Effects of Cocaine Self-Administration on Plasma Corticosterone and Prolactin in Rats

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ABSTRACT

The effects of i.v. cocaine self-administration under "naturalistic" conditions on plasma corticosterone (CORT) and prolactin (PRL) were investigated in male Sprague-Dawley rats. After the determination of plasma CORT and PRL levels under basal conditions before access to cocaine for self-administration, rats were allowed to self-administer cocaine (0.25, 0.5, 1.0, or 2.0 mg/kg/infusion i.v.) by pressing a response lever under a continuous schedule of cocaine reinforcement during five daily consecutive 10-h sessions. Plasma CORT was significantly increased and plasma PRL was significantly reduced after each of the five self-administration sessions. The effects of cocaine on plasma CORT were intake-dependent, as demonstrated by significant positive correlations between postsession plasma CORT and total cocaine intake within the preceding sessions. The effects of cocaine on PRL were also intake-dependent but only on the first day of self-administration, on which a significant negative correlation was observed between cocaine intake and postsession PRL. In contrast, significant correlations between PRL and cocaine intake were not observed during any subsequent session, apparently reflecting adaptations to cocaine-induced PRL release. Alterations in neuroendocrine homeostasis emerged over time. Reductions in presession CORT values, as well as a persistent blunting of the diurnal CORT peak, were observed. Similarly, there was a modest but significant attenuation of plasma PRL when measured 4 days after the termination of cocaine self-administration. Alterations in neuroendocrine function associated with self-administration may be related to the development of cocaine dependence and could contribute to relapse in abstinent users.

The characterization of alterations in neurobiology, physiology, and behavior that coincide with the initiation of drug-seeking behavior as well the escalation from moderate to excessive drug self-administration (SA) is a crucial step toward elucidating the underlying mechanisms involved in the onset of drug dependence. Recently, a “naturalistic” rat model of cocaine SA designed to more accurately reflect human drug-seeking behavior was developed in our laboratory for this purpose (Mantsch et al., 1999). The critical features of this model include extended (i.e., 10-h) drug access, the availability of food and water at all times during SA testing, and the use of a cocaine dose range that includes relatively high unit doses of the drug (i.e., 1.0 and 2.0 mg/kg/infusion). Using this model, we have identified a number of behavioral and physiological parameters that are altered as a result of cocaine SA, including the amount and pattern of drug intake, the locomotor and stereotypic responses to a cocaine challenge, body weight, and food and water consumption (our unpublished observations). The aim of this study was to examine the effects of cocaine SA on selected neuroendocrine measures; specifically, the effects of SA under these conditions on plasma corticosterone (CORT) and prolactin (PRL) were investigated.

The neuroendocrine effects of cocaine have been well established (for reviews, see Levy et al., 1994; Kuhn and Little, 1995; Mello and Mendelson, 1997). Cocaine stimulates the release of CORT in rats (Moldow and Fischman, 1987) and cortisol in humans (Baumann et al., 1995) and nonhuman primates (Sarnyai et al., 1996) via mechanisms that are dependent on the secretion of corticotropin-releasing factor from parvocellular neurons localized in the paraventricular nucleus of the hypothalamus (Rivier and Vale, 1987; Sarnyai et al., 1992). In contrast, cocaine has been reported to reduce plasma PRL in rats (Ravitz and Moore, 1977; Pilotte et al., 1990; Levy et al., 1992), rhesus monkeys (Mello et al., 1993), and humans (Mendelson et al., 1992; but see Baumann et al., 1995; Heesch et al., 1996), apparently by blocking the reuptake of dopamine (DA) into tuberoinfundibular DA neurons so it can inhibit lactotrophs in the anterior pituitary by binding to D2 receptors (Ben-Jonathan, 1985; Baumann and Rothman, 1993; Levy et al., 1994).

Despite what is known about the effects of experimenter-

ABBREVIATIONS: SA, self-administration; ACQ, acquiring; ACTH, adrenocorticotropic hormone; CORT, corticosterone; DA, dopamine; HPA, hypothalamic-pituitary-adrenal; NON-ACQ, nonacquiring; PRL, prolactin.
delivered cocaine on plasma CORT and PRL, the effects of self-administered cocaine on these hormones have not been adequately investigated. In rhesus monkeys, it has been demonstrated that self-administered and noncontingent “yoked” infusions of cocaine produce differential effects on plasma adrenocorticotropic hormone (ACTH) and cortisol (Broadbear et al., 1999a), suggesting that the use of SA models may be more appropriate for investigating the neuroendocrine consequences of cocaine abuse. Similar findings have recently been obtained in rats (Galici et al., 2000). Although effects of cocaine SA on plasma CORT have been characterized in rats (Goeders and Guerin, 1996; Galici et al., 2000), these effects appear to vary depending on the conditions under which cocaine is available (Mantsch and Goeders, 2000). Furthermore, the relationship of the CORT response to the amount of cocaine intake has not been characterized, and the effects of extended-access (i.e., 10-h) SA under naturalistic conditions on CORT are unknown. In contrast to CORT, the acute effects of cocaine SA on plasma or serum PRL remain to our knowledge, uncharacterized. Thus, the first aim of this study was to determine the acute effects of cocaine SA on plasma CORT and PRL.

In humans, chronic cocaine use has been reported to produce abnormalities in hypothalamic-pituitary-adrenal (HPA) function (Schluger et al., 1997; Mendelson et al., 1998) and PRL secretion (Dackis and Gold, 1985; Mendelson et al., 1989; Weiss et al., 1994). Similarly, alterations in the regulation of PRL as a result of chronic cocaine SA have been demonstrated in rhesus monkeys (Mello et al., 1994; Mello and Mendelson, 1997), and changes in HPA regulation related to cocaine SA have been characterized in rats (Mantsch and Goeders, 2000). Disruption of neuroendocrine homeostasis as a result of repeated cocaine SA could contribute to the development of dependence and the high susceptibility of abstinent users to relapse. Therefore, the second goal of this study was to identify alterations in plasma PRL and CORT that emerge as a consequence of cocaine SA using our naturalistic model.

Materials and Methods

Subjects. Forty-eight male Sprague-Dawley rats (Charles River) approximately 90 days old (325 g) at the start of the experiment were used. Rats were housed individually in a stress-minimized, temperature- and humidity-controlled environment in an American Association for the Accreditation of Laboratory Animal Care-accredited animal care facility on a reversed 12-h light/dark cycle (lights on at 7:00 PM). On arrival, rats were allowed to habituate for approximately 2 weeks. Rats were initially tested for their locomotor responses to novelty and i.p. injections of saline and cocaine (15 mg/kg; data to be reported separately) and then were trained to self-administer food pellets (45 mg; P. J. Noyes, Lancaster, NH) by pressing a response lever under a continuous schedule of reinforcement (FR1) during daily 1-h sessions. To accommodate the initiation of food-reinforced responding, each rat was food-restricted for 12 h before food training sessions until stable lever-pressing was observed (delivery of 100 reinforcers within a 1-h session). Food (standard Picolab Rodent Diet) and water were freely available at all other times during the experiment, including during the drug SA sessions. After 1 week of food training, rats were surgically implanted with jugular catheters (see later). After surgery, an additional five food-training sessions were conducted before SA testing, during which rats were connected to the swivel-leash assembly (see later). Food pellet SA training was always conducted between 8:00 and 11:00 AM. Rats were sacrificed by decapitation after brief CO2 anesthesia subsequent to the completion of the experiment for neurochemical analysis to be reported separately. All procedures were carried out in accordance with the National Institutes of Health Principles of Laboratory Animal Care (NIH publication no. 85-23) and were approved by the Rockefeller University Animal Care and Use Committee.

SA Apparatus. Modified plastic and stainless steel operant conditioning chambers encased in sound attenuating cubicles (MED Associates Inc., St. Albans, VT) were used for the food and cocaine SA components of the experiment. The operant chambers were equipped with two retractable response levers with stimulus lights located above each lever, a food pellet dispenser, and a contact lickometer device. Additionally, porcelain dishes (90 ml capacity, 77 x 32 mm) were placed into the chambers for feeding. The levers and stimulus lights were mounted on the front wall of the chamber on either side of the food pellet dispenser, and the lickometer device and food dish were located at the back of the chamber. The cubicles were equipped with an exhaust fan that provided ventilation and white noise to mask extraneous sound. Programming and data collection were performed using an IBM-compatible personal computer and interface system and Med-PC software (MED Associates).

Venous Catheterization and Drug Delivery. Rats were implanted with chronic indwelling catheters under sodium pentobarbital anesthesia (50 mg/kg, i.p.; Abbott Laboratories, Chicago, IL) after atropine sulfate pretreatment (0.05 mg/kg, i.p.; Radix Laboratories Inc., Eau Claire, WI) using procedures modified from those previously reported (Mantsch et al., 1998). A silicon catheter (0.64 mm o.d. x 0.31 mm i.d.) was inserted into the right posterior facial vein and pushed down into the jugular vein so it terminated outside the right atrium. The catheter was sutured to the vein and continued subcutaneously to the animal’s head, where it exited via a 22-gauge guide cannula (Plastics One Inc., Roanoke, VA) mounted on the skull with dental acrylic for the attachment of a polyurethane delivery line (0.51 mm i.d. x 1.52 mm o.d.) encased in a stainless steel spring leash. The delivery line was connected to a 30-ml syringe in a motor-driven pump (Razel, Stamford, CT) via a leak-proof fluid swivel (Instech Laboratories Inc., Plymouth Meeting, PA) suspended above the chamber to allow the delivery of drug solution. The swivel and leash assembly was counterbalanced to permit relatively unrestrained movement. Rats were injected with a sterile penicillin G procaine suspension (75,000 U i.m.; Anthony Products Co., Arcadia, CA) immediately before surgery and were allowed to recover for 9 days before cocaine SA sessions were conducted. Catheters were filled daily with a streptokinase solution (8333 U/ml; Sigma Chemical Co., St. Louis, MO) to eliminate blood clots and were capped whenever the leash/delivery line assembly was disconnected.

Cocaine SA. Rats had access to cocaine for SA for five consecutive daily 10-h sessions (Monday through Friday), during which they could obtain the drug by pressing a response lever under a continuous schedule of reinforcement (FR1). During SA sessions, the cocaine (i.e., right) lever was extended into the chamber and the corresponding stimulus light was illuminated. Depression of this lever resulted in an i.v. infusion of drug solution (200 μl delivered over 5.0 s) followed by a 20-s time-out period, during which the lever was retracted and the stimulus light was extinguished. A second inactive (i.e., left) lever was extended into the chamber at all times. Responding on this lever was recorded but had no programmed consequences. Rats also had access to water via a contact lickometer located in the chamber. As described earlier, food was also available during the sessions in porcelain dishes located inside the chambers. After each 10-h session, rats were returned to their home cages. Cocaine SA testing was always conducted between 7:00 AM and 7:00 PM. Rats were separated into four groups of 12. Each group corresponded to one of four doses of cocaine that were available for SA: 0.25, 0.50, 1.0, and 2.0 mg/kg/infusion. Group assignments were made according to a Latin square design. The criterion for the acquisition of cocaine SA was set as an intake of at least 4.0 mg/kg cocaine within at least four of five consecutive 2-h time-bins, and the point of acquisition was...
defined as the first of the five time-bins during which this criterion was met. For some comparisons, rats were divided into groups according to whether they had acquired cocaine SA on each of the 5 days of testing. In these cases, rats classified as having acquired SA (ACQ) had already met the criterion for acquisition either before or within the first 4 h of the 10-h test session. All other rats were classified as nonacquiring (NON-ACQ) rats.

**Determination of Plasma CORT and PRL.** Blood (approximately 750 μl) for plasma CORT and PRL determination was obtained from the catheters, collected into preheparinized 1.5-ml tubes, and immediately placed on ice. Blood was centrifuged to allow separation of plasma, which was collected and frozen at −40°C until needed. Plasma CORT was measured using the Immunochrom Double Antibody Corticosterone 125I radioimmunoassay kit (ICN BioMedical, Irvine, CA). The detection limit of this assay is approximately 2 ng/ml. For CORT determination, 10-μl aliquots of plasma samples were analyzed in duplicate. Plasma PRL was measured using the Biotrak cellular communication rat PRL 125I radioimmunoassay system Amersham Life Science (Clearbrook, IL). The sensitivity of this assay is 0.07 ng/tube (i.e., 100-μl sample). Due to the limited amount of plasma available for PRL measurement, single 100-μl sample aliquots were analyzed. Likewise, plasma ACTH concentrations were not measured due to the small volume of plasma available. Blood for basal hormone determination was obtained between 7:00 and 8:00 AM (i.e., just after lights off) and between 6:00 and 7:00 PM (i.e., just before lights on) 2 days before the start of SA testing (i.e., on Saturday). Blood samples were also obtained immediately before and after each of the five 10-h SA test sessions conducted on Monday through Friday. In addition, blood was collected on days 1 and 4 of withdrawal at time points corresponding to those used for basal hormonal determination. For the CORT radioimmunoassays, the coefficients of intra-assay and interassay variation were 7.1 and 1.6, respectively. For the PRL assays, they were 20.1 and 16.6, respectively.

**Statistical Analysis.** Data from all four cocaine dose-groups were combined for ANOVAs (see later). Repeated measures ANOVAs were used to determine the significance of the differences in plasma CORT and PRL related to the day (i.e., baseline, SA days 1 to 5, and withdrawal days 1 and 4; repeated measures) and time (i.e., AM/pre versus PM/post) of sampling. Post hoc testing was performed using the Fisher’s protected least significant difference test. The significance of the relationships between precession plasma CORT or PRL (nanograms per milliliter) to cocaine intake (milligrams per kilogram) during the preceding SA sessions was determined using linear regression analysis. These analyses were performed individually for each dose on each day of SA testing as well as for all doses and days combined. Likewise, linear regression analyses were used to determine the relationships between precession CORT and PRL and cocaine intake during the subsequent and preceding sessions. Unpaired Student’s *t* tests were used to determine the significance of the differences in cocaine intake, postsession CORT, and postsession PRL between ACQ and NON-ACQ rats. For all statistical analyses, significance was defined as *P* < .05.

**Results**

**Cocaine SA.** Because no significant overall effects of unit dose on plasma CORT or PRL were found, data from all four dose-groups were combined for ANOVA. It is likely that the lack of significant dose effects was largely due to individual variability in cocaine intake at each of the four available cocaine doses. Forty-six of the 48 rats successfully completed the experiment. One rat from the 0.25 mg/kg/infusion dose group died immediately after surgery and one rat from the 0.50 mg/kg/infusion dose group was eliminated due to catheter leakage, resulting in a total of 11 for these two groups. Neither lethality nor convulsions were observed during cocaine SA testing. The mean total cocaine intakes (milligrams per kilogram) for all four cocaine doses combined during each of the five consecutive 10-h SA sessions are shown in Fig. 1. As reported elsewhere, the mean cocaine intake progressively increased over the 5 days of testing (Mantsch et al., 1999). This increase was the result of 1) an increase in the number of rats that had acquired cocaine SA over time and 2) an escalation of cocaine intake by rats that had already acquired cocaine SA. The postacquisition escalation of cocaine intake observed under the present conditions of extended (i.e., 10-h) drug access is consistent with reports that cocaine intake increases over time in rats provided access to the drug for longer (i.e., 6-h) but not shorter (i.e., 1-h) SA periods (Ahmed and Koob, 1998). The mean daily cocaine intakes (milligrams per kilogram) at each of the four doses of cocaine individually are reported in Table 1. Dose-related alterations in the acquisition of cocaine SA and in postacquisition SA were also observed and will be reported in detail elsewhere (Mantsch et al., 1999; our unpublished observations).

**Acute Effects of Cocaine SA on Plasma CORT.** Figure 2 depicts plasma CORT under basal conditions, before and after each of the five consecutive 10-h sessions, and during withdrawal in rats that had access to cocaine i.v. SA. Repeated measures ANOVA revealed a significant effect of test day (*F* 5, 65 = 10.397; *P* < .0001) and a significant interaction between test day and time of testing (AM/pre versus PM/post; *F* 5, 450 = 16.471; *P* < .0001). Although no statistically significant overall effect of time of testing was observed, a planned comparison showed a significant time effect under basal conditions (AM versus PM) that is consistent with the well-characterized circadian patterns of CORT secretion (mean difference = 109.93; *P* < .0001). Thus, as anticipated, CORT was higher at the AM time point that corresponded to the onset of the dark (i.e., active) phase compared with the PM time point that was determined immediately before the beginning of the light (i.e., inactive) phase.

Cocaine SA markedly increased plasma CORT. Compared with basal values (PM), postsession CORT was significantly elevated after each of the five consecutive daily SA sessions (*P* < .0001 for each comparison). In addition, on days 3, 4,
TABLE 1

Daily relationships (r values) between cocaine intake (mg/kg over the entire 10-h session) and postsession plasma PRL and CORT determined on each of the 5 consecutive days of testing for groups of rats provided access to one of the four doses of cocaine for SA.

The r values were determined using linear regression analysis. Intakes represent the total cocaine intake during the 10-h SA session, immediately after which blood samples were obtained for hormonal determinations. Values are mean ± S.E.

<table>
<thead>
<tr>
<th>Cocaine Intake (mg/kg/infusion)</th>
<th>Baseline</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRL Intake</td>
<td>CORT</td>
<td>r Value</td>
<td>PRL Intake</td>
<td>CORT</td>
<td>r Value</td>
</tr>
<tr>
<td>0.25</td>
<td>6.06 ± 1.84</td>
<td>15.96 ± 3.65</td>
<td>3.05 ± 0.66</td>
<td>18.09 ± 4.33</td>
<td>1.86 ± 0.56</td>
<td>−0.019</td>
</tr>
<tr>
<td></td>
<td>7.35 ± 14.60</td>
<td>141.35 ± 46.83</td>
<td>0.786 ± 97.88</td>
<td>97.88 ± 3.05</td>
<td>0.693 ± 114.29</td>
<td>0.401</td>
</tr>
<tr>
<td>0.5</td>
<td>6.48 ± 1.60</td>
<td>26.32 ± 5.66</td>
<td>2.17 ± 0.75</td>
<td>25.07 ± 4.75</td>
<td>2.50 ± 0.74</td>
<td>−0.254</td>
</tr>
<tr>
<td></td>
<td>64.26 ± 19.55</td>
<td>113.25 ± 34.52</td>
<td>0.46</td>
<td>165.18 ± 36.78</td>
<td>0.762</td>
<td>160.72 ± 36.14</td>
</tr>
<tr>
<td>1.0</td>
<td>6.20 ± 1.42</td>
<td>41.83 ± 7.65</td>
<td>2.05 ± 1.15</td>
<td>47.33 ± 7.67</td>
<td>1.43 ± 0.46</td>
<td>−0.650</td>
</tr>
<tr>
<td></td>
<td>39.96 ± 17.46</td>
<td>191.57 ± 43.72</td>
<td>0.809</td>
<td>126.45 ± 20.50</td>
<td>0.827</td>
<td>212.30 ± 25.81</td>
</tr>
<tr>
<td>2.0</td>
<td>7.49 ± 2.4</td>
<td>62.55 ± 10.76</td>
<td>0.88</td>
<td>70.36 ± 7.72</td>
<td>2.17 ± 0.77</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td>34.51 ± 10.32</td>
<td>157.99 ± 37.68</td>
<td>0.845</td>
<td>184.16 ± 24.30</td>
<td>0.339</td>
<td>238.47 ± 31.38</td>
</tr>
</tbody>
</table>

a Significant positive correlation (P < .05).
b Significant negative correlation (P < .05).
and 5 of SA, postsession CORT was significantly increased compared with presession (AM peak) values \((P < .001\) for each comparison). Increases in postsession CORT relative to baseline were observed at each of the four cocaine doses and on all 5 days of testing (see Table 1). On day 1 of withdrawal, PM plasma CORT was restored to basal pre-SA values and remained within this range on withdrawal day 4. Rats were also divided into ACQ and NON-ACQ groups on each of the 5 test days, according to whether they had acquired SA (see earlier). Table 2 shows cocaine intake and postsession CORT in ACQ and NON-ACQ rats on days 1 and 5 of SA. On each of the 5 test days, postsession plasma CORT was significantly higher in ACQ rats compared with NON-ACQ rats \((P < .05)\).

**Relationships between Cocaine Intake and Postsession CORT**. When data from all 5 SA days were combined, a significant positive correlation was observed between the amount of cocaine intake (milligrams per kilogram) during the experimental sessions and postsession plasma CORT (nanograms per milliliter; Table 3; \(r = 0.505; P < .0001\)). This correlation was also significant when determined on days 1 through 4 of SA individually \((r = 0.595, 0.569, 0.589, \) and \(0.393, \) respectively; \(P < .05\) for each) but not on day 5 of testing \((r = 0.305; P = .056)\).

The relationships between cocaine intake and postsession plasma CORT for each of the four doses of cocaine determined on each of the 5 days of SA testing are presented in Table 1. Positive correlations between cocaine intake and postsession CORT were observed on all days of testing, regardless of dose.

The relationships between cocaine intake and postsession CORT in ACQ and NON-ACQ rats on days 1 and 5 of SA are displayed in Table 2. Significant positive correlations between intake and postsession CORT were observed in both ACQ and NON-ACQ rats on day 1 of testing. However, on day 5, a positive correlation was observed only in NON-ACQ rats.

**Effects of Cocaine SA on “Basal” CORT.** By day 3 of cocaine SA, reductions in presession/AM CORT emerged (Fig. 2). Presession plasma CORT was significantly reduced before the sessions on days 3, 4, and 5 of SA testing compared with basal values and plasma concentrations measured before the session on day 1 \((P < .05)\). These reductions in AM CORT persisted into withdrawal and were observed on withdrawal days 1 and 4 \((P < .05\) versus basal for each). On day 1 of withdrawal, AM CORT was also significantly reduced compared with the first day of SA \((P < .01)\). However, by day 4 of withdrawal, AM concentrations were returning to presession values and were no longer significantly different from values measured before SA day 1. On day 1 of withdrawal, the circadian fluctuation of CORT was blunted as demonstrated by the lack of a significant difference between AM and PM CORT values \((P = .058)\). By day 4 of withdrawal, this circadian fluctuation had begun to “normalize,” as reflected by a significant AM versus PM difference \((P < .01)\).

To investigate whether the observed reductions in AM/presession plasma CORT were related to cocaine-induced CORT secretion during the previous SA sessions, linear regression analysis was used. Overall, a significant negative correlation was observed between AM/presession CORT and CORT immediately after the previous SA session \((P < .05; \) data not shown). This relationship did not emerge until days 4 and 5 of SA \((\text{post-day 4 SA versus pre-day 5 SA}; P < .05; r = -0.315)\) and failed to reach statistical significance on SA day 5/withdrawal day 1 \((r = -0.251; P = .11)\).

**Presession CORT and Cocaine Intake.** Linear regression analysis was used to examine the relationship between CORT before and after individual SA sessions. A significant positive correlation was observed between presession and

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**TABLE 2**

Relationships between cocaine intake and postsession PRL and CORT on days 1 and 5 of SA testing in rats that had acquired cocaine SA (ACQ) and rats that failed to meet the acquisition criterion (NON-ACQ)

<table>
<thead>
<tr>
<th>Self-Administration Test Day</th>
<th>Subjects</th>
<th>Cocaine Intake</th>
<th>Postsession CORT</th>
<th>CORT and Intake r Value</th>
<th>Postsession PRL</th>
<th>PRL and Intake r Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>ACQ ((n = 26))</td>
<td>54.25 ± 5.30</td>
<td>201.49 ± 28.22</td>
<td>+0.434&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.19 ± 0.34</td>
<td>-0.484&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NON-ACQ ((n = 20))</td>
<td>13.40 ± 2.38</td>
<td>80.58 ± 19.39</td>
<td>+0.493&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.23 ± 0.75</td>
<td>-0.471&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 5</td>
<td>ACQ ((n = 37))</td>
<td>74.42 ± 5.56</td>
<td>238.72 ± 22.28</td>
<td>+0.034&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.31 ± 0.47</td>
<td>+0.226&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>NON-ACQ ((n = 9))</td>
<td>19.08 ± 6.25</td>
<td>126.40 ± 42.10</td>
<td>+0.858&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.80 ± 0.79</td>
<td>-0.758&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant \((P < .05)\) versus NON-ACQ rats.

<sup>b</sup> Significant correlation \((P < .05)\).
postsession CORT only on day 1 of testing ($r = 0.463; P < .01$), when many of the rats self-administered low amounts of cocaine. The relationships between presession CORT and cocaine intake during the subsequent SA session were also determined. Overall, presession CORT failed to predict cocaine SA during the subsequent session, as demonstrated by the lack of significant correlations between presession CORT and cocaine intake when determined for all test days combined or on individual test days. However, when rats were divided into groups according to the dose of cocaine that was available for SA, positive correlations were observed between presession CORT and the amount of cocaine self-administered on days 1 ($r = 0.595$) and 3 ($r = 0.603$) of testing but only at the 0.25 mg/kg/infusion cocaine dose. On day 3 of testing, this correlation was statistically significant ($P < .05$). However, on day 1 of low-dose SA, the correlation failed to reach statistical significance ($P = .053$).

**Acute Effects of Cocaine SA on Plasma PRL.** The effects of cocaine SA on plasma PRL are depicted in Fig. 3. Repeated measures ANOVA revealed significant effects of test day ($F_{7,33} = 3.503; P < .005$) and time of testing (AM/pre versus PM/postsession; $F_{1,53} = 26.167; P < .001$) and a significant interaction between test day and time of testing ($F_{7,371} = 5.125; P < .0001$). In contrast to plasma CORT, no circadian fluctuation of plasma PRL was observed under basal conditions. Cocaine SA resulted in substantial decreases in plasma PRL. When measured immediately after each of the five daily SA sessions, PRL was significantly reduced relative to presession and basal values ($P < .001$ for each comparison). These reductions were observed at each available dose of cocaine and on each individual day of testing (see Table 1). On day 1 of SA, postsession PRL was significantly lower in ACQ rats compared with NON-ACQ rats (see Table 2).

PM PRL was restored to basal values on day 1 of withdrawal, at which time it was also significantly elevated compared with each of the 5 SA days ($P < .005$). Interestingly, when tested on day 4 of withdrawal, PM PRL was reduced relative to baseline values ($P < .05$), although it was not significantly different from withdrawal day 1. Thus, it appears that modest reductions in basal PRL emerged over time during withdrawal. No significant time-related alterations in AM/presession PRL were observed.

A significant negative correlation between cocaine intake and postsession plasma PRL was observed on day 1 of SA (Table 3; $r = -0.507; F = 13.115; P = .0009$). However, this correlation failed to reach significance on any of the SA days thereafter. Day 1 negative correlations were strongest at the two highest cocaine doses (i.e., 1.0 and 2.0 mg/kg/infusion; see Table 1). Over time, these correlations disappeared as demonstrated by an overall day-5 $r$ value of 0.080 and day-5 $r$ values of $-0.033$ and $+0.348$ at the 1.0 and 2.0 mg/kg/infusion doses of cocaine, respectively. The shift in $r$ values from days 1 to 5 of SA was most apparent in rats provided access to the highest (i.e., 2.0 mg/kg/infusion) dose of cocaine (Table 1). Furthermore, on day 1 of SA, negative correlations between cocaine intake and postsession PRL were observed in both ACQ and NON-ACQ rats (see Table 2). However, when determined on day 5, a significant negative correlation was observed in NON-ACQ rats, whereas the $r$ value in ACQ rats was actually positive although not statistically significant ($r = 0.226$).

This lack of a significant correlation between cocaine intake and postsession PRL appeared to be in part the result of a failure of high cocaine exposure to continue to reduce plasma PRL after repeated SA in some rats. Table 4 shows presession and postsession PRL and cocaine intake on days 1 and 5 of SA in representative rats. Several rats self-administered large amounts of cocaine yet still displayed high plasma levels of PRL. Also, on day 5 of SA, no differences in postsession PRL were observed between ACQ and NON-ACQ rats despite large differences in cocaine intake (Table 2).

In all cases, presession PRL failed to predict cocaine SA during the subsequent session, as indicated by the lack of significant correlations between presession PRL and cocaine intake on any of the SA test days.

**Table 3.** Relationships between within-session cocaine intake and plasma CORT and PRL determined from blood obtained immediately after the 10-h SA sessions.

<table>
<thead>
<tr>
<th>Postsession Plasma</th>
<th>All Days</th>
<th>SA Day 1</th>
<th>SA Day 2</th>
<th>SA Day 3</th>
<th>SA Day 4</th>
<th>SA Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORT (ng/ml)</td>
<td>$F = 70.22$</td>
<td>$F = 22.41$</td>
<td>$F = 19.64$</td>
<td>$F = 20.67$</td>
<td>$F = 6.92$</td>
<td>$F = 3.90$</td>
</tr>
<tr>
<td>PRL (ng/ml)</td>
<td>$F = 1.14$</td>
<td>$F = 13.11$</td>
<td>$F = 0.82$</td>
<td>$F = 0.01$</td>
<td>$F = 0.39$</td>
<td>$F = 0.24$</td>
</tr>
</tbody>
</table>

$a$ Significant positive correlation, $P < .05$.  
$b$ Significant negative correlation, $P < .05$.  

---

**Fig. 3.** Mean ± S.E. plasma PRL (nanograms per milliliter) determined from blood obtained under basal conditions (BAS), immediately before and after each of the five daily consecutive cocaine SA sessions (SA1–SA5), and on days 1 and 4 of withdrawal (WD1 and WD4). a, PM/postsession significantly less than basal ($P < .05$). b, PM/postsession significantly less than same-day AM/presession ($P < .001$).
Differences in cocaine intake between these groups. Significantly greater in ACQ rats compared with NON-ACQ rats on each day of testing, presumably due to CORT was significantly greater in ACQ rats compared with or not they had met the criterion for acquisition, postsession CORT was observed in NON-ACQ rats on day 5, there was no significant correlation in ACQ rats. It may be that the lack of significant day-5 correlations in all animals considered together and in the subset of ACQ rats was the result of a "ceiling effect" of cocaine on plasma CORT, because most rats were self-administering amounts of cocaine well above those capable of producing maximal CORT effects. However, several rats had high cocaine intake yet displayed relatively low plasma CORT levels. Thus, adaptations within the HPA axis may have developed as a result of earlier cocaine exposure. Reductions in the HPA effects of cocaine with repeated administration have been previously reported in rats (Zhou et al., 1996) and humans (Mendelson et al., 1998). The characterization of adaptations that emerge as a result of cocaine SA will require further investigation.

A progressive reduction in presession plasma CORT was observed relative to basal values. This effect developed over time and was apparent during withdrawal as a persistent disruption in the circadian fluctuation of CORT. This progressive reduction in presession/peak CORT is consistent with earlier findings and may be the result of enhanced negative feedback on the HPA axis in part as a result of alterations in type II glucocorticoid receptors, most notably in the hippocampus (Mantsch and Goeders, 2000). Evidence for the involvement of negative feedback mechanisms in this effect is provided by the finding that a modest, but statistically significant, negative correlation between presession CORT and CORT measured after the previous SA session emerged over time.

A number of studies have demonstrated that plasma CORT at the time of initial low-dose psychomotor stimulant exposure is an important determinant of whether rats will initiate SA (Piazza et al., 1991; Goeders and Guerin, 1996; Mantsch et al., 1998). In this study, modest but significant positive correlations were observed between plasma CORT immediately before the SA sessions and the amount of cocaine intake during the sessions but only at the lowest (i.e., 0.25 mg/kg/infusion) cocaine dose and only during the initial SA sessions. These findings are consistent with reports that CORT facilitates low-dose cocaine SA but has little effect on the SA of higher doses of the drug (Mantsch et al., 1998).

### Discussion

The results of this study demonstrate that cocaine SA acutely increases plasma CORT and reduces plasma PRL in rats. These findings are consistent with a number of reports describing the neuroendocrine effects of cocaine (for reviews, see Levy et al., 1994; Kuhn and Little, 1995; Mello and Mendelson, 1997). Moreover, this study clearly shows that self-administered cocaine produces effects on plasma CORT and PRL that are directly related to the amount of drug self-administered.

**Effects of Cocaine SA on CORT.** It is well established that experimenter-delivered cocaine increases plasma CORT, ACTH, and β-endorphin in rats (Moldow and Fischman, 1987; Rivier and Vale, 1987; Zhou et al., 1996). Likewise, it has been reported that cocaine infusions stimulate the release of ACTH and cortisol in cocaine-dependent humans (Mendelson et al., 1992; Baumann et al., 1995) and in nonhuman primates (Sarnyai et al., 1996; Broadbear et al., 1999a,b).

Less is known about the effects of self-administered cocaine on the HPA axis. Broadbear et al. (1999a,b) demonstrated that self-administered cocaine dose-dependently increases plasma cortisol and ACTH levels in male rhesus monkeys. Others have reported that self-administered cocaine increases plasma CORT in rats (Goeders and Guerin, 1996; Galici et al., 2000). Interestingly, self-administered infusions of cocaine appear to produce greater effects on HPA activity than do noncontingent infusions of the drug (Broadbear et al., 1999b; Galici et al., 2000). Thus, experiments in which cocaine is self-administered may provide more accurate information regarding its effects on the HPA axis compared with studies in which cocaine delivery is independent of the behavior of the subjects.

This study demonstrates cocaine intake-related increases in plasma CORT relative to basal concentrations determined before SA. Plasma CORT increased linearly with the amount of cocaine self-administered during the preceding experimental session. In this regard, significant positive correlations were observed between drug intake and postsession plasma CORT on each day of SA testing with the exception of day 5. When rats were separated into groups according to whether or not they had met the criterion for acquisition, postsession CORT was significantly greater in ACQ rats compared with NON-ACQ rats on each day of testing, presumably due to differences in cocaine intake between these groups. Significant positive correlations between cocaine intake and postsession plasma CORT were observed on day 1 of testing in both ACQ and NON-ACQ rats. However, although a significant positive correlation between cocaine intake and postsession CORT was observed in NON-ACQ rats on day 5, there was no significant correlation in ACQ rats. It may be that the lack of significant day-5 correlations in all animals considered together and in the subset of ACQ rats was the result of a "ceiling effect" of cocaine on plasma CORT, because most rats were self-administering amounts of cocaine well above those capable of producing maximal CORT effects. However, several rats had high cocaine intake yet displayed relatively low plasma CORT levels. Thus, adaptations within the HPA axis may have developed as a result of earlier cocaine exposure. Reductions in the HPA effects of cocaine with repeated administration have been previously reported in rats (Zhou et al., 1996) and humans (Mendelson et al., 1998). The characterization of adaptations that emerge as a result of cocaine SA will require further investigation.

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### Table 4

<table>
<thead>
<tr>
<th>Rat</th>
<th>Presession PRL PRL</th>
<th>Cocaine Intake</th>
<th>Postsession PRL</th>
<th>PRL</th>
<th>Cocaine Intake</th>
<th>Postsession PRL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/ml</td>
<td>mg/kg</td>
<td>ng/ml</td>
<td></td>
<td>ng/ml</td>
<td>mg/kg</td>
</tr>
<tr>
<td>T54e1.1</td>
<td>12.94</td>
<td>82.0</td>
<td>0.01</td>
<td></td>
<td>9.40</td>
<td>120.0</td>
</tr>
<tr>
<td>T65e1.1</td>
<td>3.12</td>
<td>74.0</td>
<td>0.02</td>
<td></td>
<td>3.75</td>
<td>118.0</td>
</tr>
<tr>
<td>T51e1.1</td>
<td>3.69</td>
<td>38.5</td>
<td>0.00*</td>
<td></td>
<td>2.41</td>
<td>55.5</td>
</tr>
<tr>
<td>T29e1.1</td>
<td>5.66</td>
<td>66.0</td>
<td>0.24</td>
<td></td>
<td>2.71</td>
<td>104.0</td>
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<tr>
<td>T49e1.1</td>
<td>4.01</td>
<td>30.0</td>
<td>0.20</td>
<td></td>
<td>6.96</td>
<td>114.0</td>
</tr>
<tr>
<td>T47e1.1</td>
<td>3.70</td>
<td>27.5</td>
<td>0.00*</td>
<td></td>
<td>4.87</td>
<td>73.5</td>
</tr>
<tr>
<td>T64e1.1</td>
<td>6.28</td>
<td>48.0</td>
<td>0.01</td>
<td></td>
<td>19.63</td>
<td>109.0</td>
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<tr>
<td>T65e1.1</td>
<td>3.42</td>
<td>7.0</td>
<td>3.75</td>
<td></td>
<td>16.12</td>
<td>49.0</td>
</tr>
<tr>
<td>T29e1.1</td>
<td>6.81</td>
<td>32.0</td>
<td>4.72</td>
<td></td>
<td>10.60</td>
<td>36.75</td>
</tr>
<tr>
<td>T43e1.1</td>
<td>8.85</td>
<td>3.0</td>
<td>4.39</td>
<td></td>
<td>4.56</td>
<td>3.0</td>
</tr>
</tbody>
</table>

* Plasma PRL was undetectable.
Interestingly, predictable individual differences in the acquisition of psychomotor stimulant SA are also only observed at lower doses (Piazza et al., 1989; Mantsch et al., 1999). Thus, variability in HPA function may underlie individual differences in predisposition to initially engage in cocaine-seeking behavior.

Effects of Cocaine SA on PRL. Previous studies have demonstrated that single (Ravitz and Moore, 1977; Levy et al., 1992) or multiple (Pilote et al., 1990) experimenter-delivered cocaine infusions reduce plasma PRL in rats. In rhesus monkeys (Mello et al., 1993) and humans (Heesch et al., 1996) with a history of cocaine exposure, cocaine challenges have been shown to reduce serum PRL. However, it has also been reported that cocaine has no effect on serum PRL in humans compared with vehicle administration (Mendelson et al., 1992; Baumann et al., 1995). In this study, we demonstrate inhibitory effects of self-administered cocaine on plasma PRL relative to basal and presession values on each day of testing. Furthermore, on day 1 of testing, postsession PRL was significantly lower in ACQ rats relative to NON-ACQ rats. However, over time, the differences in postsession PRL between ACQ and NON-ACQ rats diminished, to the point that PRL was almost identical between these groups after day 5 of SA despite large differences in cocaine intake.

On day 1 of testing, an overall significant negative correlation was observed between postsession PRL and the amount of self-administered cocaine. This day-1 negative correlation was present in both ACQ and NON-ACQ rats and appeared to be driven by the higher (i.e., 1.0 and 2.0 mg/kg infusion) cocaine doses at which stronger correlations were observed. After day 1 of testing, PRL was still reduced after SA, but clear cocaine intake–PRL relationships were no longer observed. By day 5, there was no significant correlation between cocaine intake and PRL overall or in ACQ rats. In fact, in ACQ rats, there actually was a nonsignificant positive correlation between day-5 intake and postsession PRL. In contrast, a significant negative correlation was still observed in NON-ACQ rats. Thus, it appears that the effects of cocaine on PRL secretion were altered in a manner directly related to cocaine SA. This uncoupling of the relationship between cocaine intake and PRL may have been due to an adaptation to the effects of cocaine in some rats. In Table 4, it can be seen that some rats (e.g., T45, T65, and T51e1.1) displayed reductions in plasma PRL on day 1 of testing but failed to show reductions in PRL on day 5, despite self-administering substantial amounts of cocaine (i.e., up to 120 mg/kg). In other rats (e.g., T29, T49, and T47e1.1), cocaine-induced reductions in PRL were observed on both days 1 and 5. Interestingly, the change in PRL responsiveness in individual rats was not predicted by the total amount of cocaine self-administered over the course of testing. However, this change did appear to be related to the self-administered dose of cocaine. Rats that were provided access to 2.0 mg/kg infusion cocaine displayed very low postsession PRL levels on day 1 of testing that were negatively correlated with cocaine intake. Over the course of testing, postsession PRL gradually increased in these rats so that it was almost four times the day-1 value when measured immediately after the day-5 SA session, despite an almost 2-fold increase in cocaine intake. Importantly, PRL was still reduced below presession and basal values in these rats. The negative correlation between cocaine intake and postsession PRL that was observed in these rats on day 1 of SA disappeared by day 2. By days 4 and 5 of testing, this correlation was actually positive.

In humans, abstinence from chronic cocaine use has been associated with the development of hyperprolactinemia under both basal and stimulated conditions (Dackis and Gold, 1985; Mendelson et al., 1988; Weiss et al., 1994). However, normal (e.g., Swartz et al., 1990) and reduced (e.g., Gawin and Kleber, 1985) PRL concentrations during cocaine abstinence have also been reported. Female rhesus monkeys display increases in basal PRL (Mello and Mendelson, 1997) and exaggerated PRL rebound after repeated DA challenges (Mello et al., 1994) subsequent to chronic SA. In this study, no overall hyperprolactinemia was observed. However, increases in presession PRL did emerge in some rats (see Table 4: T64, T58, and T23e1.1). These effects were time-dependent, because PRL was slightly, but significantly, reduced at the PM time point 4 days after the cessation of SA.

Conclusions. In summary, the present findings demonstrate that cocaine SA is associated with alterations in both CORT and PRL. Cocaine acutely disrupts these systems and appears to produce persistent disturbances in neuroendocrine homeostasis. In this study, rats were only tested during the acquisition phase of SA; therefore, their exposure to cocaine was limited (i.e., subacute). Additional studies in which rats have chronic access to cocaine are required to determine the magnitude and the persistence of cocaine-induced alterations within these systems and the relevance of such changes to escalations in cocaine-seeking behavior and to relapse.

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