Effects of Self-Administered Cocaine on Plasma Adrenocorticotropic Hormone and Cortisol in Male Rhesus Monkeys

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ABSTRACT

This study was designed to examine the effects of self-administered cocaine on hypothalamic-pituitary-adrenal (HPA) axis activity in rhesus monkeys. Initially, basal release of cortisol and adrenocorticotropic hormone (ACTH) was measured in singly housed male and female monkeys (n = 9) over a 24-h period using plasma samples obtained from indwelling venous catheters. Basal cortisol and ACTH levels in both male and female rhesus monkeys demonstrated a circadian pattern of release, with peak levels for cortisol (19.60 ± 2.16 μg/dl) and ACTH (19.63 ± 2.56 pg/ml) measured at 6:00 AM. The nadir for ACTH (6.27 ± 0.62 pg/ml) occurred at 6:00 PM, preceding the cortisol nadir (5.55 ± 1.21 μg/ml) at 9:00 PM. The reinforcing effects of saline, 0.01, 0.03, 0.1, and 0.3 mg/kg/injection cocaine were then evaluated using a fixed-ratio 30, time-out 10-min schedule of reinforcement in seven male monkeys. Blood was sampled before, during, and after self-administration sessions. Self-administration of cocaine produced dose-dependent increases in cortisol and ACTH. One dose of cocaine (0.03 mg/kg/injection), although reliably self-administered, did not produce a significant increase in HPA axis activity. These results indicate that although cocaine dose-dependently increases HPA axis activity, the HPA effect is more likely a consequence of overall cocaine intake than it is an indicator of cocaine doses that are sufficient to maintain self-administration behavior.

Since the original observation that cocaine administration resulted in activation of the hypothalamic-pituitary-adrenal (HPA) axis via a corticotropin-releasing hormone (CRH)-mediated mechanism in the rat (Rivier and Vale, 1987; Moldow and Fischman, 1987), a number of published studies have attempted to address the significance of this effect. Activation of the HPA axis by cocaine is not unique to the rat; the same has been reported in rhesus monkeys (Sarnyai et al., 1996) as well as in human subjects (Mendelson et al., 1989; Vescovi et al., 1992; Heesch et al., 1995). In humans, the rise in plasma adrenocorticotropic hormone (ACTH) subsequent to i.v. administered cocaine has been shown to mirror the time course of cocaine’s distribution in the blood (Scholar et al., 1998), both of which precede the slower and more sustained cortisol response (Wilkins et al., 1992). Scholar and coworkers’ results also indicate that in experienced cocaine users, the administration of cocaine rapidly triggers the release of CRH, which in turn stimulates the rise in plasma ACTH.

Manipulations that use surgical or pharmacological means to decrease HPA activity in rats also produce a notable increase in both the dose and time needed for the acquisition of cocaine self-administration (Goeders and Guerin, 1994). These manipulations also decrease ongoing self-administration behavior (Goeders and Guerin, 1996a) as well as reduce the likelihood of a resumption in drug-maintained responding after a period of abstinence or saline extinction (Piazza et al., 1994; Piazza and le Moal, 1998). In related studies, rats that were designated as “high responders” after being prescreened for their locomotor and corticosterone responses to either a novel environment or an i.p. amphetamine injection also showed an increased propensity to self-administer amphetamine (Piazza et al., 1989, 1991) or cocaine (Goeders and Guerin, 1996) at smaller doses. Collectively, the results of these studies have led to speculation that individuals whose HPA axis is highly responsive to a stressor may possess a physiological profile that renders them more vulnerable to drug abuse (Piazza and le Moal, 1996; Goeders, 1997).

ABBREVIATIONS: CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropic hormone; FR, fixed-ratio; TO, time-out; AUC, area under curve.
Although it is generally accepted that passive cocaine administration stimulates activity of the HPA axis, there are very little published data demonstrating the effects of self-administered cocaine on HPA activity. The only exception was in a study done by Goeders and Guerin (1996b) where single samples of blood were obtained from rats before and after self-administration of different doses of cocaine. Mean plasma corticosterone levels after cocaine self-administration were reported to be significantly elevated above levels measured before cocaine exposure for all of the doses of cocaine that were tested. However, there was no indication as to whether this increase was dose-dependent in terms of either the dose of cocaine that was available, or the number of infusions that were taken.

Complex logistics are involved in studying the relationship between cocaine administration and the corresponding HPA response. Environmental factors such as handling of the animals, needle poke in the case of both i.p. cocaine administration and peripheral blood sampling, and relocation to a testing enclosure may each produce substantial HPA activation quite apart from any effect produced by the cocaine (Elvidge et al., 1976; Blank et al., 1983; Reinhardt et al., 1990; Capitanio et al., 1996). These kinds of manipulations may actually blunt the response to cocaine as a prior increase in baseline ACTH and glucocorticoids may reduce HPA reactivity to subsequent stimulation via negative feedback (Keller-Wood and Dallman, 1984; Dallman et al., 1994), as well as obscure differences between cocaine and saline controls. This is not to say that all extra-hypothalamic CRH release is inhibited in the same way; however the apparent sensitivity of the HPA system to extraneous stimuli highlights the need for use of familiar procedures that minimize disturbance to the subject.

In the present study, we initially examined the basal release of ACTH and cortisol and its circadian rhythmicity in male and female monkeys, some of whom were experienced with cocaine self-administration and some of whom had no such experience. Using these data as a reference, we evaluated the HPA response to self-administered cocaine in the morning and afternoon in male rhesus monkeys with extensive histories of cocaine self-administration. To minimize both disturbance to the monkeys as well as experimenter-induced increases in ACTH and cortisol, testing took place in their home cages and blood was sampled from behind each cage via the same indwelling venous catheter as was used for drug infusions.

Materials and Methods

Subjects

Seven adult male rhesus monkeys (Macaca mulatta), weighing between 9.0 and 14 kg, and three adult females, weighing between 5.0 and 7.4 kg, were used in this study. These monkeys were individually housed in stainless steel cages measuring 83.3 × 76.2 × 91.4 cm deep (Bryan Research Equipment Corporation, Bryan, TX) located in a laboratory that contained a total of 24 monkeys. The monkeys were fed 8 to 14 Purina Monkey Chow biscuits twice daily to maintain adult body weight and water was available ad libitum. The laboratory was illuminated from 7:00 AM to 10:30 PM. 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. After placement in the vein, the catheter was guided s.c. to the mid-scapular region where it exited the monkey. The external portion of the catheter was protected inside the cage by a flexible stainless steel arm, with one end of the arm attached to the double-layer polyester jacket (Lomir, New York, NY) worn by the monkey and the other bolted to the rear of the cage.

Circadian Rhythm Study. Six male and three female monkeys were subjects for this study. All monkeys had been acclimated to the jacket and flexible arm apparatus over a period of months or years. Five monkeys had a history of cocaine self-administration, two monkeys had received cocaine passively on one or more occasions, and two monkeys were cocaine-naive. Ketamine, used for sedation, was the only psychoactive drug noted in the histories of the latter two monkeys. For those monkeys conditioned to cocaine self-administration, testing was suspended in this phase of the study. Blood (1.4 ml) was sampled at 3-h intervals over a 24-h period from the rear of the cage via the venous catheter. Each monkey was exposed to this sampling procedure once only. Sample collection and handling is described below.

Cocaine Self-Administration Study. Seven adult male monkeys (five of whom were in the circadian study) were subjects for this study. Four of the seven monkeys had 1 or more years experience with the self-administration of different doses of cocaine under the fixed-ratio (FR) 30 time-out (TO) 10 min. The remaining three subjects were more recently trained and had 1- to 6-months’ experience with cocaine self-administration under the FR 30 TO 10 min schedule. Each monkey had blood samples taken between one and twelve times (four on average) at each dose of cocaine or saline.

Apparatus

Each cage had a 15- × 20-cm panel fixed to the right wall of the cage. 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 time-out, all stimulus lights were extinguished and responding had no programmed consequences.

The experiment was controlled by IBM/PS2 computers located in an adjacent room. The computers were programmed using Med Associates software (Georgia, VT).

Procedure

Circadian Rhythm Study. Blood sampling for the study of basal release of ACTH and cortisol took place on two separate occasions. Five monkeys were tested on the first occasion and four on the second. Blood (1.4 ml) was sampled at 3-h intervals, beginning at 9:00 AM and ending with the 9:00 AM sample the following day. Self-administration testing for the monkeys in this study was suspended for the duration of the basal sampling period, although the other monkeys in the room were tested as usual. The blood sample handling procedure is described below.

Self-Administration Study. Drug self-administration sessions were scheduled twice daily for 130 min starting at approximately 10:00 AM and 4:00 PM. There was a maximum of 13 infusions available in each session. The effect of cocaine self-administration on ACTH and cortisol was measured primarily before, during, and after the morning session, although on a few occasions the HPA response to cocaine was measured during the evening session for comparison. All seven monkeys were tested with saline, 0.01, 0.03, 0.1, and 0.3 mg/kg/injection cocaine on an FR 30 TO 10 min schedule of reinforcement. The criteria required for a stable baseline of self-administration behavior were response rates of greater than one response per second for 0.03 mg/kg/injection cocaine, and delivery of the maximum number of injections available during the session (13 injec-
Blood was sampled no more than two or three times per week. A sample of venous blood was drawn via the catheter 5 to 30 min before the morning 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 rate slowed during the session). Blood samples continued to be drawn at 15 min postsession, and at hourly intervals for the next 3 h, making a total of nine blood draws. When sampling took place during the evening session, blood continued to be drawn at the same intervals as during the morning session, making a total of 13 blood draws for the day. Each blood sample (1.1–1.4 ml) was placed in a 2 ml Vacutainer (Becton Dickinson & Co., Franklin Lakes, NJ) containing 0.04 ml of 7.5% EDTA and immediately placed on ice. After each blood sample, 1.5 to 3 ml of 30 U/ml heparin saline solution was infused into the catheter and, when sampling was done during sessions in which cocaine was available, a volume of the cocaine solution equal to the catheter volume was injected (0.6–1.5 ml). Samples were centrifuged at 5,000 RPM and 4°C for 5 min and then the plasma (0.7 ml) was pipetted into 2-ml Cryovials (Corning Costar Corp., Cambridge, MA) 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 measured using radioimmunoassay kits (cortisol: Diagnostic Products Corporation, Los Angeles, CA; ACTH: Nichols Institute Diagnostics, San Juan Capistrano, CA). In 90% of cases, single determinations were made for both cortisol and ACTH levels from each blood sample. Ten percent of samples were assayed in duplicate to determine inter- and intra-assay variability. Each cortisol and ACTH kit was used to measure approximately 100 samples. For the cortisol assay, the range of detection was 0.2 to greater than 50 μg/dl. Interassay variability was 4 to 6.5%, and intra-assay variability was 3 to 5%. For the ACTH assay, the range of detection was 0.5 to 1500 pg/ml. Interassay variability was 7%, and intra-assay variability was 3%.

Data Analysis

Circadian Rhythm Study. The plasma cortisol and ACTH levels over the 24-h sampling period were averaged for each sampling time according to gender and then analyzed for gender differences. In the absence of a sex difference, the data for male and female monkeys were averaged and analyzed as described below.

Self-Administration Study. Rate of responding during each session was defined as the number of responses emitted while the red stimulus light was on divided by the number of seconds that the light was illuminated (responses/s). The total dose of cocaine (mg/kg) for each monkey was the number of infusions taken during the session multiplied by the dose per infusion (mg/kg/injection). Plasma cortisol (μg/dl) and ACTH (pg/ml) levels are shown in raw form as well as after normalization to area under curve (AUC) data. AUC values provide an estimate of the cortisol or ACTH release relative to basal levels during the self-administration session. AUC values were 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/dl) 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 or two-way ANOVA and post hoc pairwise comparisons using the Tukey HSD test of significance (p < .05) were carried out using Statistica (v.5.0, Statsoft, Tulsa, OK). When experiments were replicated within subjects, the mean response for each subject was used in calculating treatment effects across subjects.
was no difference in basal ACTH levels between the two groups (data not shown). Six of the nine monkeys showed an additional cortisol peak at 12:00 PM, whereas eight of the nine monkeys showed an increase in ACTH at this time.

**Cocaine Self-Administration.** The response rate and cocaine intake from the cocaine self-administration study are shown in Fig. 2. The response rate for the session was lowest when saline was available for self-administration (0.3 ± 0.1 responses/s) and increased as a function of the available dose of cocaine (0.01–0.1 mg/kg/injection). The ascending arm of the dose-response curve was generated, with the peak rate of responding (3.5 ± 0.5 responses/s) maintained by 0.1 mg/kg/injection cocaine. There was a slight, nonsignificant rate decrease at 0.3 mg/kg/injection cocaine (2.6 ± 0.4 responses/s), the largest dose that was tested. Total drug intake (mg/kg) was positively related to the dose of cocaine that was available for self-administration. One-way ANOVA was used to examine the effects of cocaine dose on both rate of responding and total drug intake (rate: df = 4, F = 20.52, p < .001; intake: df = 4, F = 5177, p < .001), indicating that increasing the dose of cocaine led to significantly increased rates of responding and cocaine intake. The results of subsequent pairwise comparisons are shown in the legend to Fig. 2.

Cocaine self-administration produced dose-dependent increases in plasma cortisol and ACTH levels (Fig. 3, top and bottom, and Table 1). Presession, as well as peak within-session plasma ACTH and cortisol levels, are presented for each dose of cocaine (Table 1). The peak plasma level of cortisol generally occurred later in the session than the ACTH peak (Table 1). Plasma levels of ACTH and cortisol decreased during the 3 h after the morning self-administration session, taking between 1 and 3 h to return to presession levels. Plasma cortisol levels obtained from samples 2 to 6 taken during the self-administration session were examined for effects of dose and sampling time using 2-way ANOVA. Dose (df = 4, F = 5.07, p < .005), sampling time (df = 4, F = 10.48, p < .001) and dose × sampling time (interaction: df = 16, F = 2.79, p < .001) all had significant effects on plasma cortisol. Plasma ACTH levels were similarly examined. Sampling time (df = 4, F = 22.23, p < .001) and dose × sampling time (interaction: df = 16, F = 2.40, p < .005) both had significant effects on plasma ACTH. Post hoc pairwise comparisons evaluating the effect of both dose and sampling time for cortisol and ACTH are summarized in the legend to Fig. 3.

The cortisol and ACTH response to 0.3 mg/kg/injection cocaine when it was self-administered during the morning or afternoon sessions is compared in Fig. 4. Several monkeys showed a diminished HPA response to 0.3 mg/kg injection cocaine self-administration during the afternoon session, despite the fact that response rates and cocaine intake did not differ between morning and afternoon sessions (data not shown). The greater sensitivity of the HPA axis to cocaine during the morning session corresponds with higher basal levels of cortisol and ACTH, whereas during the evening session, basal HPA activity is approaching its nadir (see Fig. 1).

The AUC data for plasma ACTH and cortisol release is shown in Fig. 5. These values represent the cumulative release of ACTH and cortisol above pre-session levels during the cocaine (or saline) self-administration sessions. Between-subjects ANOVA, measuring the effect of cocaine dose on cortisol or ACTH AUC, did not reveal any statistical difference among the monkeys in their HPA response to cocaine or saline self-administration. Subsequent 1-way ANOVA revealed a significant effect of cocaine dose on both cortisol...
The results of post hoc pairwise comparisons are described in the legend to Fig. 5.

**Discussion**

The results of the circadian study presented here demonstrate a diurnal rhythmicity in the release of ACTH and cortisol in both male and female rhesus monkeys. This basal release constituted a stable baseline from which the HPA axis response to self-administered cocaine could be measured. Males and females were no different with respect to the timing of the peak, nadir, or magnitude of the plasma ACTH and cortisol levels. Four of the nine monkeys tested had no self-administration history. These four tended to have higher cortisol levels; however, ACTH levels for both groups were the same. This difference may reflect either drug-taking history or the novelty of the experimental procedure, as three of these monkeys had undergone their first catheter placement surgeries within 2 weeks of the circadian study. Comparison of the male and female data is complicated by the fact that all of the female monkeys in our study also belonged to the group that lacked self-administration history.

Our circadian data showed a second, smaller peak at 12:00 PM for the majority of subjects. Additional peaks in ACTH and cortisol have been reported in humans (Slag et al., 1981; Goldman et al., 1985) and were attributed to meal ingestion, particularly high protein lunches. The unexpected rise in ACTH and cortisol seen in our data is unlikely to be due to food ingestion as the monkeys were fed at 8:30 AM and 2:30 PM daily. The only other activity likely to have disrupted basal release of cortisol and ACTH was the 2-h 10-min self-administration session scheduled daily from 10:00 AM until 12:10 PM. Although the monkeys in this study had their self-administration sessions suspended during the sampling period, the remaining monkeys in the room were tested as usual. A recent study by Caggiula and coworkers (1998) reported that rats showed a conditioned elevation of plasma corticosterone in response to environmental cues associated with nicotine delivery. The fact that a self-administration session did take place during the sampling time makes the possibility of a conditioned HPA axis response the most likely.

**TABLE 1**

<table>
<thead>
<tr>
<th>Cocaine</th>
<th>Time of ACTH Peak</th>
<th>ACTH (±S.E.M.)</th>
<th>Time of Cortisol Peak</th>
<th>Cortisol (±S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg/injection</td>
<td></td>
<td>pg/ml</td>
<td></td>
<td>µg/dl</td>
</tr>
<tr>
<td>0 (presession)</td>
<td>8th infusion</td>
<td>6.56 - 10.32</td>
<td>&lt; pre-session</td>
<td>9.43 - 11.89</td>
</tr>
<tr>
<td>0 (saline)</td>
<td>13th infusion</td>
<td>11.82 ± 2.2</td>
<td>13th infusion</td>
<td>11.20 ± 1.00</td>
</tr>
<tr>
<td>0.01</td>
<td>8th infusion</td>
<td>19.76 ± 3.82</td>
<td>15 min postsession</td>
<td>14.48 ± 1.73</td>
</tr>
<tr>
<td>0.03</td>
<td>8th infusion</td>
<td>20.95 ± 2.59</td>
<td>13th infusion</td>
<td>15.00 ± 1.57</td>
</tr>
<tr>
<td>0.1</td>
<td>8th infusion</td>
<td>22.12 ± 2.99</td>
<td></td>
<td>20.19 ± 0.97</td>
</tr>
<tr>
<td>0.3</td>
<td>8th infusion</td>
<td>29.16 ± 2.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(df = 4, F = 12.82, p < .001) and ACTH AUC (df = 4, F = 2.84, p < .05). The results of post hoc pairwise comparisons are described in the legend to Fig. 5.

**Fig. 4.** Effect of self-administered cocaine (0.3 mg/kg/injection) on plasma cortisol (µg/dl) and ACTH (pg/ml) during morning or afternoon self-administration sessions in five subjects. The HPA response to cocaine was more robust in some monkeys during the “AM SESSION”, when basal cortisol and ACTH levels were at a peak, than during the “PM SESSION” when basal HPA activity was approaching its nadir (see Fig. 1). Rates of responding as well as cocaine intake during the morning and afternoon sessions were comparable for each monkey. Other details as for Fig. 2.
levels did not produce a significant elevation in HPA activity above levels produced by saline self-administration. In fact, under these schedule conditions there was no difference in the rates of responding maintained by 0.03, 0.1, and 0.3 mg/kg/injection cocaine, although the intake of cocaine differed markedly, as did the ACTH and cortisol response. This is evidence for a dissociation between doses of cocaine that clearly maintained responding versus doses that clearly elevated HPA activity. So although HPA activation by cocaine appeared dose-dependent, a dose of cocaine that maintains self-administration behavior does not necessarily produce significant HPA activation. This point is discussed further below.

It is difficult to ascertain from other published work in this field whether a dose of cocaine will produce the same HPA response when it is injected by the investigator or self-administered by the subject. When cocaine was injected i.v. into rats, it produced a dose-dependent increase in plasma ACTH (Rivier and Vale, 1987) and corticosterone (Moldow and Fischman, 1987). The one paper that directly addressed cocaine self-administration and activation of the HPA axis in the rat did not provide an estimate of cocaine intake (Goeders and Guerin, 1996b). This prevents a comparison of the results of these two studies. The only work of this kind in rhesus monkeys examined the plasma ACTH and cortisol response to a single infusion of 0.4 and 0.8 mg/kg cocaine in males (Sarnyai et al., 1996) and females (Sarnyai et al., 1995). Although female monkeys showed no change in HPA function in response to cocaine, increases in ACTH and cortisol levels in male monkeys were proportional to the cocaine-induced level of behavioral activation. It is noteworthy that the pre-infusion cortisol and ACTH levels in most of the monkeys in the study by Sarnyai et al. (1996) are far greater (cortisol is 2-fold higher and ACTH is 4-fold higher) than in the monkeys in the present study, perhaps reflecting procedural differences between the studies. A consequence of these high baseline values may have been a blunting of cocaine’s effect on HPA activity. Sarnyai gave 0.4 and 0.8 mg/kg i.v. in a single infusion, whereas in the present study, monkeys self-administered cocaine incrementally with the total dose (0.13, 0.39, 1.3, and 3.9 mg/kg) being injected over the course of a 2-h session. Sarnyai found that the subgroup of monkeys that were responsive to cocaine had increases in cortisol and ACTH release after the 0.8 mg/kg but not after the 0.4 mg/kg dose. In the present study, the dose of cocaine that resulted in a cumulative intake of 0.39 mg/kg (0.03 mg/kg/injection) did not lead to a significant increase in ACTH and cortisol release, despite the fact that this dose of cocaine maintained high rates of responding. The earliest indication in the session that cocaine was increasing HPA axis activity beyond saline levels was after the 4th infusion of 0.3 mg/kg/injection (total dose = 1.2 mg/kg over 30 min), and after the 8th infusion of 0.1 mg/kg/injection (total dose = 0.8 mg/kg over 70 min). Thus it is possible that the dose of i.v. cocaine that marks the threshold for activation of the HPA axis lies between 0.4 and 0.8 mg/kg. If this were the case, it could be anticipated that doses of cocaine that, either singly or cumulatively, do not reach this range, would not activate the HPA axis. This casts some doubt on any suggestion that glucocorticoid release may be integral to cocaine’s reinforcing effects.

The results presented here are in agreement with previous reports that cocaine administration is correlated with in-
creased HPA activity. However, in contrast to previous studies, the data presented here were collected from awake, minimally disturbed monkeys, behaving in their home cages, and with control over cocaine delivery. Under these conditions, male and female monkeys exhibited similar, normal diurnal variation in basal ACTH and cortisol release. In male monkeys with an extensive cocaine self-administration history, plasma ACTH and cortisol levels increased as a function of cocaine intake, indicating that self-administered cocaine dose-dependently increased HPA activity. Nevertheless, there was evidence that small, but nevertheless reinforcing, doses of cocaine failed to produce significant activation of the HPA axis. Thus, the procedure used in this study for examining cocaine's effect on HPA function demonstrated a low, stable cortisol and ACTH baseline upon which a clear effect of cocaine was measured.

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References


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