Cocaine-Reinforced Responding in Rhesus Monkeys: Pharmacological Attenuation of the Hypothalamic-Pituitary-Adrenal Axis Response

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ABSTRACT
Intravenously self-administered cocaine produces a dose-dependent release of adrenocorticotropic hormone (ACTH) and cortisol in male rhesus monkeys. This study investigated whether the acute disruption of cortisol and/or ACTH release had any effect on ongoing cocaine-maintained responding. Four hypothalamic-pituitary-adrenal (HPA) axis inhibitors were examined: etomidate and ketoconazole, both of which are cortisol synthesis inhibitors; astressin, a peptidic corticotropin-releasing factor (CRF) antagonist that binds CRF1 receptors predominantly in the pituitary gland; and dexamethasone, a highly selective glucocorticoid receptor agonist whose long-lasting effects reduce or abolish the endogenous release of ACTH and cortisol. The reinforcing effects of a range of cocaine doses, with or without pretreatment with an HPA inhibitor, were evaluated using a fixed ratio 30 time-out 10-min schedule of reinforcement in six male monkeys. Blood was sampled before, during, and after self-administration sessions. Self-administration of cocaine increased plasma cortisol and ACTH. Pretreatment with etomidate and ketoconazole dose-dependently inhibited the cocaine-induced rise in cortisol and, at the highest doses, produced a compensatory increase in ACTH release. Astressin and dexamethasone attenuated or abolished cocaine-induced cortisol and ACTH release. Despite the efficacy exhibited by these pretreatments and the variety of mechanisms by which they inhibited the HPA axis, there was no evidence for any change in cocaine-reinforced behavior (response rate or infusion number), an indication that acute changes in the ACTH or cortisol response to cocaine do not play a direct role in modulating cocaine-seeking behavior under these behavioral circumstances.

Cocaine produces a robust and dose-dependent activation of the hypothalamic-pituitary-adrenal (HPA) axis in rats (Rivier and Vale, 1987; Saphier et al., 1993), rhesus monkeys (Sarnyai et al., 1996; Broadbear et al., 1999a,b), and humans (Baumann et al. 1995; Heesch et al., 1995; Ward et al., 1998). This raises the question of whether the HPA axis response is necessary for the manifestation of cocaine’s effects or is simply a correlate or consequence of the actions of cocaine.

Currently, it is hypothesized that exposure to physically or psychologically stressful events may render an individual more sensitive to the rewarding effects of drugs such as cocaine (Piazza and le Moal, 1996, 1998; Goeders, 1997). This hypothesis is supported by studies where prior exposure to stressful stimuli, such as an aggressive intruder or unsignaled mild footshock, results in an enhancement of behaviors pertaining to acquisition (Piazza et al., 1990; Haney et al., 1995; Goeders and Guerin, 1996b) and maintenance of cocaine self-administration (Miczek and Mutschler, 1996), and to reinstatement of drug-seeking in rats following extinction of operant behavior (Erb et al. 1998).

Activation of the HPA axis is a physiological response to stressful events. Corticosterone (or cortisol), a glucocorticoid, is a primary hormonal end-product of HPA activation. Corticosterone levels correlate with the degree of stress-induced behavioral activation (Piazza et al., 1991) and are predictive of subsequent drug-seeking behavior (Piazza et al., 1991; Goeders and Guerin, 1996a). Indeed, administration of exogenous corticosterone to simulate stress-induced plasma levels produces the same facilitation of self-administration behavior (Piazza et al., 1991). Also of note is evidence that corticosterone may be rewarding in its own right (Piazza et al., 1993; Deroche et al., 1993).

The hypothesis that glucocorticoids facilitate the rewarding effects of drugs has been examined further by evaluating how the absence of corticosterone affects cocaine-related behavior. Here, the converse of the facilitatory effect was demonstrated, using surgical means such as adrenalectomy.

ABBREVIATIONS: HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor; FR, fixed ratio; TO, time-out; AUC, area under curve.
in a laboratory that contained a total of 24 monkeys. The monkeys were fed 8 to 12 Purina (St. Louis, MO) Monkey Chow biscuits twice daily to maintain normal adult weight, and water was available ad libitum. Each monkey had an indwelling venous catheter in a femoral, internal, or external jugular vein. Catheters were inserted during aseptic surgery under ketamine (10 mg/kg) and xylazine (2 mg/kg) anesthesia. Following placement in the vein, the catheter was guided s.c. to the midscapular region where it exited the monkey. The external portion of the catheter was protected inside the cage by a flexible stainless steel tether, with one end attached to a double-layer polyester jacket (Lomir, New York, NY) worn by the monkey and the other bolted to the rear of the cage.

Three subjects had several months of experience with the self-administration of different doses of cocaine under a fixed-ratio 30, time-out 10 min (FR 30 TO 600 s) schedule of cocaine delivery. The other three subjects had several years of history that also included prior experience with other classes of drugs under different delivery schedules.

Animals used in these studies were maintained in accordance with the University of Michigan Committee on Animal Care and Guidelines of the Committee on the Care and Use of Laboratory Animal Resources, National Health Council (Department of Health, Education and Welfare, ISBN 0-309-05377-3, revised 1996).

**Apparatus.** Each cage had a 15- × 20-cm panel fixed to its right wall. Each panel had three stimulus lights, two red and one central green light, placed above two response levers. The red stimulus light over the right lever signaled drug availability. Drug delivery was contingent on the monkey emitting the required response (30 lever presses). The green center light was illuminated for the duration of the drug infusion, 1 ml over 5 s. During each 10-min TO, all stimulus lights were extinguished and responding had no programmed consequences.

The experiment was controlled by IBM/PS/2 computers located in an adjacent room. The computers were programmed using Med Associates software (Georgia, VT).

**Procedure.** Drug self-administration sessions were scheduled twice daily for 130 min starting at approximately 10 AM and 4 PM. There was a maximum of 13 infusions available in each session. The criteria required for a stable baseline of self-administration behavior were response rates of greater than 1 response/s for 0.03 mg/kg injection cocaine, and delivery of the maximum number of injections available during the session (13 injections). In addition, when saline was available for self-administration, response rates were required to be <20% of the rates measured for 0.03 mg/kg injection cocaine, with total saline injections numbering <13. Monkeys were also required to show a reliable decrease in saline-maintained response rate and infusion number on saline sessions that immediately followed cocaine sessions. Saline was substituted for cocaine in 25 to 50% of sessions. For each monkey, there were 3 to 12 test days on which blood samples were drawn (5 on average) for saline or each dose of cocaine.

Blood was sampled during the morning session, twice a week on average. Saline and 0.01, 0.03, 0.1, and 0.3 mg/kg injection cocaine were made available to all monkeys on different occasions. On test days, a sample of venous blood was drawn via the catheter 5 to 30 min before the session, and then again after the 1st, 4th, 8th, and 13th infusions (or at approximately 5, 30, 70, and 130 min after the session began if the monkey’s response and infusion rate slowed during the session). Blood samples were also drawn at 15 min postsession and at hourly intervals for the next 3 h, making a total of nine blood samples. On mornings when a pretreatment was administered, an additional blood sample was obtained just before the start of the self-administration session to determine whether the pretreatment affected basal cortisol and ACTH levels. Each blood sample (1.1-1.4 ml) was placed in a 2-ml Vacutainer (Becton Dickinson, Franklin Lakes, NJ) containing 0.04 ml of 7.5% EDTA and immediately placed on ice.

After the collection of each blood sample, 1.5 to 3 ml of 30 U/ml heparin saline solution was infused into the catheter and, when

Materials and Methods

**Subjects.** Six adult male rhesus monkeys (Macaca mulatta), weighing between 9.0 and 13 kg, were used in this study. One monkey (1583) had been orchiectomized. These monkeys were individually housed in stainless steel cages measuring 83.3 × 76.2 × 91.4 cm deep (Bryan Research Equipment Corp., Bryan, TX) located in a laboratory that contained a total of 24 monkeys. The monkeys

(Goeders and Guerin, 1996a) as well as pharmacological intervention to lower corticosterone levels. Adrenalectomy prevents untrained rats from acquiring self-administration behavior (Goeders and Guerin, 1996a) and prevents reinstatement of self-administration behavior following extinction by saline substitution (Erb et al., 1998). Ketocazol (Goeders et al. 1998) and metyrapone (Piazza et al., 1994; Goeders and Guerin, 1996a) are examples of reversible, pharmacological glucocorticoid synthesis inhibitors that also appear to produce dose-dependent reductions in self-administration behavior. These data support the suggestion that corticosterone may not only facilitate the effects of psychomotor stimulants such as amphetamine and cocaine, but may in fact be necessary for activation of the central mechanisms associated with drug-seeking (Goeders and Guerin, 1996a; Goeders, 1997).

Although there are data that support the hypothesis for direct participation of the HPA axis in the reinforcing effect of cocaine in rats, there are no published studies examining whether manipulation of the HPA axis influences self-administration behavior in any other species. In earlier studies, we found that self-administered cocaine produced a reliable, dose-dependent increase in HPA activity (Broadbear et al., 1999a,b). In the present study we evaluated whether the acute disruption of cortisol and/or ACTH release had any effect on ongoing cocaine-maintained responding in male rhesus monkeys. Four HPA axis inhibitors were examined. Etoimidate, an i.v. active anesthetic, and ketoconazole, an antifungal, both inhibit several enzymatic steps in the cortisol synthesis pathway in the adrenal gland (Loose et al., 1983; Alloio et al., 1985; Albertson et al., 1988). Ketoconazole may also be a glucocorticoid receptor antagonist (Loose et al., 1983; Feldman, 1986). Astressin, a peptidic corticotropin-releasing factor (CRF) antagonist with limited ability to penetrate the central nervous system when administered peripherally (Martins et al., 1996), inhibits HPA axis activity by binding to CRF receptors in the anterior lobe of the pituitary gland (Gulyas et al., 1995). Dexamethasone is a highly selective glucocorticoid receptor agonist whose long-lasting effects reduce or abolish endogenous release of adrenocorticotropic hormone (ACTH) and cortisol in rhesus monkeys for 24 h or more (Kalin et al., 1981).

In this study, we compared the effectiveness with which etomidate, ketoconazole, astressin, and dexamethasone attenuated the cortisol and ACTH responses to self-administered cocaine. Monkeys were acutely pretreated with these compounds to investigate whether either basal or cocaine-induced HPA axis activity is necessary for maintaining cocaine-reinforced behavior. Rates of cocaine-maintained responding and cocaine intake were measured to determine whether there were any behavioral changes that correlated with the degree to which ACTH and/or cortisol plasma levels were attenuated.
sampling was done during sessions in which cocaine was available, a volume of the cocaine solution equal to the cathether volume (0.6–1.5 ml) was injected after the heparin saline solution. Samples were centrifuged at 5000 rpm for 5 min at 4°C and then the plasma (0.7 ml) was pipetted into 2-ml Cryovials (Corning Glass, Corning, NY) and stored at −80°C until assay. Samples were sent on dry ice to Washington University (St. Louis, MO) where ACTH and cortisol levels were determined using radioimmunoassay kits (cortisol: Diagnostic Products, Los Angeles, CA; ACTH: Nichols Institute Diagnostics, San Juan Capistrano, CA).

Pretreatment Studies. Etomidate (Amidate, Abbott Laboratories, North Chicago, IL) was purchased in 2 mg/ml vials and administered i.v. to monkeys at 0.1, 0.3, and 1.0 mg/kg using a 15- to 45-min pretreatment, as some of these doses produced a brief anesthesis and behavioral disruption. Etomidate pretreatment was given before sessions where saline or 0.01, 0.03, or 0.3 mg/kg/injection cocaine were available. Ketoconazole (Nizoral; Janssen Pharmaceutica N.V., Beerse, Belgium) was dissolved in sterile water at a concentration of 56 mg/ml. The cloudy solution was cleared by the addition of several drops of 11.3 M HCl. Ketoconazole was administered i.v. at 3.2, 10, and 32 mg/kg with a pretreatment time of 30 to 60 min before sessions where saline or 0.01, 0.03, 0.1, or 0.3 mg/kg/injection cocaine were available. Astressin (Neurocrine, San Diego, CA) was dissolved in sterile water at 5 mg/ml immediately before use. Astressin was administered i.v. at 0.1, 0.3 and 1.0 mg/kg 15 min before the initiation of a session in which 0.3 mg/kg/injection cocaine was available. Dexamethasone (Gensia Pharmaceuticals Inc., Irvine, CA) was purchased in 10 mg/ml vials and was administered at a dose of 0.5 mg/kg via i.m. injection at 8 PM on the evening before the 10 AM 0.3 mg/kg/injection cocaine self-administration sessions on days 1 and 2 after pretreatment.

Data Analysis. Rate of responding during each session was determined from the number of responses emitted during the time the red stimulus light was on divided by the number of seconds that the light was illuminated (responses per second). Plasma cortisol (µg/dl) and ACTH (pg/ml) levels are shown in raw form as well as after normalization to area under curve (AUC) values. AUC values are an estimate of the total cortisol or ACTH release relative to basal (presession sample) levels during the self-administration session. AUC values are calculated according to the trapezoidal rule (e.g., Tallarida and Murray, 1987). The presession plasma level before each experiment was used as the reference for the calculation of the AUC for cortisol (µg·min/ml) or ACTH (pg·min/ml). The AUC was calculated from the six samples taken before, during, and 15 min after the session.

Statistics. All data are presented as mean ± S.E.M. One-way ANOVA and post hoc pairwise comparisons using the Tukey honest significant difference test of significance (p < .05) were carried out using Statistica (version 5.0; Statsoft, Tulsa, OK). Where experiments were replicated within subjects, the mean response for each subject was used in calculating treatment effects across subjects. One subject (1583) showed an atypical cortisol response to the pretreatment compounds at the highest dose of cocaine (0.3 mg/kg/injection), as he did not show the fall in cortisol levels measured in the other subjects. However, his ACTH response to pretreatment did not differ from those of the other subjects. Monkey 1583 was also the only orchietomized subject. Where different, monkey 1583’s cortisol data were not included in the data analysis.

ETOMIDATE PRETREATMENT

Fig. 1. Plasma cortisol and ACTH levels from samples taken before, during, and after self-administration of 0.01, 0.03, and 0.3 mg/kg/injection cocaine on an FR 30 TO 10-min schedule of cocaine delivery. Etomidate (0.1, 0.3, and 1.0 mg/kg) was administered i.v. before the session. Etomidate (n = 3–6) dose-dependently decreased the cortisol response to cocaine, whereas the largest dose (1.0 mg/kg) increased ACTH levels. *p < .05, etomidate versus no pretreatment; **p < .005, 1.0 etomidate versus no pretreatment and 0.1 etomidate dose; ***p < .01, 1.0 etomidate versus all other treatments.
significant change in either the rate of cocaine-maintained different doses of self-administered cocaine, there was no changes had dissipated during the 15- to 45-min pretreatment period before the start of the session. Despite the effectiveness of etomidate in disrupting the HPA axis response to different doses of self-administered cocaine, there was no significant change in either the rate of cocaine-maintained responding or the number of cocaine infusions delivered (Tables 1 and 2). Details of subsequent post hoc pairwise comparisons for both cortisol and ACTH are given in the legends to Fig. 1 and Tables 1 and 2. The two subjects that were pretreated with 0.3 mg/kg etomidate before sessions in which saline was available for self-administration both increased their rate of saline-maintained responding, as well as the number of saline injections taken relative to the other treatment conditions. This effect was neither dose-dependent nor related to any change in HPA activity (Table 2).

**Etomidate.** Pretreatment with etomidate before sessions in which cocaine was available produced a dose-dependent attenuation of cortisol release (Fig. 1; Tables 1 and 2), with some doses of etomidate reducing cortisol release to levels below those produced when saline was available for self-administration (Tables 1 and 2). Plasma cortisol levels obtained from samples 2 to 6 taken during the 0.3 mg/kg injection cocaine self-administration session were examined for effects of etomidate dose and sampling time using ANOVA. Etomidate dose (df = 3, F = 3.08, p < .06), sampling time (df = 4, F = 16.31, p < .001), and dose × sampling time (interaction: df = 12, F = 4.42, p < .001) all affected plasma cortisol (Fig. 1, rightmost panel). The effect of etomidate on ACTH levels was striking. At the largest dose (1.0 mg/kg), etomidate pretreatment precipitated a significant cocaine-induced increase in ACTH. ACTH levels were examined for effects of etomidate dose (NS) and sampling time (df = 8, F = 5.01, p < .001) and dose × sampling time (interaction: df = 24, F = 1.65, p < .05). The infusion of 0.3 and 1.0 mg/kg etomidate produced behavioral changes in the most of the monkeys, consistent with its clinical use as an anesthetic, with the 0.3 mg/kg dose causing a glazed appearance in five of six subjects and ataxia in four subjects. At 1.0 mg/kg, etomidate produced glazed appearance, ataxia, and complete, brief anesthetization in all six subjects. All behavioral changes had dissipated during the 15- to 45-min pretreatment period before the start of the session. Despite the effectiveness of etomidate in disrupting the HPA axis response to different doses of self-administered cocaine, there was no significant change in either the rate of cocaine-maintained

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**Table 1** Summary of effects of etomidate, ketoconazole, astressin or dexamethasone pretreatment on HPA axis activity and high dose-cocaine self-administration behavior compared with HPA response when saline was available for self-administration (±S.E.M.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Subjects</th>
<th>Cortisol AUC</th>
<th>ACTH AUC</th>
<th>No. of Cocaine/ Saline Injections</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 cocaine</td>
<td>6</td>
<td>1,175 ± 78*</td>
<td>2,598 ± 321</td>
<td>12.75 ± 0.17</td>
<td>2.38 ± 0.45</td>
</tr>
<tr>
<td>+ 0.1 etomidate</td>
<td>5</td>
<td>887 ± 338*</td>
<td>1,981 ± 262</td>
<td>12.6 ± 0.4</td>
<td>1.95 ± 0.62</td>
</tr>
<tr>
<td>+ 0.3 etomidate</td>
<td>6</td>
<td>171 ± 39</td>
<td>2,756 ± 1,536</td>
<td>12.2 ± 0.58</td>
<td>2.13 ± 0.83</td>
</tr>
<tr>
<td>+ 1.0 etomidate</td>
<td>5</td>
<td>-402 ± 198*</td>
<td>15,378 ± 11,232</td>
<td>11.33 ± 0.84</td>
<td>1.59 ± 0.71</td>
</tr>
<tr>
<td>Saline alone</td>
<td>6</td>
<td>-124 ± 54</td>
<td>504 ± 129</td>
<td>8.87 ± 0.58*</td>
<td>0.20 ± 0.06*</td>
</tr>
<tr>
<td>0.3 cocaine</td>
<td>5</td>
<td>1,194 ± 84*</td>
<td>2,714 ± 344</td>
<td>12.75 ± 0.20</td>
<td>2.31 ± 0.44</td>
</tr>
<tr>
<td>+ 3.2 ketoconazole</td>
<td>5</td>
<td>603 ± 251*</td>
<td>1,782 ± 693</td>
<td>12.2 ± 0.37</td>
<td>1.33 ± 0.53</td>
</tr>
<tr>
<td>+ 10 ketoconazole</td>
<td>5</td>
<td>318 ± 217</td>
<td>1,573 ± 437</td>
<td>12.2 ± 0.58</td>
<td>2.27 ± 0.75</td>
</tr>
<tr>
<td>+ 32 ketoconazole</td>
<td>4</td>
<td>89 ± 140*</td>
<td>13,420 ± 7,171*</td>
<td>12.25 ± 0.48</td>
<td>1.76 ± 0.96</td>
</tr>
<tr>
<td>Saline alone</td>
<td>5</td>
<td>-122 ± 57</td>
<td>477 ± 134*</td>
<td>8.67 ± 0.62</td>
<td>0.20 ± 0.07*</td>
</tr>
<tr>
<td>0.3 cocaine</td>
<td>4</td>
<td>1,262 ± 75*</td>
<td>2,822 ± 359*</td>
<td>12.68 ± 0.25</td>
<td>2.22 ± 0.56</td>
</tr>
<tr>
<td>+ 0.1 astressin</td>
<td>4</td>
<td>526 ± 94*</td>
<td>1,396 ± 590*</td>
<td>13 ± 0</td>
<td>2.07 ± 0.49</td>
</tr>
<tr>
<td>+ 0.3 astressin</td>
<td>4</td>
<td>315 ± 36*</td>
<td>1,254 ± 338*</td>
<td>12.75 ± 0.25</td>
<td>1.65 ± 0.49</td>
</tr>
<tr>
<td>+ 1.0 astressin</td>
<td>4</td>
<td>123 ± 29*</td>
<td>689 ± 221*</td>
<td>13 ± 0</td>
<td>2.65 ± 0.68</td>
</tr>
<tr>
<td>Saline alone</td>
<td>4</td>
<td>-139 ± 68</td>
<td>529 ± 156</td>
<td>8.00 ± 0.73*</td>
<td>0.18 ± 0.08*</td>
</tr>
</tbody>
</table>

* Versus saline (p < .05).
† Versus no pretreatment and 0.1 etomidate pretreatment (p < .005).
‡ Versus all treatment conditions (p < .05).
§ Versus no pretreatment (p < .01).
¶ Versus 3.2 and 10 ketoconazole pretreatment (p < .05).
‖ Versus no pretreatment (p < .005).
¶¶ Versus no pretreatment and 0.1 astressin pretreatment (p < .05).
mg/kg ketoconazole. All behavioral changes had dissipated during the 30- to 60-min pretreatment period before the start of the session. Ketoconazole pretreatment did not change the rate of cocaine-maintained responding or the number of cocaine infusions delivered over a range of cocaine doses (Tables 1 and 2). The one exception occurred at the lowest dose of cocaine (0.01 mg/kg/injection), where there was a reduction in the number of injections taken following pretreatment with the lowest dose of ketoconazole (3.2 mg/kg). Our only explanation for this is that two of the three monkeys (the only subjects to show a reduction in both rate of responding and cocaine infusions) were given ketoconazole 20 and 23 min before the initiation of the session, whereas the pretreatment time used in all of the other experiments was 30 to 60 min. This effect on cocaine-maintained responding (0.01 mg/kg injection) was not evident in these subjects following a higher dose of ketoconazole (10 mg/kg; Table 2).

**Astrressin.** Pretreatment with astrressin dose-dependently reduced plasma cortisol released in response to self-administered cocaine (Fig. 3), with 1.0 mg/kg astrressin resulting in a cocaine-induced cortisol release similar to the HPA response when saline was available for self-administration (Table 1). Statistical analysis revealed a significant effect of astrressin pretreatment (df = 3, F = 4.62, p < .04), with the largest dose (1.0 mg/kg) producing an attenuation of cocaine-induced cortisol release over the entire sampling period (p < .05). There was a sampling order effect (df = 8, F = 34.05, p < .001) and a sampling order × treatment interaction (df = 24, F = 2.45, p < .005). All three astrressin doses significantly attenuated the cortisol AUC for self-administered cocaine (Table 1). Unlike etomidate and ketoconazole, astrressin also dose-dependently attenuated the cocaine-induced rise in ACTH (Fig. 3 and Table 1). Treatment with 1.0 mg/kg astrressin significantly inhibited ACTH release relative to no pretreatment (p < .05). There was a sampling order effect (df = 8, F = 22.75, p < .001) and a sampling order × treatment interaction (df = 24, F = 2.11, p < .01). Two monkeys vomited immediately following both the 0.3 and 1.0 mg/kg infusions of astrressin, but all behavioral effects dissipated during the 15-min pretreatment period. Pretreatment with astrressin had no effect on the rate of responding maintained by 0.3 mg/kg/injection cocaine or on the number of cocaine infusions delivered (Table 1).

**Dexamethasone.** Ten-hour pretreatment with 0.5 mg/kg dexamethasone effectively reduced ACTH and cortisol to levels at or below the level of detection of the assay. ACTH and cortisol levels were still suppressed 34 h later (Fig. 4). Dexamethasone blunted the cocaine-induced release of ACTH (df = 2, F = 14.08, p < .01) and cortisol (df = 2, F = 43.43, p < .0005). The AUC data for ACTH and cortisol release during the 0.3 mg/kg/injection cocaine self-administration session is shown in Table 1. Despite the almost complete attenuation of HPA responsiveness for 2 days after dexamethasone treatment, the number of cocaine infusions and the rate of responding remained unchanged (Table 1).

**Monkey 1583.** The only orchietomized male, had an atypical cortisol response to 0.3 mg/kg/injection cocaine following pretreatment with etomidate, ketoconazole, or astrressin. Instead of pretreatment producing an attenuation of cortisol release, as was seen with the other subjects, monkey 1583’s cortisol response to self-administered cocaine remained unchanged (p < .04; data not shown). This was not evident with

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**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Subjects</th>
<th>Cortisol AUC μg · min/dl</th>
<th>ACTH AUC pg · min/ml</th>
<th>No. of Cocaine/Saline Injections</th>
<th>Rate responses/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03 cocaine</td>
<td>5</td>
<td>515 ± 83b</td>
<td>1814 ± 201c</td>
<td>13 ± 0</td>
<td>2.49 ± 0.28</td>
</tr>
<tr>
<td>+ 0.1 etomidate</td>
<td>5</td>
<td>44 ± 161b</td>
<td>1323 ± 424</td>
<td>12.8 ± 0.20</td>
<td>3.01 ± 0.96</td>
</tr>
<tr>
<td>+ 0.3 etomidate</td>
<td>5</td>
<td>3 ± 141b</td>
<td>3008 ± 863</td>
<td>12.5 ± 0.2</td>
<td>3.02 ± 0.46</td>
</tr>
<tr>
<td>+ 1.0 etomidate</td>
<td>5</td>
<td>51 ± 162b</td>
<td>2338 ± 978</td>
<td>12.75 ± 0.25</td>
<td>2.53 ± 1.04</td>
</tr>
<tr>
<td>Saline alone</td>
<td>5</td>
<td>-56 ± 62</td>
<td>666 ± 187</td>
<td>8.80 ± 0.79</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>0.01 cocaine</td>
<td>3</td>
<td>349 ± 98b</td>
<td>1629 ± 205</td>
<td>11.7 ± 0.35</td>
<td>2.31 ± 0.60</td>
</tr>
<tr>
<td>+ 0.1 etomidate</td>
<td>3</td>
<td>232 ± 160</td>
<td>1404 ± 319</td>
<td>12.7 ± 0.33</td>
<td>2.18 ± 1.04</td>
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<tr>
<td>+ 0.3 etomidate</td>
<td>3</td>
<td>-168 ± 106b</td>
<td>1470 ± 206</td>
<td>11.0 ± 1.53</td>
<td>1.31 ± 1.10</td>
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<tr>
<td>+ 1.0 etomidate</td>
<td>3</td>
<td>-176 ± 56b</td>
<td>7525 ± 4476a</td>
<td>13 ± 0</td>
<td>3.41 ± 1.03</td>
</tr>
<tr>
<td>Saline alone</td>
<td>3</td>
<td>-50 ± 93</td>
<td>794 ± 255</td>
<td>7.00 ± 1.12</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>-88 ± 92</td>
<td>424 ± 143</td>
<td>7.24 ± 2.65</td>
<td>0.11 ± 0.05</td>
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<tr>
<td>+ 0.1 etomidate</td>
<td>2</td>
<td>-31 ± 227</td>
<td>150 ± 412</td>
<td>6.50 ± 0.50</td>
<td>0.05 ± 0.01</td>
</tr>
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<td>2</td>
<td>-3 ± 236</td>
<td>543 ± 309</td>
<td>11.0 ± 0.5</td>
<td>0.25 ± 0.04</td>
</tr>
<tr>
<td>+ 1.0 etomidate</td>
<td>2</td>
<td>-501 ± 84b</td>
<td>505 ± 378</td>
<td>8.5 ± 0.5</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>0.1 cocaine</td>
<td>2</td>
<td>1158 ± 343a</td>
<td>3470 ± 1000</td>
<td>13 ± 0</td>
<td>3.04 ± 0.57</td>
</tr>
<tr>
<td>+ 10 ketoconazole</td>
<td>2</td>
<td>-189 ± 8b</td>
<td>1109 ± 140</td>
<td>13 ± 0</td>
<td>2.27 ± 0.78</td>
</tr>
<tr>
<td>Saline alone</td>
<td>2</td>
<td>-88 ± 92</td>
<td>424 ± 143</td>
<td>7.24 ± 2.65</td>
<td>0.11 ± 0.05</td>
</tr>
<tr>
<td>0.03 cocaine</td>
<td>3</td>
<td>557 ± 87a</td>
<td>1897 ± 195</td>
<td>13 ± 0</td>
<td>2.93 ± 0.29</td>
</tr>
<tr>
<td>+ 3.2 ketoconazole</td>
<td>3</td>
<td>-41 ± 239b</td>
<td>1006 ± 61</td>
<td>13 ± 0</td>
<td>3.89 ± 1.18</td>
</tr>
<tr>
<td>+ 10 ketoconazole</td>
<td>3</td>
<td>-133 ± 341b</td>
<td>2907 ± 825</td>
<td>13 ± 0</td>
<td>4.49 ± 1.52</td>
</tr>
<tr>
<td>Saline alone</td>
<td>3</td>
<td>-50 ± 93</td>
<td>794 ± 255</td>
<td>7.00 ± 1.12</td>
<td>0.10 ± 0.03</td>
</tr>
<tr>
<td>0.01 cocaine</td>
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<td>349 ± 98a</td>
<td>1629 ± 205</td>
<td>11.7 ± 0.35</td>
<td>2.31 ± 0.60</td>
</tr>
<tr>
<td>+ 3.2 ketoconazole</td>
<td>3</td>
<td>-37 ± 113b</td>
<td>1031 ± 138</td>
<td>10.0 ± 1.73</td>
<td>1.36 ± 1.26</td>
</tr>
<tr>
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<td>-45 ± 77b</td>
<td>1680 ± 819</td>
<td>13 ± 0</td>
<td>2.65 ± 1.18</td>
</tr>
<tr>
<td>Saline alone</td>
<td>3</td>
<td>-50 ± 93</td>
<td>794 ± 255</td>
<td>7.00 ± 1.12</td>
<td>0.10 ± 0.03</td>
</tr>
</tbody>
</table>

a Different from saline (p < .05).
b Significant effect of pretreatment (p < .05).
c Saline different from all other conditions (p < .05).
lower doses of cocaine following pretreatment with etomidate or ketoconazole, probably because the HPA response to lower doses of cocaine was less robust. Despite the apparent lack of sensitivity of the adrenal gland of monkey 1583 to the pharmacological inhibition of cortisol release, his ACTH response to the various treatments was similar to the other subjects. The characteristics of cocaine-reinforced responding for monkey 1583, including rate, intake, and sensitivity to dose, did not differ from the other monkeys in this study.

**Discussion**

The increases in plasma ACTH and cortisol that occurred with i.v. self-administration of cocaine are in agreement with our previous study (Broadbear et al., 1999a). In this earlier study, a range of cocaine doses (0.01, 0.03, 0.1, and 0.3 mg/kg/injection) and saline were made available for self-administration, resulting in a cocaine-dose-dependent increase in HPA axis activity. We found that the smaller cocaine doses (0.01 and 0.03 mg/kg/injection) supported self-administration behavior without significantly increasing ACTH or cortisol plasma levels. The findings presented in this current study focused on inhibition of the HPA response to a range of cocaine doses, although our earlier observations indicated that only 0.1 and 0.3 mg/kg/injection cocaine led to statistically significant elevations in ACTH and cortisol levels in this behavioral paradigm.

Acute i.v. pretreatment with etomidate, ketoconazole, and astressin produced a dose-dependent attenuation of the cortisol response to cocaine. The largest dose for each of the pretreatment compounds was sufficient to abolish the cocaine-induced rise in cortisol, maintaining cortisol at preession levels or below, similar to occasions in which saline was available for self-administration. Etomidate and astressin shared similar potency in reducing cortisol release and were at least 30 times more potent than ketoconazole. Despite the fact that each of these compounds acutely inhibited the cocaine-induced release of cortisol, there was no evidence for any change in ongoing cocaine-maintained behavior.

Our results contrast with a similar study using rats, in which ketoconazole dose-dependently reduced the number of low-dose cocaine infusions without affecting food-maintained responding (Goeders et al., 1998). Although in rats ketoconazole appeared to disrupt responding maintained by smaller doses of cocaine (0.125 and 0.25 mg/kg/injection), its effectiveness against a larger cocaine dose (0.5 mg/kg/injection) was difficult to ascertain, as rats trained at this dose of cocaine showed no change in overall self-administration behavior following either ketoconazole treatment or saline substitution, perhaps indicating a greater resistance to extinction for this dose of cocaine than at lower training doses (Goeders et al., 1998). In addition, in rats the effect of self-administered cocaine on corticosterone was not dose-dependent, although at the doses of cocaine for which ketoconazole reduced responding, corticosterone levels were also decreased. Therefore, the findings of Goeders et al. (1998) and the present study diverge, as attenuation of basal (with dexamethasone) or cocaine-induced cortisol release in rhesus monkeys did not change self-administration behavior at any dose of cocaine.

In agreement with the present findings are two studies by Ward et al. (1998, 1999) in which human subjects with a history of cocaine use were either pretreated with oral ketoconazole (0, 600, or 1200 mg) 1 h before smoking cocaine (0, 12, or 50 mg; Ward et al., 1998) or pretreated with 2 mg of dexamethasone 10 h before smoking cocaine (Ward et al., 1999). Blood levels of cocaine, cortisol, and ACTH were mea-
sured, as well as subjective ratings and cardiovascular effects. Ketoconazole pretreatment attenuated the cortisol response to cocaine without changing ACTH release. Ketoconazole (1200 mg) was also associated with reductions in the cardiovascular response to 50 mg of cocaine but did not produce any changes in the subjective ratings for cocaine, except to lower anxiety ratings before cocaine administration. Dexamethasone pretreatment produced slight increases in cardiovascular measures, enhanced the cocaine-induced increase in heart rate, and abolished the cocaine-induced HPA activation. However, although dexamethasone pretreatment in combination with the high dose of cocaine did enhance some subjective ratings (e.g., “The dose was of high quality” and “The dose was potent”), it did not affect the positive ratings such as “I feel high” and “I feel a good drug effect” produced by cocaine administration (Ward et al., unpublished data). Recent work by Mantsch and Goeders (1999), in agreement with Ward et al. (1998), found that ketoconazole did not block the discriminative effects of cocaine in rats (Ward et al., 1999).

Previous studies evaluating the effects of etomidate on HPA axis function have largely focused on etomidate's clinical use as a sedative hypnotic (Wagner et al., 1984; Allolio et al., 1985; Fellows et al., 1985; Crozier et al., 1997). There are no reports of etomidate having been used to attenuate the cortisol response to cocaine or other self-administered drugs. The use of etomidate to induce and/or maintain anesthesia does suppress the postsurgical cortisol response (Wagner et al., 1984; Fellows et al., 1985), and measurement of the intermediate products of adrenal steroid synthesis has shown that the mechanism for etomidate's effect on cortisol synthesis is inhibition of mitochondrial P-450 enzymes, particularly 11-β-hydroxylase (Wagner et al., 1984; Allolio et al., 1985). Ketoconazole, as an antimycotic, has a very different clinical application. Both ketoconazole and etomidate, however, share an imidazole structure that is probably the basis for the similarity of their actions on steroidogenesis. Large doses (600–1200 mg/day) are required therapeutically to inhibit adrenal steroid synthesis (Pont et al., 1984; Allolio et al., 1985; Fellows et al., 1985; Crozier et al., 1997) and bind glucocorticoid receptors (Loose et al., 1983; Feldman, 1986). The compensatory increase in ACTH following the inhibition of steroid synthesis (Pont et al., 1984; Allolio et al., 1985; Fellows et al., 1985; Crozier et al., 1997) was also observed in the present study at doses of etomidate (1.0 mg/kg) and ketoconazole (32 mg/kg) that maximally suppressed the cortisol response to cocaine. Despite the effectiveness of the cortisol synthesis blockade at these doses, neither ketoconazole nor etomidate had any effect on ongoing cocaine-reinforced responding over a range of cocaine doses, either in...
terms of the frequency of lever pressing or the number of infusions earned. There is one report that ketoconazole was ineffective in rats that lacked experience with saline substitution (Goeders et al., 1998), but this is not an issue in the present study, as the cocaine dose was varied and saline was frequently substituted.

Pretreatment with larger doses of etomidate and ketoconazole produced a concomitant increase in ACTH levels because of their inhibition of glucocorticoid feedback. Is there any evidence of a role for ACTH in cocaine-reinforced responding? There is some correlative data suggesting a relationship between cocaine's effects and ACTH release in humans (Scholar et al., 1998; Ward et al., 1998). Ward and colleagues found a close correlation between plasma ACTH and cocaine's cardiovascular effects, and both the Ward and Scholar studies reported a temporal correlation of cocaine and ACTH peak plasma levels and subjective effects. Neither study was designed to evaluate the causality of these relationships. However, the use of the peripheral CRF antagonist, astressin, in the present study may shed some light on the role of ACTH in cocaine's effects. Astressin dose-dependently attenuated the ACTH response to self-administered cocaine, yet there was no change in response rate or infusion number. Furthermore, basal plasma ACTH levels wereabolished by dexamethasone pretreatment without producing any change in cocaine-maintained behavior. Dexamethasone occupied and activated glucocorticoid receptors to such an extent that the HPA axis was effectively inactivated for several days. Yet despite the evidence for maximal glucocorticoid activation, along with the absence of ACTH release, and perhaps by extrapolation, CRF release, there was no change in cocaine-directed behavior. Our findings of a lack of effect of dexamethasone on cocaine-reinforced behavior are similar to results reported by Ward et al. (1999) described earlier.

Only a few studies have addressed the role of the HPA axis in maintaining ongoing cocaine-seeking behavior (Goeders and Guerin, 1996a; Miczek and Mutschler, 1996; Goeders et al., 1998). However, there is a lack of agreement between rodent and primate studies. Rodent work has focused on the importance of a precocaine elevation in plasma corticosterone, estimated to be in the vicinity of 150 pg/ml or more (Goeders and Guerin, 1996b), which perhaps serves to increase receptiveness of the rat to cocaine's effects (for review, see Goeders, 1997; Piazza and le Moal, 1998). Certainly, corticosterone seems critical for all aspects of cocaine and amphetamine self-administration (acquisition, maintenance, and relapse) in rats, although several studies have provided evidence that CRF, not corticosterone, may be of key importance in relapse to cocaine (Erb et al., 1998) or heroin self-administration in rats (Shaham et al., 1996, 1997). In primates, the presence of basal levels of ACTH and cortisol appears to be unimportant for continued cocaine-seeking behavior, as does the increase in HPA axis activity in response to cocaine administration. However, these findings do not rule out a role for the effects of CRF release on central CRF receptors and the subsequent release of neuropeptides in the central nervous system, as we have not measured CRF in plasma or cerebrospinal fluid and it is doubtful that astressin penetrates the central nervous system following i.v. administration.

This study demonstrates the relative effectiveness with which etomidate, ketoconazole, astressin, and dexamethasone attenuate the plasma ACTH and cortisol response following cocaine self-administration in rhesus monkeys. Despite the fact that these pretreatments selectively reduced cortisol and/or ACTH release in cocaine-maintained monkeys, there was no behavioral evidence of any change in cocaine's reinforcing effects. Hence, for rhesus monkeys with a history of cocaine self-administration, it appears that acute changes in the ACTH or cortisol response to cocaine do not play a direct role in modulating cocaine-seeking behavior under these experimental circumstances.

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References


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