Effect of Ovarian Hormones and Estrous Cycle on Stimulation of the Hypothalamo-Pituitary-Adrenal Axis by Cocaine

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ABSTRACT

Cocaine is known to exert sexually dimorphic HPA axis effects in rats and to disrupt estrous cyclicity and/or fertility in rats, nonhuman primates, and humans. The present studies investigated the reciprocal interactions between ovarian hormones and HPA axis responses to cocaine. Thirty minutes after injection, cocaine (15 mg/kg i.p.) increased serum ACTH and corticosterone more in cycling than ovariectomized females or male rats. ACTH and corticosterone were highest in proestrus when estradiol was elevated. Cocaine did not alter serum estradiol in females or testosterone in males but did stimulate progesterone secretion in both sexes. Cocaine-stimulated progesterone secretion was significantly greater in females than in males or ovariectomized females, and greater in proestrus than diestrous 1 rats. Cocaine stimulated corticosterone and progesterone secretion in sham-adrenalectomized, but not adrenalectomized rats, indicating that the adrenal gland and not the ovary is the source of cocaine-stimulated progesterone. Estrogen influenced cocaine-stimulated progesterone secretion more than corticosterone, suggesting different release mechanisms for the two steroids in the adrenal. These results suggest that adrenally derived progesterone could contribute to cocaine-induced physiological changes, including inhibited gonadotropin release.

Cocaine induces larger physiological responses in female than male rats. This laboratory has shown that cocaine increased serum ACTH more in female than male rats (Kuhn and Francis, 1996). We have also shown that cocaine stimulated locomotion more in female rats (Bowman and Kuhn, 1996; Walker et al., 2000a). Several reports indicate that acquisition, maintenance, and reinstatement of cocaine self-administration are also greater in female than male rats (Roberts et al., 1989; Lynch and Carroll, 1999, 2000). Fast scan cyclic voltammetry has recently been used to show that basal dopamine release and uptake are 30 to 40% greater in the striatum of female rats (Walker et al., 2000b) and that cocaine increased dopamine overflow more in female striatum (Walker and Kuhn, 1997). Pharmacokinetic differences do not account for these sexually dimorphic behavioral, neurochemical, and neuroendocrine responses to cocaine in rats (Bowman et al., 1999).

Patterns of cocaine use in humans also exhibit sex differences. More men than women in the United States use cocaine (U.S. Department of Health and Human Services, 1993) although certain aspects of cocaine taking and addiction are more severe in women. Prevalence of cocaine dependence was actually shown to be higher in adolescent females than adolescent males (Kandel et al., 1997). Two reports of clinical treatment of cocaine abuse reported more severe cocaine use patterns in females at the time of intake (Kosten et al., 1993; Robbins et al., 1999). McCance-Katz et al. (1999) reported that women consumed cocaine by more addictive routes (as did Kosten et al., 1993) and progressed to dependence more rapidly than men. Audio and videotapes of cocaine preparation and use elicited more self-reported cocaine craving in female users (Robbins et al., 1999). Significantly more female than male crack users reported emergency room visits following crack use (Dudish and Hatsukami, 1996). Scant evidence exists describing the impact of circulating levels of gonadal hormones on cocaine responses in humans. Women administered cocaine intranasally had greater peak plasma cocaine levels in their follicular than luteal menstrual phase although subjective drug effects were the same in both phases (Lukas et al., 1996). Intravenous cocaine administration produced equivalent cocaine concentrations in follicular and luteal phase women, however (Mendelson et al., 1999). Because so little is known about how gonadal hormones influence cocaine effects, the present studies were designed to test the hypothesis that fluctuating ovarian hormone levels would affect neuroendocrine responses to cocaine in rats.

Neuroendocrine function clearly influences behavioral effects of stimulants (Goeders, 1997). Marinelli et al. (1997) have shown that adrenalectomy attenuated cocaine-stimulated locomotion and corticosterone replacement restored it.

ABBREVIATIONS: ACTH, adrenocorticotropin; HPA, hypothalamo-pituitary-adrenal; RIA, radioimmunoassay; CRF, corticotropin-releasing factor.
suggesting that corticosterone influences the behavioral response. Corticosterone seems to mediate, at least partially, the stress-induced sensitization of locomotor effects of cocaine and amphetamine (Deroche et al., 1992; Rouge-Pont et al., 1995). Plasma corticosterone also plays a role in cocaine reinforcement. Corticosterone has been reported to influence acquisition (Mantsch et al., 1998), maintenance (Deroche et al., 1997), and reinstatement (Piazza et al., 1994; Mantsch and Goeders, 1999) of cocaine self-administration. Thus, the enhanced HPA axis effects of cocaine in female rats could contribute to enhanced behavioral reactivity.

Conversely, cocaine influences neuroendocrine function. Cocaine disrupts reproductive function in female humans, monkeys, and rats. Menstrual cycle disruptions in women and monkeys include amenorrhea, luteal phase dysfunction, and anovulation (Mello and Mendelson, 1997). Rats developed irregular estrous cycles after 7 days of repeated cocaine injections (King et al., 1990, 1993). The mechanism of these disruptions may be through cocaine’s direct stimulation of luteinizing hormone release (Mello et al., 1990a, 1993; Mendelson et al., 1992) or indirect through putative changes in ovarian hormone concentrations that could alter feedback control of gonadotropin release. Cocaine has been reported to increase plasma levels of progesterone in rats (Quinones-Jenab et al., 2000). One purpose of the present study was to investigate cocaine effects on ovarian steroids at each phase of the estrous cycle as a possible mediator of its reproductive function perturbations.

The present studies postulated that estrous cycle phase and therefore ovarian hormones would modulate pituitary ACTH and adrenal corticosterone responses to cocaine. Such effects would have implications for sex- and menstrual cycle-related differences in human cocaine use. Another thrust of this work was to determine whether cocaine alters plasma levels of ovarian steroids because such an effect would have repercussions for control of gonadotropin secretion. Thus, this work has investigated cocaine effects and reciprocal interactions of the HPA and hypothalamo-pituitary-gonadal axes.

Materials and Methods

Subjects. Adult male and female Sprague-Dawley rats were purchased from Charles River Laboratories (Raleigh, NC). Female rats were adrenalectomized, ovariectomized, or sham operated by the supplier before shipment. Rats were segregated by sex and were housed in plastic cages under a 12-h light/dark cycle with lights on at 6:00 AM. Food and water were provided ad libitum. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 865-23, Bethesda, MD) and approved by the Institutional Animal Care and Use Committee.

Experimental Rationale and Protocol. On the day before the experiment all animals were moved from the main vivarium to local animal quarters, weighed, and left in place for the experiment. Injections for all experiments began between 7:30 and 8:30 AM. Thirty minutes after injection all rats were decapitated rapidly after removal from the home cage and trunk blood was collected and allowed to clot on ice. Sera were then isolated by centrifugation (3000g for 15 min) and frozen at −80°C until assayed.

The first experiment investigated the effects of estrous cycle stage and ovarian hormones on cocaine-stimulated ACTH and corticosterone secretions. Estradiol and progesterone concentrations fluctuate over a 4- or 5-day estrous cycle in rats (Butcher et al., 1974). Estradiol and progesterone concentrations in serum were also measured in these animals to determine whether cocaine affected gonadal hormone secretion. Ovariectomized females were included to determine hormone effects when estradiol and progesterone concentrations are very low. Male controls were tested to ensure the previously reported sex difference was confirmed (Kuhn and Francis, 1996). Females at known estrous stages, ovariectomized females, and intact males were injected with 15 mg/kg cocaine i.p. or its saline vehicle (1 ml/kg) and decapitated 30 min later.

Because the first experiment found cocaine changed serum concentrations of progesterone in males, dose-response effects of cocaine on male adrenal and gonadal hormones were investigated. Male rats in the dose-response experiment were injected with saline, 5, 10, or 20 mg/kg cocaine i.p. and killed 30 min later. Serum concentrations of progesterone, corticosterone, testosterone, and estradiol were determined in these rats. ACTH values from these rats have been reported previously (Kuhn and Francis, 1996).

The final experiment investigated cocaine effects on serum progesterone in adrenalectomized female rats because the previous results suggested a nonovarian source of the stimulation. Female rats were adrenalectomized by the supplier before shipment at 60 days of age. These rats were given 0.85% saline to drink. Estrous cycle stage was monitored in adrenalectomized and sham-adrenalectomized rats until the day of proestrus could be reliably predicted. These rats were injected with saline (1 ml/kg) or cocaine (15 mg/kg i.p.) and killed on the morning of proestrus (19–40 days following surgery). Proestrus stage was confirmed by vaginal lavage immediately following decapitation.

Estrous Cycle Monitoring. Estrous cycle stage was monitored by analysis of cell types in vaginal lavages. Daily vaginal lavages of cycling and ovariectomized female rats were collected in the morning for at least eight consecutive days and allowed to dry on microscope slides. Slides were then fixed with ethanol and stained with toluidine blue. Identification of cell types was made microscopically according to published methods (Long and Evans, 1922). Only rats that could be reliably staged for estrous cycle stage were used. Two ovariectomized females were disqualified from the study because histological analysis showed evidence of estrous cycling (Cooper et al., 1993).

Hormone Measurements. The serum concentrations of ACTH, corticosterone, estradiol, and progesterone were measured by radioimmunoassay (RIA) in all rats. Testosterone concentrations were assayed only in male rats. The RIA for each hormone used kits from Diagnostic Products Corporation (Los Angeles, CA). Double-antibody kits were used for ACTH and estradiol. Inter- and intra-assay coefficients of variation for all the hormone assays were determined and ranged from 4.2 to 10.0%.

Drugs. Cocaine hydrochloride was provided by Research Triangle Institute through an arrangement with National Institute on Drug Abuse. Cocaine solutions were prepared the day of the experiment in distilled water. The RIA for each hormone used kits from Diagnostic Products Corporation (Los Angeles, CA). Double-antibody kits were used for ACTH and estradiol. Inter- and intra-assay coefficients of variation for all the hormone assays were determined and ranged from 4.2 to 10.0%.

Data Analysis and Statistics. RIA standard curves were analyzed and unknown hormone concentrations were determined by nonlinear regression using Prism 3.02 (GraphPad Software, Inc., San Diego, CA). Two-factor ANOVA was used to determine effects of estrous cycle and cocaine and their interactions on hormone concentrations. To determine the main effect of estrous cycle only the data from the four estrous stages were used. To determine the effect of sex, data from the estrous stages were combined and compared with those from males using a two-factor ANOVA [sex by cocaine (cocaine or saline)]. To determine the effect of ovariectomy, data from the estrous stages were combined and compared with those from ovariectomized females using a two-factor ANOVA [surgical status (sham or ovariectomy) by dose (cocaine or saline)].

To analyze further the estrous cycle-related effects of cocaine on corticosterone and progesterone secretions, the Δ amount of cocaine-stimulated hormone secretion was also determined by subtracting the mean of the saline-treated rats at the same estrous stage from cocaine-treated rats.
the hormone concentration of each cocaine-treated cycling female. Changes in Δ corticosterone and progesterone across groups were analyzed by one-factor ANOVA.

The cocaine dose-response experiment was analyzed by two-way ANOVA (cocaine treatment and sex). Three-way ANOVA was used to determine main effects and interactions of treatment, sex, and time in the time course experiment. The effects of adrenalectomy and sex were determined by two-way ANOVA.

All ANOVAs were performed using the GLM procedure of NCSS 2000 (NCSS Statistical Software, Kaysville, UT). Main effects were considered significant if \( p < 0.05 \). Newman-Keuls multiple comparison test was used for post hoc comparisons when significant interactions were found.

Results

Effects of Estrous Cycle, Ovariectomy, and Cocaine on HPA-Axis and Ovarian Hormones

**ACTH.** The effects of saline or cocaine injection (15 mg/kg) on serum concentrations of ACTH in cycling and ovariectomized females, and intact male rats are shown in Fig. 1A. The range of cocaine effects on ACTH was from more than a 5-fold stimulation compared with saline in proestrous females to 2-fold in males and ovariectomized females. When data from all cycling females were summed and compared with males, ACTH concentrations were significantly greater in females than males \( [F(1,114) = 4.04, p < 0.05] \). Cocaine increased ACTH in both sexes \( [F(1,114) = 14.33, p < 0.001] \). Estrous cycle significantly affected serum ACTH in saline- and cocaine-treated rats together \( [F(3,85) = 6.0, p < 0.001] \). Cocaine stimulated ACTH secretion most in proestrous females [cycle by treatment interaction \( [F(3,85) = 3.6, p < 0.02] \)]. ACTH concentrations were less in ovariectomized than all sham-operated, cycling females together \( [F(1,106) = 3.98, p < 0.05] \). The average ACTH concentrations in all cycling female and ovariectomized rats administered cocaine were \( 161 \pm 22 \) versus \( 89 \pm 17 \) pg/ml, respectively.

**Corticosterone.** Figure 1B shows the effects of saline or cocaine injection (15 mg/kg) on serum concentrations of corticosterone in the same rats as in Fig. 1A. Cocaine administration increased serum corticosterone in all groups of rats \( [F(1,135) = 103, p < 0.001] \). Cocaine increased corticosterone more in cycling females than males [sex and sex by cocaine \( (p \text{ values} < 0.007) \). Likewise, ovariectomy attenuated cocaine stimulation of corticosterone secretion \( [F(1,107) = 10.2, p < 0.002] \). Estrous cycle significantly affected serum corticosterone in cycling females \( (p < 0.001) \). Unlike its effect on ACTH, cocaine increased corticosterone similarly at each cycle stage.

The inset graph in Fig. 1B displays the amount of cocaine-stimulated or Δ corticosterone (cocaine-injected corticosterone value – saline mean of same group). This graph shows the changes in serum corticosterone more clearly by correcting for the different basal levels on different days of the cycle. Since cocaine increased corticosterone concentrations similarly at each cycle stage, Δ corticosterone was not significantly higher at any particular estrous stage. Δ corticosterone was lower in males than all the other groups \( (p < 0.05) \) and this contributed to the significant main effect of group \( [F(5,68) = 4.93, p < 0.001] \).

**Estradiol.** Serum concentrations of the ovarian hormones estradiol and progesterone were also determined in the rats from Fig. 1. As expected a profound surge in serum estradiol was observed on the morning of proestrus (Fig. 2A). Estradiol concentrations varied across the phases of the estrous cycle \( [F(3,82) = 80.3, p < 0.001] \). Cocaine did not affect estradiol concentrations in cycling female rats \( (p = 0.19) \). Estradiol concentrations were significantly greater in intact females relative to both ovariectomized females and males \( (21 \pm 3, 3 \pm 1, 6 \pm 1 \) pg/ml, respectively, \( p \text{ values} < 0.001) \).

**Progesterone.** Cocaine dramatically increased progesterone concentrations in cycling female rats and modestly in ovariectomized females and intact males (Fig. 2B). Progesterone concentrations were highest during diestrus in saline-injected rats and cocaine stimulated progesterone secretion most in proestrus females. Estrous cycle significantly affected progesterone concentrations \( [F(3,85) = 4.6, p < 0.005] \) and cocaine increased progesterone concentrations in cycling females \( [F(1,85) = 32.8, p < 0.001] \). Progesterone concentrations were higher in cycling females than males \( (p < 0.001) \) and ovariectomy significantly decreased cocaine-stimulated progesterone secretion \( (p < 0.001) \). Cocaine significantly increased progesterone concentrations in the ovariectomized females and males \( (p \text{ values} < 0.01 \text{ by } t \text{ tests}) \).

To show the magnitude of the cocaine-induced progesterone secretion more clearly these data were also analyzed as
the amount of cocaine-stimulated or Δ progesterone (cocaine-injected progesterone value – saline mean of same group). Data from males and ovariectomized females were included with data from each estrous stage to demonstrate sex and ovarian hormone effects. The inset in Fig. 2B confirmed that cocaine stimulated progesterone secretion robustly in all cycling females and slightly in ovariectomized females and males. The effect of cocaine differed between treatment groups \[F(5,67) = 5.9, p < 0.001\] and post hoc analysis indicated that Δ progesterone was significantly greater in proestrus than diestrous 1, ovariectomized females, and males \(p < 0.05\).

**Cocaine Dose-Response Effects on HPA-Axis Hormones and Testosterone in Male Rats.** The neuroendocrine effects of cocaine were further investigated in male rats. Table 1 indicates that 30 min postinjection none of the three cocaine doses altered serum testosterone or estradiol. Cocaine increased serum progesterone in males in a dose-related manner \[F(3,97) = 11.4, p < 0.001\]. Progesterone values in males injected with 10 or 20 mg/kg cocaine were greater that those of the saline control and 5-mg/kg groups \(p < 0.05\). Cocaine increased serum corticosterone in a similar dose-related manner \[F(3,103) = 4.1, p < 0.009\], except that only 20 mg/kg cocaine induced significant increases above those of the vehicle and 5-mg/kg groups \(p < 0.05\).

**Effect of Adrenalectomy**

**ACTH.** Because the preceding experiment showed that cocaine-stimulated progesterone secretion was greatest during proestrus, all rats in this experiment were injected on the morning of proestrus to look for attenuation of this cocaine effect. Figure 3A shows that ACTH concentrations were markedly elevated in adrenalectomized rats. The loss of corticosterone negative feedback on CRF neurons in the hypothalamus led to 14-fold greater serum ACTH concentrations in adrenalectomized relative to sham-operated females following saline injection. Thus, surgery significantly affected ACTH \(p < 0.001\). Cocaine increased serum ACTH in shams \[F(1,18) = 7.86, p < 0.02\]. Cocaine did not increase ACTH in the adrenalectomized females, probably because secretion was already either maximal or near-maximal.

**Corticosterone.** Figure 3B shows that serum corticosterone was significantly lower in adrenalectomized female rats \[F(1,34) = 51.9, p < 0.001\]. Cocaine significantly increased serum corticosterone overall \[F(1,34) = 12.8, p < 0.002\]. Cocaine did not affect corticosterone in adrenalectomized rats as indicated by a significant interaction of surgery and dose \[F(1,34) = 12.8, p < 0.002\]. As expected, the adrenals mediate the effect of cocaine on serum corticosterone.

**Progesterone.** Figure 3C indicates that cocaine increased progesterone in sham but not in adrenalectomized females. Cocaine significantly increased serum progesterone \[F(1,36) = 5.33, p < 0.03\]. Unlike corticosterone, the adrenals are not the only source of progesterone and thus adrenalectomy did not significantly alter basal serum progesterone concentrations \(p = 0.19\).

**Estradiol.** Figure 3D shows that estradiol concentrations were significantly decreased in the sera of adrenalectomized females on the morning of proestrus \[F(1,36) = 13.0, p < 0.001\]. In contrast to the effect on progesterone, cocaine did not affect estradiol concentrations \(p = 0.10\).

**Discussion**

The present studies show that the HPA response to cocaine is strongly influenced by activational effects of ovarian hor-

TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Testosterone (ng/dl)</th>
<th>Estradiol (pg/ml)</th>
<th>Progesterone (ng/ml)</th>
<th>Corticosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>262 ± 21</td>
<td>3.3 ± 0.4</td>
<td>2.5 ± 0.3</td>
<td>131 ± 10</td>
</tr>
<tr>
<td>5 mg/kg COC</td>
<td>254 ± 22</td>
<td>4.1 ± 0.7</td>
<td>2.8 ± 0.4</td>
<td>141 ± 10</td>
</tr>
<tr>
<td>10 mg/kg COC</td>
<td>249 ± 25</td>
<td>3.8 ± 0.8</td>
<td>4.7 ± 0.6*</td>
<td>162 ± 11</td>
</tr>
<tr>
<td>20 mg/kg COC</td>
<td>246 ± 20</td>
<td>3.7 ± 0.7</td>
<td>6.4 ± 0.7*</td>
<td>176 ± 9*</td>
</tr>
</tbody>
</table>

* Significantly different than vehicle and 5 mg/kg groups, \(p < 0.05\).
mones. Estrous cycle stage influenced ACTH responses to cocaine and ovariectomy attenuated serum concentrations of ACTH and corticosterone following cocaine. These studies also revealed an unexpected finding that cocaine stimulated secretion of progesterone in both male and female rats. Cocaine-stimulated progesterone secretion was estrogen responsive because it was greatest during the high-estrogen state of proestrus and was attenuated by ovariectomy. The source of cocaine-stimulated progesterone secretion was shown to be the adrenal gland because adrenalectomy completely abolished cocaine-stimulated progesterone secretion.

**Sex, Cycle, and Ovariectomy Effects on ACTH and Corticosterone.** The presently reported sex differences in cocaine-stimulated ACTH and corticosterone are similar to previous reports of sex differences in basal HPA activity (Lesniewska et al., 1990; Chisari et al., 1995) and cocaine-stimulated ACTH secretion (Kuhn and Francis, 1996). In rats, HPA axis responses to many stimuli, including stressors and drugs, are sexually dimorphic with females exhibiting greater responses than males (Le Mevel et al., 1979; Aloisi et al., 1994; Handa et al., 1994a; Rivier, 1994; Ogilvie and Rivier, 1997). However, the endocrine basis of these differences is not always clear.

The variations in cocaine-stimulated HPA axis activation with estrous cycle stage and ovariectomy support a role for ovarian steroids in the greater HPA responses of females to cocaine in the present study. Females in proestrus exhibited 2- to 3-fold greater cocaine-induced ACTH secretion than at any other phase of the cycle, and ovariectomy caused a marked drop in ACTH response. Variations in HPA reactivity across the estrous cycle in response to other stimuli are not observed consistently. Viau and Meaney (1991) found greater peak ACTH and corticosterone responses to stress without any basal differences. In contrast, no cycle-related differences were found in other reports of HPA activity stimulated by restraint stress or foot-shock (Carey et al., 1995; Rivier, 1999). Studies of estrous cycle effects on basal HPA axis function are equally conflicting. Carey et al. (1995) have shown that both ACTH and corticosterone are higher on the afternoon of proestrus than estrus and diestrus II. Atkinson and Waddell (1997) used a serial blood sampling technique and found similar results for cyclic variations of corticosterone. However, these authors did not find sex or estrous cycle-related differences in absolute levels of ACTH.

The present studies revealed disparate effects of gonadal steroids on ACTH and corticosterone release. Cocaine had differential effects on ACTH, but not corticosterone, across phases of the estrous cycle. Serum corticosterone was highest...
at proestrus for both saline and cocaine-treated rats; however, only cocaine-stimulated ACTH secretion varied across cycles. These disparate effects of estradiol on cocaine-stimulated ACTH and corticosterone and the conflicting results obtained in studies of sex difference or estrous cycle effects on HPA reactivity to stimuli may reflect multiple sites of gonadal steroid action. Stimulatory effects of estradiol (Handa et al., 1994a) and inhibitory effects of testosterone (Bingsman et al., 1994; Handa et al., 1994b; Viau and Meaney, 1996) are widely reported. However, these gonadal steroid effects likely reflect actions at many sites, ranging from hippocampal sites mediating feedback inhibition, hypothalamic inputs to CRF neurons, production of CRF itself, and pituitary sensitivity to CRF (Bohler et al., 1990). For example, sex differences in response to cocaine could reflect sex differences in the dopaminergic, serotonergic, or noradrenergic inputs to the hypothalamus that contribute to the HPA response to cocaine as well as sex differences in corticotropin-releasing hormone and ACTH release (Borowsky and Kuhn, 1991).

The adrenal cortex is another site for steroid modulation. Nowak et al. (1995) have reported that estradiol enhanced basal but not ACTH-stimulated corticosterone release from dispersed rat adrenocortical cells. This finding nicely matched the present result that corticosterone was very high in saline-treated proestrous females and that Δ corticosterone did not show cyclic, estradiol-related changes. Furthermore, this same study indicated that testosterone attenuated ACTH-stimulated but not basal corticosterone release. This finding is also reflected in the present data that Δ corticosterone was lowest in intact males. Further studies are needed to define the particular sites for gonadal steroid mediation of cocaine-stimulated ACTH and corticosterone secretions.

Mediation of Cocaine-Stimulated Progesterone Secretion. Cocaine-stimulated progesterone secretion was an unexpected finding of this study. This effect was more robust in intact than ovariectomized females or males. Cocaine-stimulated progesterone secretion (Δ progesterone) was greater when estradiol peaked at proestrus than when estradiol was low at diestrus 1, in ovariectomized and male rats. These results implicate ovarian hormones in this phenomenon. However, the presence of cocaine-stimulated progesterone secretion in ovariectomized and male rats led us to investigate the adrenal as the site of origin.

The adrenal gland was shown to be the source of cocaine-stimulated progesterone secretion because it was eliminated in adrenalectomized rats. As expected, cocaine did not stimulate corticosterone secretion in adrenalectomized rats, and ACTH secretion was extremely high due to the loss of feedback inhibition. The source of the minimal level of corticosterone in our adrenalectomized rats is unclear, although cross-reacting steroid precursors in the RIA might be responsible. Cocaine stimulation of progesterone secretion was absent in adrenalectomized females, confirming our hypothesis that the adrenal was the source of cocaine-stimulated progesterone secretion. The progesterone concentration in saline-treated adrenalectomized rats was equal to that found in shams at proestrus, the cycle stage in which progesterone is low. Progesterone in ovariectomized rats was about 5-fold lower than at proestrus, indicating that the ovary contributes more than the adrenal to total plasma progesterone (Fajer et al., 1971). Progesterone is a major secretory product of the ovary but is primarily considered a precursor steroid in the synthesis of gluco- and mineralocorticoids in the adrenal gland. Progesterone is released from the adrenal gland (Short, 1960; Holzbauer et al., 1969; Fajer et al., 1971). Feder and Ruf (1969) reported that systemic injections of ACTH increased plasma progesterone concentrations enough to stimulate lordosis in ovariectomized, estrogen-primed rats and guinea pigs. This finding suggests that adrenally derived progesterone is functionally significant.

The present findings that estrous cycle and ovarian hormones influence adrenal secretion of corticosterone and progesterone are consistent with several observations in the literature. Telegdy et al. (1967) have shown that progesterone secretion by the adrenals of female dogs decreased after ovariotomy and increased with estrone replacement. Bartosik et al. (1971) have shown that progesterone concentration in adrenal vein blood is less in ovariectomized than intact rats treated with pregnant mares' serum. The primary mechanism for estrogen to affect adrenal cortex function is indirect via effects in the hypothalamus and/or pituitary to increase plasma ACTH (Viau and Meaney, 1991; Kuhn and Francis, 1996). However, estradiol also directly stimulated corticosterone secretion in dispersed adrenocortical cells (Nowak et al., 1995). Thus, estrogen may influence adrenal corticosterone and progesterone secretion directly and indirectly.

The present studies also indicated that adrenal function is necessary for normal ovarian function, because serum estradiol was lower overall in cocaine- and saline-injected adrenalectomized rats. Although the adrenalectomized rats were cycling normally, it seems plausible that without adrenal progesterone to sharpen the luteinizing hormone surge (Feder et al., 1971; Shaikh and Shaikh, 1975; Aono et al., 1976) some broadening and attenuation of the estradiol surge in subsequent cycles may be expected. A simpler explanation is that the adrenals secrete estradiol. In fact, high concentrations of estradiol have been measured in adrenal venous blood (Shaikh and Shaikh, 1975).

The adrenal cortex may have distinct mechanisms for the production/release of progesterone and corticosterone. The present results show that Δ progesterone, but not Δ corticosterone, varied across the estrous cycle. Progesterone and corticosterone concentrations have been reported to increase during the morning of proestrus but by mid-afternoon their secretion rates diverge and by evening, progesterone concentrations continue to increase while corticosterone declines (Feder et al., 1971; Brown et al., 1976). Progesterone and corticosterone secretions also diverge in response to paced mating. Serum progesterone was higher in females allowed to pace their mating relative to those that did not pace (Frye et al., 1996). In contrast, paced and nonpaced mating induced equivalent, moderate increases in serum corticosterone. Paced mating, and by association increased adrenal progesterone secretion, are associated with enhanced reproductive success (Frye and Erskine, 1990; Frye et al., 1996). Thus, similar to paced mating, cocaine seems to affect the estrogen-responsive pathway that permits preferential release of progesterone relative to corticosterone. Further support for the notion that progesterone release is estrogen-responsive is that Δ progesterone, but not Δ corticosterone, in ovariectomized females is less than in cycling females. Because progesterone is known to increase sexual receptivity in female...
rats (Tennent et al., 1980; Erskine et al., 1985) it seems plausible that this mechanism would be activated during proestrus and estrus, the high estrogenic and sexually receptive phases of the cycle, respectively. Our finding that cocaine-stimulated progesterone is greater during proestrus than diestrus 1 is consistent with the selective effect of cocaine on the estrogen-responsive, progesterone release pathway.

In conclusion, activation effects of ovarian steroids clearly influence HPA axis responses to cocaine in the rat that may mediate some aspects of behavioral responses to cocaine, including cocaine self-administration. These results are highly relevant because ovarian steroids may influence subjective effects of drugs of abuse in human females (Mello et al., 1990b). Estradiol was shown to influence preferentially certain sites in the HPA response to cocaine. The novel effect reported here that cocaine stimulates progesterone secretion illustrates how an HPA response to cocaine may feedback on the hypothalamic-pituitary-gonadal axis and possibly contribute to the reported reproductive toxicity.

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