Boyajian and Leslie, 1987; Winter and Rabin, 1992). To explore the pharmacological specificity of yohimbine-induced reinstatement of drug seeking, we first examined the degree to which the α_2-adrenoceptor agonist clonidine could reverse yohimbine-induced reinstatement of responding. Our results show that clonidine dose-dependently attenuated yohimbine-induced reinstatement of cocaine seeking, with significant effects observed at a dose as low as 0.1 mg/kg. It is unlikely that this attenuation of yohimbine's effect by clonidine was the result of a generalized suppression of behavior induced by the combined drugs. In this regard, McKearney (1983)

showed that combined treatment with clonidine and yohimbine at doses identical to those used in our study did not induce marked changes in lever pressing by squirrel monkeys responding under operant schedules of either food presentation or electric shock titration. We further demonstrated that another α_2-adrenoceptor antagonist RS-79948, which has very low affinity at α_1-adrenoceptors, 5-HT_{1A} receptors, D_2 receptors, and imidazoline receptors (Clark *et al*, 1989; Hume *et al*, 1996; Milligan *et al*, 1997), induced reinstatement of cocaine-seeking behavior equivalent to that induced by yohimbine. Collectively, these results suggest that yohimbine-induced reinstatement of cocaine-seeking behavior is mediated by its interaction at α_2-adrenoceptors.

It is also possible that a generalized stimulant-like effect of yohimbine contributed in some way to its ability to reinstate cocaine-seeking behavior. In squirrel monkeys, however, yohimbine at doses comparable to those we tested only decreased operant responding maintained by food presentation (McKearney, 1983). Furthermore, in our observational study yohimbine did not increase locomotion or decrease resting. It is also unlikely that yohimbine induced reinstatement of cocaine-seeking behavior by mimicking either discriminative stimulus or reinforcing effects of cocaine. In this regard, yohimbine does not reproduce the discriminative stimulus effects of cocaine in rats and pigeons (Wood *et al*, 1985; Johanson and Barrett, 1993) and induces conditioned place aversion rather than conditioned place preference in rats (File, 1986).

In our initial experiments with yohimbine, response-contingent presentations of the cocaine-paired stimulus were restored during test sessions. Therefore, we examined the possibility that yohimbine might induce reinstatement of cocaine seeking by enhancing cocaine cue-induced reinstatement of drug seeking. Liu and Weiss (2002), for example, reported that stress and drug cues can interact to augment reinstatement of drug-seeking behavior in rats trained to self-administer alcohol. It is unlikely, however, that the effects of yohimbine in our study reflected primarily an augmentation of drug cue-induced reinstatement of drug seeking, because priming with yohimbine induced comparable reinstatement of cocaine seeking both in the presence and absence of the cocaine-paired stimulus.

Similar neurobiological mechanisms may underlie yohimbine- and electric footshock-induced reinstatement of drug seeking. For example, clonidine has been shown to attenuate footshock-induced reinstatement of drug seeking in rats (Erb *et al*, 2000), just as it attenuates yohimbine-induced reinstatement of drug seeking in monkeys. In addition, Shaham and Stewart (1996) reported that dopaminergic mechanisms, which are thought to be critically involved in drug priming-induced reinstatement (Self and Nestler, 1998; Khroyan *et al*, 2000), played only a permissive role in footshock-induced reinstatement of drug seeking in rats. Similarly, in our study the dopamine receptor antagonist flupenthixol (0.03 mg/kg, i.m.) did not significantly attenuate yohimbine-induced reinstatement of cocaine seeking at a dose previously shown to produce maximal inhibition of cocaine-induced reinstatement of drug seeking under identical testing conditions (cf Khroyan *et al*, 2000). Although additional studies are required to investigate more fully the potential role of dopamine in yohimbine-induced reinstatement of cocaine seeking, our findings provide no evidence that the dopaminergic system plays a critical role in yohimbine-induced reinstatement of cocaine seeking. Collectively, these findings suggest a common role of α_2-adrenoceptor mechanisms in the reinstatement of drug-seeking behavior induced by different types of stressors. They also suggest that α_2-adrenoceptors may be viable targets for developing pharmacotherapies to counteract the relapse-inducing consequences of stress.

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