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Effects of the CRF_1 antagonist antalarmin on cocaine self-administration and discrimination in rhesus monkeys

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Abstract

Cocaine stimulates the rapid release of ACTH, and by inference, CRF in several species, suggesting that the HPA "stress" axis may contribute to the abuse-related effects of cocaine. The effects of a systemically-active CRF_1 receptor antagonist, antalarmin, on cocaine self-administration and cocaine discrimination were examined in rhesus monkeys. Antalarmin's acute (1–10 mg/kg, IV) and chronic (3.2 mg/kg IV) effects on IV cocaine self-administration were studied. The acute effects of 3.2 mg/kg IV antalarmin on the cocaine self-administration dose–effect curve (0.001–0.10 mg/kg/inj) were also examined. The acute effects of antalarmin (5 and 10 mg/kg, IM) on the cocaine discrimination dose–effect curve (0.013–1.3 mg/kg) were examined. Antalarmin did not significantly decrease the reinforcing or the discriminative stimulus effects of cocaine. Acute antalarmin administration produced a dose-dependent but non-significant decrease in self-administration of 0.01 mg/kg/inj cocaine but did not alter the cocaine dose–effect curve. Chronic daily antalarmin treatment did not significantly decrease cocaine-maintained responding. Antalarmin did not significantly alter either the cocaine discrimination dose–effect curve or the time course of the cocaine-training dose. Antalarmin (10 mg/kg) produced sedation, suggesting that it was centrally active, however, it did not attenuate cocaine's abuse-related effects in rhesus monkeys.

Keywords: Antalarmin; CRF_1 antagonist; Cocaine discrimination; Cocaine self-administration; HPA axis

1. Introduction

There is general agreement that activation of the hypothalamic-pituitary-adrenal (HPA) "stress" axis contributes to a number of psychiatric disorders (Gold et al., 1988a,b; Holsboer, 2000; Koob, 1999; Owens and Nemeroff, 1999, 1991). "Stress" is also a pervasive concomitant of drug abuse and is implicated in relapse (Bossert et al., 2005; Shaham et al., 1998, 1997; Sinha, 2001) as well as co-morbid mood and substance abuse disorders (Farrell et al., 1998; Sinha, 2001, 2006; Volkow, 2001). Clinical reports suggest that anxiety and depression associated with HPA axis activation can be attenuated with antagonists of the CRF_1 receptor (Grammatopoulos and Chrousos, 2002; Heidbreder and Hagan, 2005; Holsboer, 2003; Kehne and De Lombaert, 2002).

One neurobiologic index of HPA axis activation is an elevation in circulating levels of adrenocorticotropic hormone (ACTH) and cortisol/corticosterone. Hypothalamic corticotropin-releasing-factor (CRF) induces ACTH release from the anterior pituitary, and this is followed by an increase in cortisol/corticosterone from the adrenal. Interestingly, these HPA endocrine correlates of acute "stress" are also temporally concordant with the acute effects of cocaine administration in both clinical and preclinical studies (Broadbear et al., 1999a,b; Mendelson et al., 2002, 2003). Cocaine consistently stimulates rapid release of ACTH in cocaine abusers, and ACTH stimulation is significantly correlated with ratings of subjective feelings of "high" and "rush" (Mendelson et al., 2002, 2003; see for review Mello and Mendelson, 2002). These findings were interpreted to suggest that stimulation of hypothalamic CRF (inferred from increases in ACTH and cortisol) may contribute to the reinforcing effects of cocaine (Mello and Mendelson, 2002; Mendelson et al., 2002, 2003) as well as alcohol (Mendelson et al., 1992) and nicotine (Mendelson et al., 2005, 2003).

This hypothesis is consistent with data from a series of studies of the interactions between HPA axis hormones and the

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behavioral effects of cocaine in rodents (Goeders, 1997, 2002a, b; Marinelli and Piazza, 2002; Piazza and LeMoal, 1996). For example, the adrenal hormone corticosterone is critically important for the acquisition and maintenance of cocaine self-administration in rats (Deroche et al., 1997; Goeders and Guerin, 1996; Mantsch and Katz, in press; Mantsch et al., 2000). Moreover, administration of the CRF_1 antagonist, CP-154,526, significantly decreased IV cocaine self-administration by rats, with minimal effects on food-maintained responding (Goeders and Guerin, 2000). In addition, the CRF_1 antagonists CP-154,526 and alpha-helical CRF attenuated cocaine-induced conditioned place preference and locomotor activity in rats, as well as cocaine-related increases in extracellular dopamine levels in the nucleus accumbens and the ventral tegmental area (Lu et al., 2003). Stress reliably induces reinstatement of cocaine-seeking in rodents (Shaham et al., 2000), and this effect was also blocked by the CRF_1 receptor antagonists CP-154,526 (Shaham et al., 1998) D-Phe CRF $_{12–14}$ (Erb et al., 1998) and alpha-helical CRF (Wang et al., 2005). Taken together, these clinical and preclinical findings led us to examine the effects of a CRF_1 antagonist on the abuse-related effects of cocaine in rhesus monkeys.

Antalarmin is a non-peptidic, systemically-active, CRF_1 receptor antagonist (Webster et al., 1996) that is a close analog of CP-154,526 (Chen, 2006; Seymour et al., 2003). Antalarmin is reported to alleviate behavioral responses to "stress" in rhesus monkeys and in rats. For example, antalarmin reduced fear reactions to social aggression in rhesus monkeys (Ayala et al., 2004; Habib et al., 2000), and responses to pharmacologically-induced and social stress in rats (Deak et al., 1999; Ghitza et al., in press; Zorrilla et al., 2002. Acute withdrawal from cocaine is associated with increased levels of corticotropin-releasing factor (CRF) in the central nucleus of the amygdala in rodents, and administration of a CRF antagonist reduced anxiety-like behavior measured by "defensive burying" (Koob et al., 2004). Antalarmin also reversed conditioned place aversion in morphine-dependent rats after naloxone precipitated opioid withdrawal (Stinus et al., 2005). Antalarmin alone did not induce either place preference or place aversion in morphine-dependent rats (Stinus et al., 2005). These behavioral data are consistent with findings from an endocrine study that antalarmin attenuates CRF-stimulation of ACTH release in rhesus monkeys (Broadbear et al., 2004). This is the first study of the effects of the CRF_1 antagonist antalarmin on cocaine self-administration and cocaine discrimination in rhesus monkeys. These studies were designed to further examine the role of the HPA axis in the abuse-related effects of cocaine.

2. Methods

2.1. Experiment I: antalarmin effects on cocaine self-administration

2.1.1. Subjects

Three rhesus monkeys (*Macaca mulatta*) (two males and one female) weighing 6.2–9.3 kg were used in studies to assess acute and chronic effects of antalarmin on cocaine self-administration. All monkeys had prior exposure to drugs (primarily dopaminergic and opioid compounds) and operant behavioral procedures. Monkeys were individually housed, and water was freely available. Their diet consisted of 6–7 biscuits/day (Purina Jumbo monkey chow Jumbo #5037), supplemented with fresh fruit twice daily, and up to 25 1 gm food pellets (Precision Primate Pellets Formula L/I Banana Flavor, P.J. Noyes Co., Lancaster, NH) were delivered during daily sessions of food-reinforced responding (see below). A 12 hr light/12 hr dark cycle was in effect with lights on from 7 AM to 7 PM.

Animal maintenance and research were conducted in accordance with the guidelines provided by the Committee on Laboratory Animal Resources. The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was periodically monitored by consulting veterinarians. Monkeys had visual, auditory and olfactory contact with other monkeys throughout the study. Operant food self-administration procedures provided an opportunity for environmental manipulation and enrichment (Line et al., 1989).

2.1.2. Apparatus

Experimental sessions were conducted in each monkey's home cage. The front wall contained an operant response panel (28×28 cm^2) that included three circular response keys (5.1 cm in diameter) arranged 2.5 cm apart horizontally. Each key could be transilluminated by red, green, or yellow stimulus lights (Superbright LEDs, St. Louis MO). A food-pellet dispenser (Model G5210, Ralph Gerbrands Co., Arlington, MA) was mounted above each cage to deliver 1 g banana-flavored food pellets (Precision Primate Pellets, P.J. Noyes Co., Lancaster, NH) to a food receptacle located below the response panel. Two syringe pumps (Model B5P-1E, Braintree Scientific, Braintree, MA; or Model 98021, Harvard Apparatus, South Natick, MA) for IV delivery of saline or drug solutions were located above the cage. The schedules of reinforcement were controlled and data were collected with IBM-compatible computers and interface systems (MED Associates, Inc., Georgia, VT) located in a separate room.

2.1.3. Surgical procedures

For IV drug administration, a double-lumen silicone rubber catheter (0.71 mm inside diameter; 2.2 mm outside diameter) was chronically implanted into a jugular or femoral vein under aseptic conditions. Details of the surgical procedure have been described previously (Mello and Negus, 1998). One catheter lumen was used for administration of IV cocaine and the second lumen was used for administration of IV antalarmin or saline. Saline (0.1 ml) was delivered over 1 s every 20 min from 10 AM each day to 9 AM the next day to maintain catheter patency. Each monkey wore a nylon vest attached to a flexible stainless steel cable to protect the IV catheter. The distal end of the catheter and cable were attached to a fluid swivel (Lomir Biomedical, Montreal, Canada), which was connected to the syringe pumps. Catheter patency was periodically evaluated by IV ketamine administration (5 mg/kg). If ketamine produced a

loss of muscle tone within 10 s, the catheter was judged to be patent.

2.1.4. Behavioral procedures

Cocaine self-administration sessions were conducted for 2 h (from 11am–1pm) seven days per week. At the onset of each session, the center response key was illuminated with green stimulus lights, and completion of the response requirement [a fixed-ratio 10 (two monkeys) or a fixed-ratio 30 (one monkey)] resulted in the delivery of the available cocaine dose in a volume of 0.1 ml delivered in 1 s. Completion of the FR response requirement also initiated a 60-s timeout, during which the stimulus lights were turned off, and responding had no scheduled consequences (i.e., FR10 TO 60 s or FR30 TO 60 s). Between 1:00 and 2:00 PM, monkeys were observed for signs of unusual behavior and/or sedation. Responses to the approach of the investigator and acceptance or rejection of preferred food treats were noted.

At 3:00 PM, a session of food-maintained responding began each day and ended after 1 h or delivery of 25 1 g food pellets, whichever occurred first. The schedule of food reinforcement was identical to the schedule of cocaine self-administration for each monkey. The food session provided an opportunity for environmental enrichment (Line et al., 1989) and was a behavioral indicator of the monkey's overall health status. (Data from the food session were collected but are not included in the general data analysis).

2.1.5. Training and testing procedures

Prior to testing with antalarmin, a baseline cocaine self-administration dose-effect curve was determined. On a test day, either saline or a test dose of cocaine (0.001–0.1 mg/kg/injection) was substituted for the maintenance dose (0.032 mg/kg/inj cocaine). Dose was manipulated by exchanging the syringe in the syringe pump that contained the maintenance dose of cocaine with a new syringe that contained the test dose of cocaine. To minimize the effects of any cocaine that might be infused during this exchange, placement of new syringes always occurred between 4 and 6 PM, at least 3 h after the most recent cocaine self-administration session, and at least 17 h before the test session. If the subject earned fewer than 20 injections on the test day, then the maintenance dose of 0.032 mg/kg/inj cocaine was reinstated during the next session, and if necessary for subsequent sessions, until the subject again earned more than 20 injections/session. If the subject earned more than 20 injections during a test session, then saline was substituted for cocaine on the next day, and if necessary for subsequent days, until self-administration declined to fewer than 20 injections/session. The maintenance dose of 0.032 mg/kg/inj cocaine was then reinstated for at least one day and until the subject again earned more than 20 injections/session. Thus, testing always occurred after at least one day of self-administration at rates lower than 20 injections/session (during saline substitution or availability of a low cocaine dose) followed by at least one day of responding at rates greater than 20 injections/session during availability of the maintenance dose of 0.032 mg/kg/inj cocaine. Cocaine doses were tested in an irregular order, and each dose was tested twice in each monkey.

Following determination of the cocaine self-administration dose–effect curve, test sessions with antalarmin or its vehicle were conducted using both an *acute* and a *chronic* dosing procedure. For *acute* dosing studies, vehicle or antalarmin (1–10 mg/kg) was administered IV 30 min before a single cocaine self-administration session during which a test dose of 0.01 mg/kg/inj cocaine was available. This unit dose of cocaine was selected because it was at the peak of the group cocaine self-administration dose–effect curve. This range of antalarmin doses was selected on the basis of an endocrine study of IV antalarmin effects on CRF-stimulated ACTH and cortisol release in rhesus monkeys (Broadbear et al., 2004). In that report, 1.0 and 3.2 mg/kg, IV antalarmin antagonized CRF-stimulation of ACTH and 10 mg/kg IV antalarmin stimulated ACTH and cortisol and produced sedation (Broadbear et al., 2004).

For *chronic* treatment studies, either vehicle or 3.2 mg/kg, IV antalarmin was administered 30 min prior to daily cocaine self-administration sessions for 7 consecutive days. A unit dose of 0.01 mg/kg/inj cocaine was available during these sessions. Between tests with chronic vehicle and chronic antalarmin administration, training sessions with saline and/or 0.032 mg/kg/inj cocaine availability were conducted as described above.

To assess acute antalarmin effects on the full cocaine self-administration dose–effect curve, 3.2 mg/kg, IV antalarmin was administered 30 min before cocaine self-administration maintained over a dose range of 0.001–0.10 mg/kg/inj. Between test days, training sessions with either saline or 0.032 mg/kg/inj cocaine were conducted as described above.

2.1.6. Data analysis

The principal dependent variable was the number of injections delivered per session. The acute effects of saline and antalarmin on cocaine self-administration were analyzed with a one-way analysis of variance with Dunnets multiple comparisons post-tests. The chronic effects of saline and antalarmin were analyzed with a two-way analysis of variance and Bonferroni post-tests.

2.2. Experiment II: antalarmin effects on cocaine discrimination

2.2.1. Subjects

Four male rhesus monkeys (*M. mulatta*) weighing between 7.0–9.0 kg were studied. Each monkey was maintained on a diet of 7–12 biscuits (Purina Monkey Chow Jumbo #5037) and one piece of fresh fruit per day. During the week, all food was delivered after the experimental session, whereas on weekends, food was delivered between 9 AM and noon. Water was freely available at all times. A 12-hr light/dark cycle was in effect, with lights on from 7 AM to 7 PM.

2.2.2. Apparatus

Each monkey was housed individually in a well-ventilated, stainless steel chamber (56 × 71 × 69 cm). The home cages of all monkeys included an operant panel (28 × 28 cm) mounted on the front wall. Three round translucent response keys (5.1 cm in diameter) were arranged 2.5 cm apart in a horizontal row 9 cm

from the top of the operant panel. Each key could be transilluminated by red or green stimulus lights (Superbright LED's). An externally mounted pellet dispenser (Gerbrands, Model G5310, Arlington, MA) delivered 1 gm fruit-flavored food pellets (Precision Primate Pellets Formula L/I Banana Flavor, P. J. Noyes Co., Lancaster, NH) to a food receptacle mounted on the cage beneath the operant response panel. Operation of the operant panels and data collection were accomplished with IBM-compatible computers and interface systems (Med Associates; St. Albans, VT) located in a separate room.

2.2.3. Discrimination training

Discrimination training was conducted 5 days per week during daily sessions composed of multiple cycles. Each cycle consisted of a 15-min time-out period followed by a 5-min response period. During the timeout, all stimulus lights were off, and responding had no scheduled consequences. During the response period, the right and left response keys were transilluminated red or green, and monkeys could earn up to 10 food pellets by responding under a FR 30 schedule of food presentation. For two monkeys, the left key was illuminated green, and the right key was illuminated red, and the colors of the response keys were reversed for the other two monkeys. The center key was not illuminated at any time, and responding on the center key had no scheduled consequences. If all 10 available food pellets were delivered before the end of the 5-min response period, the response key lights were turned off, and responding had no scheduled consequences for the remainder of the 5-min period.

On training days, monkeys were given an IM injection of either saline or 0.40 mg/kg cocaine 5-min after the beginning of each time-out period (i.e., 10 min before the response period). Following saline administration, responding on only the green key (the saline-appropriate key) produced food, whereas following administration of 0.40 mg/kg cocaine, only responding on the red key (the drug-appropriate key) produced food. Responses on the inappropriate key reset the FR requirement on the appropriate key. Sessions consisted of 1 to 5 cycles, and if the training dose of cocaine was administered, it was administered only during the last cycle. Thus, training days consisted of 0 to 5 saline cycles followed by 0 to 1 drug cycles.

During each response period, 3 dependent variables were determined using the following equations.

1) Percent injection-appropriate responding prior to delivery of the first reinforcer.

$$\frac{\text{Injection} - \text{appropriate responses emitted prior to 1st reinforcer}}{\text{Total responses emitted prior to delivery of 1st reinforcer}} \times 100$$

2) Percent injection-appropriate responding for the entire response period.

$$\frac{\text{Injection} - \text{appropriate responses emitted during response period}}{\text{Total responses emitted during response period}} \times 100$$

3) Response rate

$$\frac{\text{Total responses emitted during response period}}{\text{Total time response keys transilluminated}}$$

Monkeys were considered to have acquired cocaine discrimination when the following three criteria were met for 7 of 8 consecutive training sessions: 1) the percent injection-appropriate responding prior to delivery of the first reinforcer was greater than or equal to 80% for all cycles; 2) the percent injection-appropriate responding for the entire cycle was greater than or equal to 90% for all cycles; 3) response rates during saline training cycles were greater than 0.5 responses per second.

2.2.4. Discrimination testing

Once monkeys met criterion levels of cocaine discrimination, testing began. Test sessions were conducted using either a time course protocol or cumulative dosing protocol. For the time course protocol, the training dose of 0.4 mg/kg cocaine was administered, and 5 min response periods identical to those described above were conducted beginning 3, 10, 30 and 100 min after cocaine injection. For the cumulative dosing protocol, 15 min timeouts alternated with 5 min response periods as described above, and cocaine doses (0.013–1.3 mg/kg) were administered at the beginning of each timeout. Each cocaine dose increased the total dose by 1/2 log units. In both protocols, the effects of cocaine were determined alone or 30 min after pretreatment with antalarmin (5 and 10 mg/kg, IM).

Test sessions were conducted only if the three criteria listed above under "Criteria for Discrimination" were met on the training day immediately preceding the test day. Mean data from saline and drug cycles during the training day immediately preceding each test day served as the control data. If responding did not meet criterion levels of discrimination performance, then training was continued until criterion levels of performance were obtained for at least two consecutive days.

2.2.5. Data analysis

The effects of antalarmin on cocaine discrimination were evaluated with two-way ANOVA with treatment (none, 5 mg/kg antalarmin, 10 mg/kg antalarmin) as one factor and either time (for the time course studies) or cocaine dose (for the cumulative dosing studies) as the second factor.

ED50 values for cocaine were defined as the unit dose of cocaine from the cumulative dosing experiments that produced 50% cocaine-appropriate responding. ED50 values and 95% confidence limits for cocaine alone and after antalarmin treatment were determined by linear regression (PharmTools Pro, The McCary Group, Emmaus, PA). ED50 values were considered to be significantly different if 95% confidence limits did not overlap.

2.3. Drugs

Cocaine was obtained from NIDA, and antalarmin was supplied by Dr. Kenner C. Rice, Laboratory of Medicinal Chemistry, NIDDKD, NIH. Cocaine hydrochloride was dissolved in sterile saline. Antalarmin was dissolved in a vehicle of 10% ethanol, 10% emulphor and 1% lactic acid in sterile water. As noted earlier, antalarmin doses were based in part on a

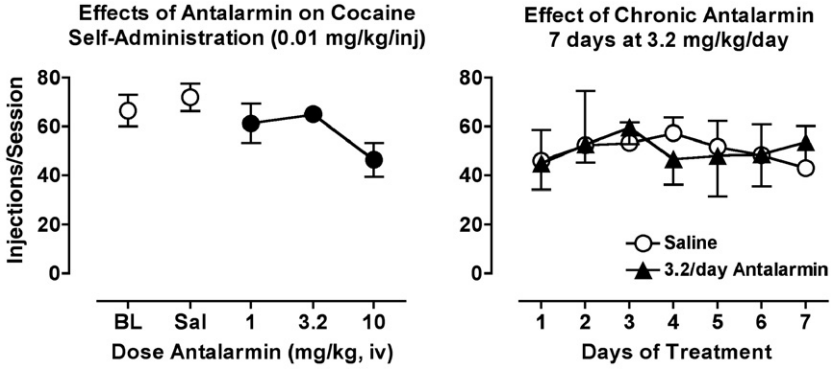

Fig. 1. Antalarmin effects on cocaine-maintained responding. The effects of saline and acute and chronic antalarmin pretreatment on cocaine-maintained responding are shown for a group of three monkeys. The number of injections per session (±SEM) is shown on the ordinates. The *acute* effects of saline and antalarmin (1, 3.2 and 10 mg/kg/day, IV) on responding maintained by 0.01 mg/kg/inj cocaine are shown in the left panel. The *chronic* effects of saline and antalarmin (3.2 mg/kg/day, IV) on responding maintained by 0.01 mg/kg/inj cocaine over seven consecutive days of treatment are shown in the right panel.

previous report that doses of 1.0 and 3.2 mg/kg, IV antagonized CRF-stimulated ACTH release in rhesus monkeys (Broadbear et al., 2004).

3. Results

3.1. Experiment I: cocaine self-administration

3.1.1. Acute and chronic effects of antalarmin on cocaine-maintained responding

Under the conditions of this study, antalarmin did not alter cocaine self-administration by rhesus monkeys. Fig. 1 (left panel) shows that acute administration of antalarmin (1–10 mg/kg, IV) did not reduce self-administration of 0.01 mg/kg/inj cocaine significantly below baseline levels. Although the highest dose of antalarmin (10 mg/kg, IV) reduced the number of cocaine injections from baseline levels of 66.5±6.43 to 46.3±6.9, this difference was not significant by one-way ANOVA. Fig. 1 (right panel) shows that chronic daily treatment with 3.2 mg/kg IV antalarmin also did not significantly change cocaine-maintained responding (0.01 mg/kg/inj). All

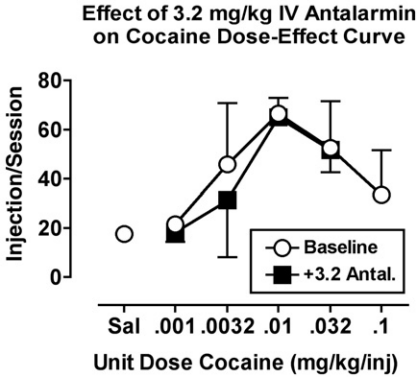

Fig. 2. Antalarmin effects on the cocaine dose–effect curve. The unit dose of cocaine (mg/kg/inj) is shown on the abscissa. The mean (±SEM) number of saline or cocaine injections during each session is shown on the ordinate. The mean number of injections self-administered when only saline was available is shown above Sal. Each data point is based on the three monkeys.

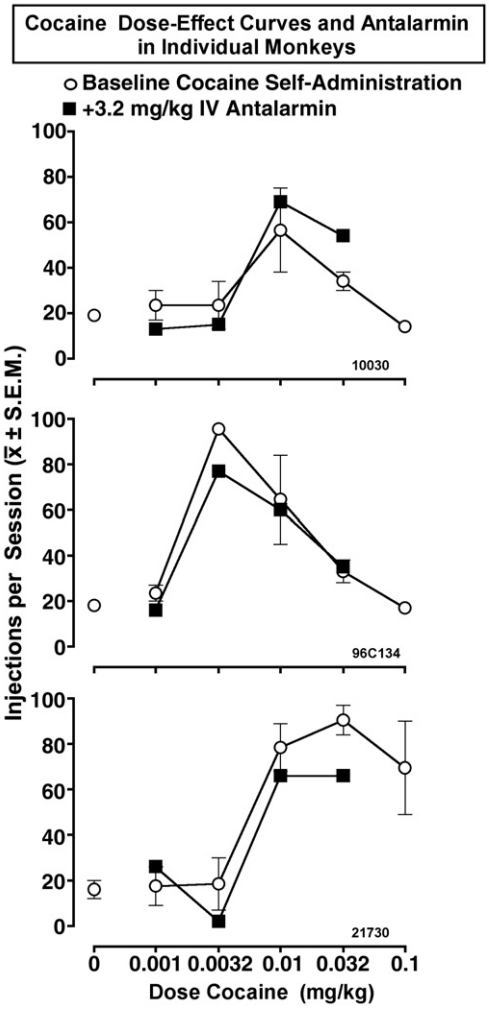

Fig. 3. Antalarmin effects on the cocaine dose–effect curve in individual monkeys. The mean (±SEM) number of saline or cocaine injections during each session is shown on the ordinates. The mean (±SEM) number of injections self-administered when only saline was available is shown above Sal. The unit doses of cocaine (mg/kg, IV) are shown on the abscissa.

monkeys appeared to be sedated after administration of 10 mg/kg, IV antalarmin. Monkeys did not react to the approach of the investigator and refused preferred food treats.

3.1.2. Effects of antalarmin on cocaine self-administration dose–effect curves

Fig. 2 shows the effects of 3.2 mg/kg, IV antalarmin on the cocaine self-administration dose–effect curve. There were no significant differences in responding at any dose of cocaine after antalarmin and vehicle control treatment. Fig. 3 shows the effects of antalarmin (3.2 mg/kg, IV) on the cocaine self-administration dose–effect curve in individual monkeys. There were no consistent changes in cocaine-maintained responding at any dose of cocaine.

3.2. Experiment II: cocaine discrimination

Fig. 4 (top panel) shows that antalarmin (5 and 10 mg/kg, IM) had no significant effect on the time course of the training dose of 0.4 mg/kg cocaine or response rates. However, 10 mg/kg, IM antalarmin produced a transient reduction in response rates by ≥50%. Fig. 5 (top panel) shows that antalarmin (5 and 10 mg/kg, IM) also had no significant effect on the cocaine discrimination dose–effect curve. Response rates were lower after antalarmin treatment than after saline treatment at all time points, but these differences were not statistically significant.

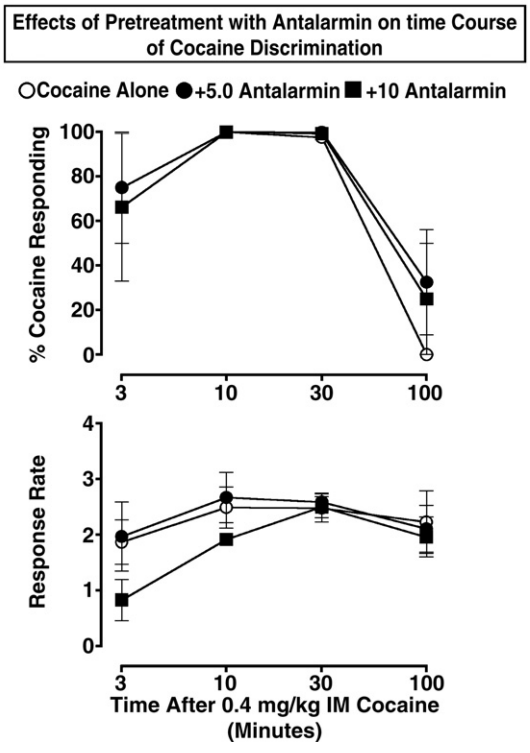

Fig. 4. Antalarmin effects on the discriminative stimulus and response rate altering effects of cocaine. The top panel shows the time course of cocaine's effects on cocaine-appropriate responding over 3 to 100 min after saline and antalarmin pretreatment (5.0 and 10 mg/kg, IM). The lower panel shows response rates at each unit dose of cocaine over 3 to 100 min. Antalarmin was administered 30 min before each test session. Each data point is based on mean data (±SEM) from four monkeys.

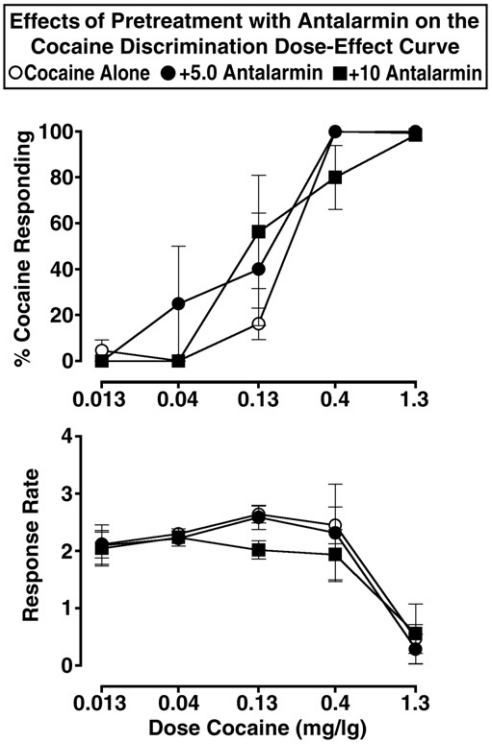

Fig. 5. The top panel shows the effects of saline and two pretreatment doses of antalarmin (5.0 and 10 mg/kg, IM) on cocaine discrimination dose–effect functions. Abscissa: dose of cocaine in mg/kg, IM; ordinate: mean percent cocaine-appropriate responding. The bottom panel shows response rates at each unit dose of cocaine after saline and antalarmin treatment. Antalarmin was administered 30 min before each test session. Each point is based on mean data (±SEM) from four monkeys.

There were no significant differences in the ED50 evaluations for cocaine's discriminative stimulus effects.

4. Discussion

Thenon-peptidic CRF_1 receptor antagonist antalarmin did not alter cocaine self-administration or cocaine discrimination in rhesus monkeys. These data are inconsistent with a previous report that another CRF_1 antagonist (CP-154, 526) selectively reduced cocaine self-administration in rats (Goeders and Guerin, 2000). These findings are also not concordant with reports that CP-154,526 and alpha-helical CRF blocked cocaine-related increases in extra cellular dopamine levels in the nucleus accumbens and the ventral tegmental area, as well as blocking acquisition of cocaine-conditioned place preference (Lu et al., 2003). CRF antagonists also block stress-induced reinstatement of cocaine-seeking in rodents (Shaham et al., 1998; Wang et al., 2005) but not cocaine-induced reinstatement (Erb et al., 1998; Shaham et al., 1997). This dissociation between the effects of CRF_1 antagonists on stress-induced but not drug-induced reinstatement is somewhat consistent with the lack of robust effects of antalarmin on cocaine discrimination in the present study. Antalarmin is structurally very similar to CP-154,526 (Chen, 2006; Seymour et al., 2003) and the extent to which differences in species (rat vs. monkey) or behavioral procedures contributed to these discrepant findings is unclear. Interestingly, antalarmin was more effective than CP-154,526 in reducing

CRF-induced locomotor activity and anxiety-like behavior measured in an elevated plus maze in rats (Zorrilla et al., 2002).

Our results agree with findings from a behavioral study in rhesus monkeys indicating that pharmacological attenuation of HPA axis activation using a non-selective peptidic CRF_1 antagonist (astressin), cortisol synthesis inhibitors (etomidate and ketoconazole) and a glucocorticoid receptor agonist (dexamethasone) failed to alter cocaine self-administration (Broadbear et al., 1999c). It was concluded that cocaine-induced changes in ACTH or cortisol do not modulate cocaine self-administration (Broadbear et al., 1999c). In studies designed to examine the temporal relationship between cocaine self-administration and HPA axis activation in rhesus monkeys, cocaine dose-dependent (0.01–0.3 mg/kg/inj) increases in ACTH and cortisol were reported (Broadbear et al., 1999a,b). When the effects of cocaine self-administration and investigator-delivered cocaine injections on HPA axis activation were compared, ACTH and cortisol levels were higher after response-contingent than non-contingent administration of equivalent doses of cocaine (Broadbear et al., 1999a).

In the present study, antalarmin did not produce significant dose-dependent effects on either cocaine self-administration or cocaine discrimination. Antalarmin also did not produce dose-related effects on the ACTH response to a social stressor in rhesus monkeys; a high dose (40 mg, PO) was less effective than a lower dose (20 mg, PO) (Habib et al., 2000). Antalarmin also did not have dose-dependent effects on endocrine endpoints (Broadbear et al., 2004). For example, doses of 1.0 and 3.2 mg/kg IV antalarmin significantly reduced CRF-stimulated ACTH release in rhesus monkeys whereas a higher dose (10 mg/kg) was less effective (Broadbear et al., 2004).

One limitation of the present study is that we cannot be certain that antalarmin was centrally active at the doses given. However, higher doses of antalarmin were not administered because some monkeys became sedated after 10 mg/kg, IV in the present study as well as in a previous study (Broadbear et al., 2004). The behavioral effects observed (sedation, refusal of preferred treats) are consistent with the interpretation that antalarmin crossed the blood brain barrier. Another limitation of this study is that we examined the effects of antalarmin on active cocaine self-administration and not on cocaine self-administration after a period of abstinence.

The contribution of HPA axis activation to the abuse-related effects of cocaine remains unclear and may be species and procedure dependent. As noted earlier in human cocaine abusers, rapid increases in ACTH and plasma cocaine were significantly correlated with ratings of subjective "high," and "rush" and these findings were interpreted to suggest that HPA axis activation may contribute to the abuse-related effects of cocaine in men (Mendelson et al., 2002, 2003). Consistent with this hypothesis, the acquisition and maintenance of cocaine self-administration in rodents appears to be critically dependent on the presence of corticosterone (Deroche et al., 1997; Goeders and Guerin, 1996; Mantsch and Katz, in press; Mantsch et al., 2000; see for review (Goeders, 1997, 2002a,b; Marinelli and Piazza, 2002). Moreover, CRF_1 antagonists selectively reduced cocaine self-administration (Goeders and Guerin, 2000) as well as cocaine conditioned place preference, cocaine-induced locomotor activity, cocaine-related increases in extracellular dopamine (Lu et al., 2003) and stress-induced reinstatement of cocaine-seeking behavior (Shaham et al., 1998; Wang et al., 2005). However, the present findings in rhesus monkeys are not consistent with hypothesis based on findings in cocaine abusers and in rats.

CRF_1 antagonists appear to be useful in treating anxiety and depression in humans (Grammatopoulos and Chrousos, 2002; Holsboer, 2003, 2000; Kehne and De Lombaert, 2002) and may eventually be effective pharmacotherapies for persons with co-morbid drug abuse and psychiatric disorders. In addition, there is emerging clinical and preclinical evidence that CRF_1 antagonists may be useful in modulating some aversive aspects of drug withdrawal (Koob et al., 2004; Stinus et al., 2005) and preventing relapse (Sinha, 2001, 2006). However, the interactions between "stress," the HPA axis and drug abuse are very complex, and interpretation of findings is determined by the behavioral endpoints measured as well as by species. As additional centrally active CRF_1 antagonists become available for evaluation, some of these issues should be clarified.

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