Effects of Saline Substitution on Responding and Plasma Corticosterone in Rats Trained to Self-Administer Different Doses of Cocaine

RACHEL L. PELTIER, GLENN F. GUERIN, NANDAKUMAR DORAIRAJ, and NICK E. GOEDERS

Departments of Pharmacology and Therapeutics (R.L.P., G.F.G., N.D., N.E.G.) and Psychiatry (N.E.G.), Louisiana State University Health Sciences Center, Shreveport, Louisiana

Received March 23, 2001; accepted June 12, 2001

This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

Research from our laboratory has explored the role of the hypothalamo-pituitary-adrenal (HPA) axis in cocaine reinforcement. These experiments were designed to determine the involvement of the HPA axis in extinction. Male Wistar rats were trained to self-administer cocaine [0.125, 0.25, or 0.5 mg/kg/infusion (inf)] and food pellets (45 mg) under a multiple, alternating schedule of reinforcement. When self-administration was stable, saline was substituted for cocaine. Blood samples were taken at the end of the sessions following cocaine self-administration, the first exposure to saline substitution (first); and once the criteria for extinction were met (final). Plasma corticosterone was measured using radioimmunoassays. Although there was a significant increase in the number of infusions obtained during the first saline substitution test by rats trained with 0.5 mg/kg/inf of cocaine, there was a decrease in infusions received when 0.125 mg/kg/inf of cocaine was tested. Following repeated exposure to the extinction conditions, responding by rats trained to self-administer all three doses of cocaine was decreased to similar levels. In addition, there were significant differences in plasma corticosterone in rats trained with different doses of cocaine. Lever-pressing behavior and plasma corticosterone varied during extinction in relation to the training dose of cocaine and according to whether the rats had been exposed to single or repeated extinction testing. These data are discussed in terms of the potential difficulties involved in interpreting the effects of compounds intended to reduce drug reinforcement.

There has been a substantial amount of published literature devoted to the drug self-administration methodology in animals as an experimental model of human drug abuse (Schuster and Johanson, 1974; Thompson and Ulna, 1977; Griffiths et al., 1980; Johanson and Schuster, 1980). More specifically, cocaine can serve as a potent reinforcer in humans as well as nonhuman animals (Johanson, 1978; Fischman, 1987; Woods et al., 1987).

One goal for investigating drug self-administration in animals is to identify pharmacological agents useful for the treatment of addiction in humans. One procedure used for these purposes involves comparing the effects of potential pharmacotherapeutic agents with the effects of substituting saline for the self-administered drug (i.e., extinction tests). Extinction is operationally defined as “withholding a reinforcer for a previously reinforced response that causes a decline in the level of that response” (Grant and Evans, 1994). In terms of self-administration, extinction occurs when vehicle (usually saline) replaces the drug that normally serves as the reinforcer. While the resulting behavioral effect that typically defines extinction is a decrease in the level of the response, extinction can also produce other effects. For example, an initial temporary increase in the level and intensity of the response, an “extinction burst”, can also be observed (Skinner, 1938).

If an effective pharmacotherapy for the treatment of drug addiction involves a compound that decreases the reinforcing efficacy of the abused substance then responding should begin to resemble that observed during extinction (i.e., saline substitution). One of the first investigators to use this procedure was Wise et al. (1978), who showed that pimozide, a dopamine receptor antagonist, produced a pattern of responding similar to extinction responding. Based on this finding, it was determined that pimozide decreased the “rewarding impact of food and other hedonic stimuli”.

The effects of pimozide on reinforcement can also be partially explained by the ability of dopamine antagonists to produce motor impairment (Phillips and Fibiger, 1979). However, comparing the effects of a treatment on drug-main-
tained responding to responding observed during extinction remains a widely accepted research tool. Despite this, there is a relatively small database specifically defining the “typical extinction curve” in drug self-administration studies.

More recently, a large quantity of extinction data has been published in manuscripts investigating animal models of relapse (reinstatement) (Gerber and Stretch, 1975; de Wit and Stewart, 1981, 1983; Erb et al., 1996, 1998; Ahmed and Koob, 1997; Mantsch and Goeders, 1999a; Lynch and Carroll, 2000). These models consist of training an animal to self-administer a drug followed by a period of extinction. Once self-administration is extinguished, drug-seeking is evaluated by measuring the reinstatement of the previously extinguished behavior. Unfortunately, a majority of these experiments either do not include the initial few saline substitution test days in the published report or only examine extinction using a single dose of the self-administered drug.

Our laboratory has demonstrated an important role for the hypothalamo-pituitary-adrenal (HPA) axis in cocaine reward. More specifically, we have demonstrated that corticosterone, a stress-related hormone, is involved in cocaine reinforcement. For example, the acquisition of cocaine self-administration does not occur unless plasma corticosterone is increased above a critical threshold (Goeders and Guerin, 1996a). In addition, ongoing, low-dose cocaine self-administration is reduced by ketocanazole, a drug that decreases the synthesis of corticosterone and serves as a glucocorticoid receptor antagonist (Goeders et al., 1998). Similarly, pretreatment with CP-154,526, a corticotropin-releasing-hormone type 1 (CRH1) receptor antagonist, also decreases cocaine self-administration (Goeders and Guerin, 2000). It is noteworthy to mention here that in both of the previously cited manuscripts (Goeders et al., 1998, 2000), the authors mention the importance of repeated exposure to saline substitution probes prior to testing.

We have recently shown that corticosterone is also critical for the stress- and cue-induced reinstatement of extinguished cocaine-seeking behavior (i.e., reinstatement; Mantsch and Goeders, 1999a,b; Clampitt et al., 2000; Goeders et al., 2000). In these reports, we demonstrated that while ketocanazole does not block the cocaine-induced reinstatement of cocaine-seeking behavior, it does block stressor- and cue-induced cocaine reinstatement.

Therefore, based on the work from our laboratory linking plasma corticosterone to cocaine reinforcement, we thought that it was reasonable to expect that the HPA axis might also be involved in the extinction process itself. Therefore, the following experiments were designed to examine the behavioral effects of saline substitution probes in rats that had been trained to self-administer different doses of cocaine. We also compared the effects of the treatments on plasma corticosterone.

Materials and Methods

Thirty-five male Wistar rats (Harlan Sprague-Dawley), 80 to 100 days old at the start of the experiments, were used. Eight rats were trained to self-administer 0.125 mg/kg/infusion of cocaine, 16 rats were trained with 0.25 mg/kg/infusion, and 11 rats were trained with 0.5 mg/kg/infusion. Rats were housed singly in cages equipped with a laminar flow unit and an air filter in a temperature- and humidity-controlled American Association for the Accreditation of Laboratory Animal Care-accredited animal care facility on a reversed 12-h light/dark cycle (lights on at 6:00 PM) with free access to water. Rats were allowed free access to food until their free-feeding body weights increased to approximately 390 to 400 g. These rats were subsequently maintained at 85 to 90% of their free-feeding body weights by presentations of food pellets (45 mg; P. J. Noyes Co., Inc., Lancaster, NH) during the behavioral sessions and/or by supplemental postsession feeding (Purina Rat Chow; Purina, St. Louis, MO) throughout the course of the experiments. All procedures were carried out in accordance with the National Institutes of Health Principles of Laboratory Animal Care (NIH publication 85-23) and were approved by the Louisiana State University Health Sciences Center-Shreveport Institutional Animal Care and Use Committee.

Rats were implanted with chronic indwelling jugular catheters under pentobarbital anesthesia (50 mg/kg i.p.) with methylamphetamine nitrate pretreatment (10 mg/kg i.p.) using previously reported procedures (Koob and Goeders, 1989; Goeders and Guerin, 1996a,b, 2000). Briefly, the catheter (0.012 i.d. × 0.025 o.d., silastic tubing) was inserted into the right posterior facial vein and terminated just outside the right atrium. The catheter was anchored to tissue in the area and continued subcutaneously to the back where it exited just posterior to the scapulae through a marlex mesh/dental acrylic/22-gauge guide cannula (Plastics One, Roanoke, VA) assembly that was implanted under the skin for leash attachment. Rats were allowed a minimum of 4 days to recover from the surgical procedure. A stainless-steel spring leash (Plastics One) was attached to the guide cannula assembly and to a counterbalanced fluid swivel suspended above the test chamber, which allowed for relatively unrestricted movement. The patency of the catheters was tested once weekly immediately following a behavioral session. If blood could be drawn through the catheter then it was considered patent. If not, the rat was injected via the catheter with methohexital sodium (1.5 mg i.v.). An immediate light anesthesia indicated that the catheter was patent.

Experimental test chambers consisted of standard plastic and stainless steel sound-attenuating operant chambers (MED Associates, Inc., St. Albans, VT). Each chamber was equipped with two response levers (MED Associates, Inc.) mounted on either side of a food pellet dispenser located on one wall of the chamber. A stimulus light was mounted above each lever. The chambers were also equipped with an exhaust fan that supplied ventilation and white noise. An IBM-compatible personal computer and interface system (MED Associates, Inc.) was used to program the procedures and collect the data.

Rats were trained to respond under a multiple, alternating schedule of food reinforcement and cocaine self-administration (Goeders et al., 1998, 2000). During the food component of the schedule, the stimulus light located above the food response lever was illuminated to indicate the availability of food reinforcement. Initially, each deprivation of the food lever resulted in the delivery of a food pellet (45 mg; P. J. Noyes Co., Inc.). A 25-s timeout followed the delivery of each pellet. During this time-out, the stimulus light was turned off and responses on the food lever were recorded but had no programmed consequences. In addition, responding on the other (i.e., cocaine) lever during the food component had no programmed consequences. The response requirement for the food lever was gradually increased to a fixed ratio 10 (FR10) schedule whereby 10 responses were required for food presentation. Following 15 min of access to food, all stimulus lights in the chamber were turned off for a 1-min time-out. Following the time-out, the stimulus light above the cocaine lever was illuminated to indicate the availability of cocaine. Initially, each lever response resulted in an infusion of cocaine (0.125, 0.25, or 0.5 mg/kg/infusion in 200 μl of 0.9% NaCl delivered over 5.6 s). A 20-s timeout period followed each infusion. The response requirement for cocaine was gradually increased to FR4. After 15-min access to cocaine and another 1-min time-out, the rats were again allowed 15-min access to the food component of the schedule. Access to food and cocaine alternated in this manner during the 2-h behavioral session. The availability of either food or

at ASPET Journals on October 17, 2017 jpet.aspetjournals.org Downloaded from
cocaína as the first behavioral component alternated daily. Stable baselines of responding occurred when the total number of cocaine and food reinforcers, as well as the number of reinforcers obtained during each 15-min bin, varied by less than 10% for three consecutive sessions.

On extinction probe test days, a saline vehicle syringe was substituted for the cocaine syringe normally present. Therefore, responses on the “cocaína” lever only resulted in infusions of saline. Cocaine was made available during the next session, and subsequent extinction probes were not conducted until the criteria for stable self-administration were met once again. Extinction was trained during discrete trials (typically on Tuesdays and Fridays) as described above, rather than during consecutive sessions, to more closely resemble the effects of the acute treatment with a test compound during self-administration. The rats were presented with these extinction probes until stable, reproducible, “extinction-like” behavior was observed. Extinction-like behavior was defined as a greater than 50% decrease in lever pressing compared with that observed during baseline self-administration with less than 20% variability in rates and patterns of responding.

Plasma corticosterone measurements were conducted following pretreatment with vehicle after the animals had met the stability criteria for cocaine/food self-administration and following the saline substitution extinction probes. Plasma samples were taken at the conclusion of the behavioral test sessions. Plasma corticosterone was determined by specific radioimmunoassay using the ImmuChem double antibody [125I]corticosterone kit (ICN Biomedicals, Cleveland, OH). Blood was obtained via the implanted catheters or, if necessary, collected from the tail following light anesthesia with methohexital sodium (5 mg i.v.; Goeders and Guerin, 1996b). It has been demonstrated by Saphier et al. (1993) that there is no difference in plasma corticosterone measured using these two methods of blood collection, provided that the samples are obtained quickly (i.e., <2 min).

Cocaine was obtained from the National Institute on Drug Abuse (Research Triangle Park, NC) and was dissolved in heparinized (2.5 units/ml) 0.9% saline. Cocaine was self-administered at doses of 0.125, 0.25, and 0.5 mg/kg/infusion and was delivered in a 200-μl volume over 5.6 s.

Data collected included the total number of infusions and food pellets obtained per session as well as the number delivered during each of the four components (i.e., bins) of the multiple schedule. Significance of the differences between the various treatments was determined with an analysis of variance (ANOVA). Two-way ANOVAs were performed on the total infusion data as well as the total food delivery data (dose × treatment), on the bin data (treatment × bin), and on the corticosterone data (dose × treatment). Post hoc analyses were performed using a Tukey’s pairwise comparisons test.

**Results**

Once stable cocaine self-administration was observed, saline was repeatedly substituted for cocaine until responding was reduced to less than 50% of baseline (Fig. 1). There were no statistically significant differences in the total number of sessions required to reach the criteria for extinction [F(2,30) = 0.701, p = 0.50], depending on the dose of cocaine that served as the reinforcer during acquisition and maintenance (i.e., 0.125, 0.25, or 0.5 mg/kg/inf). In general, however, more sessions were required to reach the extinction criteria for the rats trained with the highest dose of cocaine tested (i.e., 0.5 mg/kg/inf) than with the two lower doses. To demonstrate the differences in the development of successful extinction among rats trained with different doses of cocaine, Fig. 2 depicts extinction data from three representative rats; one rat that was trained with 0.125 mg/kg/inf of cocaine, one rat that was trained with 0.25 mg/kg/inf of cocaine, and one rat that was trained with 0.5 mg/kg/inf of cocaine.

Remarkably, as indicated in Fig. 2, the different training doses of cocaine produced very different behavioral responses during the first exposure to saline substitution (Fig. 3). A two-way ANOVA (saline exposure × dose) revealed a significant main effect of saline exposure [F(2,99) = 29.713, p < 0.001] as well as a dose × saline exposure interaction [F(4,99) = 4.848, p = 0.001]. When these data were analyzed according to cocaine dose, a one-way ANOVA (saline exposure) indicated a significant effect of saline exposure at each dose of cocaine tested: 0.125 [F(2,24) = 19.64, p < 0.001]; 0.25
Effects of saline substitution on the number of infusions obtained during the 2-h session. Data are represented as means ± S.E.M. There was a significant main effect of saline exposure \(F(2,24) = 29.713, p < 0.001\) as well as a dose × saline exposure interaction \(F_{(4,99)} = 4.848, p = 0.001\). In addition, there was a significant effect of saline exposure at each dose of cocaine tested: 0.125 \(F_{(2,24)} = 19.64, p < 0.001\); 0.25 \(F_{(2,24)} = 16.89, p < 0.001\); and 0.5 mg/kg/inf \(F_{(2,24)} = 7.46, p = 0.004\). a, significantly different from baseline (0.125: \(q = -15.22, p = 0.005\); \(q = -26.89, p < 0.001\); and 0.25: \(q = -17.9, p < 0.001\)). b, significantly different from first saline extinction (0.125: \(q = -11.67, p = 0.032\); 0.25: \(q = -12.05, p = 0.001\); 0.5: \(q = -27.5, p = 0.003\)).

**Fig. 3.** Effects of saline substitution on the number of infusions obtained during the 2-h session. Data are represented as means ± S.E.M. There was a significant main effect of saline exposure \(F(2,24) = 29.713, p < 0.001\) as well as a dose × saline exposure interaction \(F_{(4,99)} = 4.848, p = 0.001\). In addition, there was a significant effect of saline exposure at each dose of cocaine tested: 0.125 \(F_{(2,24)} = 19.64, p < 0.001\); 0.25 \(F_{(2,24)} = 16.89, p < 0.001\); and 0.5 mg/kg/inf \(F_{(2,24)} = 7.46, p = 0.004\). a, significantly different from baseline (0.125: \(q = -15.22, p = 0.005\); \(q = -26.89, p < 0.001\); and 0.25: \(q = -17.9, p < 0.001\)). b, significantly different from first saline extinction (0.125: \(q = -11.67, p = 0.032\); 0.25: \(q = -12.05, p = 0.001\); 0.5: \(q = -27.5, p = 0.003\)).

Tukey’s post hoc analyses were performed for each of the three training doses of cocaine. For the lowest training dose of cocaine (i.e., 0.125 mg/kg/inf) exposure to saline substitution during both the first and final exposure resulted in a significant decrease in responding (\(q = -15.22, p = 0.005\); \(q = -26.89, p < 0.001\), respectively). In addition, repeated exposures to extinction produced a larger decrease in responding than did the first exposure (\(q = -11.67, p = 0.032\)).

For the medium training dose of cocaine (i.e., 0.25 mg/kg/inf) exposure to repeated extinction trials resulted in a decrease in responding that was significantly different from both baseline and the first saline exposure (\(q = -17.9, p < 0.001\); \(q = -12.05, p = 0.001\), respectively). In contrast, an increase in responding during the first saline substitution test was observed in rats trained with the highest dose of cocaine (i.e., 0.5 mg/kg/inf). However, exposure to repeated extinction trials resulted in a decrease in responding that was significantly different from the first saline exposure (\(q = -27.5, p = 0.003\)).

To determine whether rats trained with different doses of cocaine exhibited different intrasession response patterns during extinction (Fig. 4), a three-way ANOVA (session × bin × dose) was performed. There was a significant effect of exposure to saline \(F_{(2,396)} = 64.83, p < 0.001\), which produced different patterns of responding across bins \(F_{(3,396)} = 32.308, p < 0.001\). There was also a saline exposure × bin interaction \(F_{(6,396)} = 8.23, p < 0.001\) and a saline exposure × dose interaction \(F_{(4,396)} = 10.58, p < 0.001\), indicating that this effect was different across bins as well as training doses of cocaine.

Behavior was maintained in these experiments using a multiple, alternating schedule of cocaine and food reinforcement. Therefore, in addition to the effects of cocaine extinction on responding on the lever previously reinforced by cocaine, the effects of saline substitution on the total number of food pellets obtained in the 2-h behavioral sessions were also examined (Fig. 5). A two-way ANOVA (session × dose) indicated a significant effect of saline exposure, i.e., baseline versus first saline exposure versus last saline exposure \(F_{(2,99)} = 8.212, p = 0.001\). While the number of responses during extinction trials was greater than that seen during baseline self-administration, Tukey’s post hoc analyses revealed that there were no differences in responding during each of these test sessions across doses.

To measure plasma corticosterone, blood was obtained at the end of the saline substitution sessions. Since we do not routinely take blood samples after daily training sessions, baseline corticosterone was determined from blood samples.
taken after a cocaine self-administration session where the rats were pretreated with vehicle (i.e., 5% Emulphor in saline) 30 min before the start of the session. To determine whether these two behavioral sessions (i.e., baseline versus vehicle pretreated) were different, a one-way ANOVA was performed on the total number of cocaine infusions obtained during the 2-h sessions. There was a significant main effect of dose \( F(2,66) = 17.08, p = 0.001 \), indicating that as the dose of cocaine was increased, the rats self-administered fewer injections (this effect is observed in the baseline data of Fig. 3). However, there was no difference in number of injections delivered between baseline self-administration and following pretreatment with vehicle \( F(1,66) = 0.207, p = 0.651 \). The effects of saline substitution on plasma corticosterone are shown in Fig. 6. A two-way ANOVA (saline exposure \( \times \) dose) revealed a significant effect of saline exposure \( F(2,99) = 5.13, p = 0.008 \), as well as a significant effect of saline exposure at the highest dose of cocaine tested (0.5 mg/kg/inf: \( F(2,21) = 5.53, p = 0.012 \)). Tukey’s post hoc analysis on the 0.5-mg/kg/inf dose of cocaine data demonstrated that the initial exposure to extinction produced a significantly greater increase in plasma corticosterone compared with either baseline or repeated saline extinction \( (q = 129.625, p = 0.014; q = -105.625, p = 0.047, \text{ respectively}) \). The results from this experiment indicate that the initial training dose of cocaine is a critical factor in determining the behavioral responses during extinction. In fact, there is a dose-related relationship between the cocaine training dose and the number of responses emitted during the first extinction test session. For example, compared with baseline responding, rats trained with the lowest dose of cocaine (i.e., 0.125 mg/kg/inf) tended to decrease responding on the cocaine lever the first time saline was substituted for cocaine, while rats trained with the highest dose of cocaine (i.e., 0.5 mg/kg/inf) generally increased responding. In contrast to the data obtained from the initial saline substitution tests, repeated exposure to extinction produced an overall decrease in behavior that was seen regardless of cocaine training dose. In fact, rats trained with all three doses of cocaine tested (i.e., 0.125, 0.25, and 0.5 mg/kg/inf) showed a similar level of responding during the final saline substitution test. When these data were more closely analyzed according to individual 15-min bins, the patterns of responding during the first saline substitution trial varied markedly across the doses of cocaine. In closer detail, rats trained with 0.125 mg/kg/inf of cocaine responded as during baseline in bin 1, followed by progressive, significant decreases in responding during the remaining three bins. Rats trained with 0.25 mg/kg/inf of cocaine responded as during baseline in the first three bins followed by a decrease in responding in the final bin. In contrast, rats trained with 0.5 mg/kg/inf of cocaine significantly increased responding during the first bin, followed by baseline-like responding in the remaining three bins.
bins. Therefore, during the initial response to saline substitution, only the rats trained with the highest dose of cocaine (i.e., 0.5 mg/kg/inf) exhibited what others have reported as a "typical extinction" response (i.e., an initial burst of responding at the beginning of the test session followed by a decrease in responding during the remainder of the test session). However, this behavior gradually changed over time following repeated exposure to extinction. Ultimately, responding during extinction was similar among groups regardless of the training dose of cocaine. Responding is typically highest during the first 15-min bin as the rats come into contact with the extinction conditions, and this behavior subsequently declines throughout the remainder of the session.

Taken together, the data obtained from both the initial saline substitution tests as well as the data from the final tests clearly demonstrate the importance of the cocaine training dose when determining the responses to saline substitutions. In addition, these data further elucidate the importance of establishing dose-effect curves for both cocaine self-administration and saline substitutions before a comparison is made between pretreatment with test compounds and "extinction-like" behaviors. This is of particular importance when the intended purpose of the experiment is to examine potential pharmacotherapeutic agents that exert their actions by decreasing the reinforcing efficacy of cocaine.

In addition to the effects of saline substitution tests on cocaine self-administration, the substitution of saline for cocaine during the multiple, alternating sessions also affected food-maintained responding. More specifically, rats obtained more food reinforcers during the saline substitution tests than during the training sessions when cocaine was available. This finding may suggest that cocaine intake disrupts lever-pressing maintained by food using this paradigm.

To assess the potential role for the HPa axis in the behavioral effects of extinction, blood samples were drawn at the end of the saline substitution tests and plasma corticosterone was measured using radioimmunoassay. Rats trained with the highest dose of cocaine (i.e., 0.5 mg/kg/inf) showed an increase in plasma corticosterone during the initial saline substitution test. However, this increase was not present during the final saline substitution test following repeated exposure to extinction. It is possible that the increase in behavior during the initial saline substitution test is a conditioned plasma corticosterone response.

There are other reports indicating that corticosterone is involved in the conditioned effects of cocaine. For example, environmental stimuli paired with the delivery of cocaine can, when presented alone, elicit a CRH-dependent increase in plasma corticosterone in rats (DeVries and Pert, 1998). Similarly, environmental cues associated with drug self-administration have been reported to increase plasma adrenocorticotropin and cortisol in cocaine-dependent individuals (Berger et al., 1996). It is likely that this type of involvement of the HPa axis is important for development of craving and/or the persistence of cocaine-seeking behavior in the absence of the drug. In this regard, it has been demonstrated in our laboratory that during daily extinction training, when responding previously reinforced by the delivery of cocaine results in no programmed consequences, postsession plasma corticosterone was elevated on day 1 of extinction when non-reinforced responding on the cocaine lever was high and was reduced on day 9 of extinction when responding was extinguished (Mantsh and Goeders, 1999b). Furthermore, we have found that pretreatment with the CRH1 receptor antagonist CP-154,526 attenuates the increased cocaine-lever responding observed during acute extinction in rats (Gurkovskaya et al., 2001). In addition, others have demonstrated that corticosterone administration reinstates cocaine-seeking behavior following extended periods of extinction (Deroche et al., 1992). Finally, data from this laboratory also suggest an important role for CRH and corticosterone in the conditioned reinstatement of cocaine-seeking behavior (Goeders et al., 2000). In these experiments, a tone and light stimulus complex were presented simultaneously with cocaine delivery during self-administration. Following extinction, the rats were tested for reinstatement. During reinstatement, responding on the cocaine lever resulted in the presentation of the light and tone stimulus (i.e., a conditioned reinforcer), but cocaine was not delivered. Pretreatment with ketoconazole (25 mg/kg i.p.) or CP-154,526 (20 mg/kg i.p.) prevented the increase in cocaine lever responding that occurred during reinstatement in the presence of the conditioned reinforcer, which provides further credibility for the role of CRH and corticosterone in the conditioned effects of cocaine (Clampitt et al., 2000; Goeders et al., 2000). The involvement of conditioned increases in plasma corticosterone in cue-elicited cocaine-seeking is consistent with the proposed role of this hormone as an important mediator of "external" influences on cocaine self-administration, but not of the direct effects of cocaine itself.

It is well documented that the activation of the HPa axis following exposure to different stressors produces an increase in plasma corticosterone (Dunn et al., 1989; Dhabhar et al., 1997; Rivier, 1999). In addition, as previously reported by our laboratory, the acquisition of cocaine self-administration does not occur unless plasma corticosterone is increased above a threshold that is critical for reward (Goeders and Guerin, 1996a). In the present study, rats trained with 0.5 mg/kg/inf of cocaine exhibited significantly higher plasma corticosterone during the first exposure to extinction than did rats trained with lower doses of cocaine. It is therefore possible that either the initial saline substitution test was more stressful for these rats, or alternatively that this effect may be due to a larger conditioned increase in plasma corticosterone associated with the self-administration of a high dose of cocaine. The ultimate corticosterone response may actually result from a combination of these and other factors.

Taken together, these data reinforce the potential difficulties that might be encountered when interpreting the effects of compounds that reduce cocaine reinforcement. It is paramount that reliable, consistent behavioral results are observed following repeated exposure to extinction before the effects of test compounds are measured. Otherwise, it is almost impossible to accurately determine whether a test compound increased or decreased cocaine reward. In addition, these data also support a role for the HPa axis in the behavioral responses to extinction in rats previously trained to self-administer cocaine.

References
Clampitt DM, Peltier RL, and Goeders NE (2000) A role for conditioned cues in
corticosterone administration sensitizes the locomotor response to amphetamine. *Brain Res* **584:**309–313.


Mantsh CR and Goeders NE (1999a) Ketoconazole does not block cocaine discrimination or the cocaine-induced reinstatement of cocaine-seeking behavior. *Pharmacol Biochem Behav* **64:**65–73.


**Address correspondence to:** Rachel L. Peltier, Ph.D., Department of Pharmacology and Therapeutics, Louisiana State University Health Sciences Center, P.O. Box 33932, 1501 Kings Hwy., Shreveport, LA 71130-3932. E-mail: rpelti@lsuhsc.edu