The Effects of Cocaine on Basal and Human Chorionic Gonadotropin-Stimulated Ovarian Steroid Hormones in Female Rhesus Monkeys

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ABSTRACT

Cocaine stimulates gonadotropin (luteinizing hormone) release from the anterior pituitary in humans and in rhesus monkeys, but its acute effects on ovarian steroid hormones are unknown. The acute effects of cocaine and placebo on estradiol and progesterone were studied in 13 drug-naive female rhesus monkeys during the mid-follicular (days 8–10) and the mid-luteal (days 21–23) phases of the menstrual cycle. Each monkey was her own control under cocaine and placebo conditions. Samples for ovarian steroid hormone analysis were collected before and at 15-min intervals for 300 min after cocaine or placebo administration. In follicular phase females, estradiol levels increased significantly within 15 min after cocaine (0.8 mg/kg i.v.) administration ($P < .008$) but did not change after placebo administration. Estradiol remained significantly above baseline for 45 min ($P < .002–0.02$). In contrast, in mid-luteal phase females, estradiol did not change after cocaine or placebo administration. Basal progesterone levels did not change after cocaine or placebo administration in either mid-follicular or mid-luteal phase females. After hCG (500 I.U. i.m.) was administered to mid-luteal phase females, cocaine (0.4 and 0.8 mg/kg i.v.) and placebo administration did not increase or decrease estradiol or progesterone. One implication of these findings is that cocaine-induced increases in follicular phase estradiol levels could disrupt folliculogenesis and contribute to the menstrual cycle abnormalities observed during chronic cocaine self-administration.

It is well established that cocaine stimulates the release of luteinizing hormone (LH) and adrenocorticotropic hormone (ACTH) in rhesus monkeys (Mello et al., 1990, 1993; Sarnyai et al., 1996) and in human males and females (Mendelson et al., 1992; Teoh et al., 1994; Heesch et al., 1996; Sholar et al., 1998). However, the acute effects of cocaine on ovarian steroid hormones are unknown. If cocaine changes basal levels of ovarian steroid hormones and disrupts ovarian steroid feedback control of gonadotropin release, this could contribute to cocaine-related menstrual cycle abnormalities observed in female rhesus monkeys (Mello et al., 1997; Potter et al., 1998). Chronic cocaine self-administration disrupted menstrual cycle duration with concomitant amenorrhea, anovulation, and luteal phase dysfunction in otherwise healthy female rhesus monkeys (Mello et al., 1997). Daily administration of 4 mg/kg i.v. cocaine during the follicular phase of the menstrual cycle (days 2–14) resulted in anovulatory cycles of abnormal duration in female rhesus monkeys (Potter et al., 1998). Both abnormally short (14–17 days) and long (54–70 days) menstrual cycles were observed (Potter et al., 1998). Lower doses of cocaine (1 or 2 mg/kg i.v.) also resulted in anovulation and disrupted subsequent menstrual cycles (Potter et al., 1999). These findings in rhesus monkeys are consistent with earlier reports that cocaine disrupts the estrous cycle in rats (King et al., 1990, 1993). These preclinical data also are consistent with clinical reports that cocaine abuse is associated with a number of reproductive disorders, including amenorrhea, anovulation, and luteal phase inadequacy, in otherwise normal women (Mello, 1998; see Mello and Mendelson, 1997, for review). However, interpretation of clinical findings is often complicated by the fact that many cocaine abusers are also polydrug abusers, and it is difficult to ascribe menstrual cycle disorders to cocaine alone (for review, see Mello and Mendelson, 1997; Mello et al., 1997).

The basic mechanisms underlying cocaine-related disruptions of the menstrual cycle are unknown (see Mello and Mendelson, 1997, for review). However, there are several ways in which cocaine-induced changes in basal levels of

ABBREVIATIONS: LH, luteinizing hormone; ACTH, adrenocorticotropic hormone; RIA, radioimmunoassay; E2, estradiol; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin.

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ovarian steroid hormones could contribute to the disruptions of the menstrual cycle observed during chronic cocaine self-administration (Mello et al., 1997). For example, if estradiol (E₂) and progesterone did not increase during the late follicular phase, this could prevent ovulation and result in anovulation or luteal phase dysfunction. Conversely, high levels of E₂ during the follicular phase may suppress follicle-stimulating hormone (FSH) and disrupt folliculogenesis, which in turn may lead to anovulation and luteal phase dysfunction (Zelegnik, 1981; Dierschke et al., 1985). Similarly, in ovariectomized females, E₂ replacement significantly reduced levels of FSH (Bassett and Zelegnik, 1990). Moreover, if estrogen levels are high during the early luteal phase, this could result in a short luteal phase (Hutchison et al., 1987).

In addition to the direct effects of cocaine on anterior pituitary hormones, it appears that the hormonal milieu may also influence the neuroendocrine effects of cocaine. For example, in the absence of ovarian steroid hormones, cocaine did not stimulate LH and ACTH in ovariectomized monkeys as it did in gonadally intact females (Mello et al., 1995; Sarnyai et al., 1995). The possible contribution of ovarian steroid hormones, rather than pituitary dysfunction, to these findings in ovariectomized monkeys was suggested by the fact that synthetic luteinizing hormone-releasing hormone and synthetic corticotropin-releasing factor each stimulated significant increases in LH and ACTH in the same subjects (Mello et al., 1995; Sarnyai et al., 1995). These data provide inferential evidence that E₂ and progesterone may be important modulators of the neuroendocrine actions of cocaine. Although the mechanisms underlying the stimulation of LH by cocaine in gonadally intact rhesus females are unclear, a cocaine-induced increase in E₂ and/or progesterone could facilitate LH release. It is well established that the periovulatory LH surge is critically dependent on an antecedent elevation in E₂ (Karsch, 1987; Hotchkiss and Knobil, 1994). In follicular phase rhesus females, progesterone can facilitate an estrogen-induced LH surge (Helmond et al., 1981) and may stimulate an LH surge in ovariectomized monkeys (Terasawa et al., 1984).

One goal of this study was to examine the effects of acute cocaine administration on basal levels of E₂ and progesterone in female rhesus monkeys during both the follicular and the luteal phases of the menstrual cycle. Because it is often difficult to accurately predict when progesterone levels are highest during the mid-luteal phase of the menstrual cycle, we also examined the effects of cocaine on ovarian steroid hormones after stimulation with human chorionic gonadotropin (hCG). We now report the effects of acute cocaine administration on basal and hCG-stimulated E₂ and progesterone levels in female rhesus monkeys.

Materials and Methods

Subjects

Thirteen experimentally naive adult female rhesus monkeys (Macaca mulatta) (4.8–7.5 kg) lived in individual cages and were maintained on ad libitum food and water. Monkeys were fed twice each day at 9:00 AM and 5:00 PM. Lab Diet Jumbo Monkey Biscuits (PMI Foods, Inc., St. Louis, MO) were supplemented with fresh fruit, vegetables, and multiple vitamins each day. Monkeys had visual, auditory, and olfactory contact with other monkeys. A variety of toys were available, and auditory and visual enrichment was provided. A 12-h light/dark cycle (lights on from 7:00 AM to 7:00 PM) was in effect throughout the study. Each monkey was adapted to placement in a standard primate restraining chair on several occasions before the studies were initiated. Successive studies of the effects of cocaine on ovarian steroid hormones were separated by at least 2 months.

Animal maintenance and research were conducted in accordance with guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Institutes of Health. This protocol was approved by the Institutional Animal Care and Use Committee. The facility is licensed by the U.S. Department of Agriculture. The health of the monkeys was periodically monitored by consultant veterinarians.

Menstrual Cycle Monitoring

Menstrual cycle regularity was monitored daily with vaginal smears to determine the onset and duration of vaginal bleeding. All endocrine study days were scheduled during the mid-follicular phase of the menstrual cycle, 8 to 10 days after the onset of menstruation, or during the mid-luteal phase of the menstrual cycle, 21 to 23 days after onset of menses. For monkeys with menstrual cycles that were usually longer or shorter than 28 days, the estimated mid-luteal phase was adjusted accordingly. Mid-luteal phase status was verified with measures of basal progesterone levels on the morning before each planned endocrine study.

Sequence of Experimental Conditions

Three experiments were conducted to evaluate the acute effects of cocaine and placebo-cocaine on ovarian steroid hormones. In experiment I, the acute effects of placebo-cocaine and cocaine (0.8 mg/kg i.v.) on E₂ and progesterone were evaluated in mid-follicular phase female rhesus monkeys on cycle days 8 to 10, when basal E₂ levels are increasing. In experiment II, the acute effects of placebo-cocaine and cocaine on ovarian steroid hormones were evaluated in mid-luteal phase female rhesus monkeys at the estimated peak levels of E₂ and progesterone. In each experiment, basal levels of E₂ and progesterone were measured 15 min before placebo or cocaine was administered. After i.v. placebo or cocaine administration, 20 samples for analysis of E₂ and progesterone were collected at 15-min intervals for 300 min. In previous studies, peak levels of cocaine in plasma were detected within 2 to 4 min after i.v. administration in female rhesus monkeys, and the half-life of cocaine was 56 to 61 min (Mendelson et al., 1999a). Accordingly, samples for analysis of plasma cocaine levels were collected at 4, 10, 20, 30, 40, 60, 120, and 180 min after cocaine administration in the present study.

In experiment III, human chorionic gonadotropin (hCG) (Synfas; Serono Laboratories, Inc., Randolph, MA) was administered to stimulate ovarian steroid hormone levels and to ensure that E₂ and progesterone levels were equivalent between subjects and across cocaine (0.4 and 0.8 mg/kg i.v.) and placebo conditions. After collection of a single baseline sample at 9:30 AM, an acute dose of hCG (500 U.I. i.m.) was given 255 min before cocaine or placebo administration. In pilot studies, an interval of about 4 h was sufficient to produce maximal stimulation of E₂ and progesterone in female rhesus monkeys during the mid-luteal phase of the menstrual cycle. After hCG administration, samples for analysis of plasma hCG, E₂, and progesterone were collected at 11:30 AM and 1:30 PM. Then, cocaine (0.4 or 0.8 mg/kg i.v.) or placebo was administered at 1:45 PM, and the first post-cocaine sample was collected at 2:00 PM. A total of 13 samples were collected at 15-min intervals for 180 min after cocaine or placebo administration.

Cocaine and hCG Dose Selection

Cocaine. The cocaine doses were selected based on the basis of our previous studies in which 0.4 and 0.8 mg/kg cocaine stimulated LH
and suppressed prolactin in gonadally intact male and female rhesus monkeys (Mello et al., 1990, 1993). Higher cocaine doses were not studied because we have found that 1.0 mg/kg i.v. cocaine produced hyperactivity and agitated behavior and because the convulsant dose range for cocaine is 3 to 8 mg/kg i.v. (Matsuzaki et al., 1976). Placebo-cocaine was a vehicle control consisting of sterile saline for injection. The acute effects of placebo-cocaine or a low (0.4 mg/kg i.v.) or a high (0.8 mg/kg i.v.) dose of cocaine on E2 and progesterone levels were evaluated. Placebo-cocaine or cocaine was administered as an i.v. bolus over 1 min. Treatments were given in an irregular order, counterbalanced across subjects.

**hCG.** hCG stimulates ovarian steroid hormone release most effectively when administered during the mid-luteal phase of the menstrual cycle (Wilks and Noble, 1983; Ottobre and Stouffer, 1984). A dose of 500 I.U. of hCG was selected on the basis of previous studies in rhesus monkeys (Wilks and Noble, 1983). In normal women studied during the mid-luteal phase of the menstrual cycle, significant increases in progesterone were not observed until 180 min after 5000 I.U. of hCG (Teoh et al., 1990). In pilot studies, we found that hCG (500 I.U. i.m.) produced high levels of both E2 and progesterone within 4 h of administration in female rhesus monkeys.

**Acute Venous Catheter Implantation and Blood Sample Collection**

Monkeys were anesthetized with ketamine hydrochloride (5–10 mg/kg i.m.). A Sur-Flo Intercath containing a 20-gauge needle (i.d. 0.80 × 51 mm; Terumo Medical Corporation, Elkton, MD) was inserted into the saphenous vein using aseptic techniques. After removal of the needle stylet, the catheter was joined to heparin-impregnated sterile silicon tubing and secured with sutures. A second catheter for i.v. infusion of saline control or cocaine solutions was implanted in the opposite leg. Each monkey was placed in a standard primate restraint chair for 2 h before sample collection began to reduce any possible stress associated with the catheter implantation procedure and to ensure that any effects of the ketamine had dissipated. After drug or saline infusion, a 0.9% NaCl solution was infused at a rate of 2 ml/h. Blood samples for E2 and progesterone analysis were collected in heparinized tubes. Blood samples for cocaine analysis were collected in tubes containing potassium oxide and sodium fluoride (2.5 mg/ml) to prevent cocaine hydrolysis by serum esterases (Jatlow and Bailey, 1975). Samples were centrifuged, and aliquots of plasma were drawn and stored at −70°C until analysis.

**Drug and Hormone Preparation**

**Cocaine.** Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD), and solutions were prepared by dissolving cocaine in sterile saline for injection U.S.P. The solution was filter-sterilized using a 0.11-μm Millipore filter (Bedford, MA). Cocaine (0.4 or 0.8 mg/kg) or an equal volume vehicle control was infused into the saphenous vein of the leg opposite the effusion catheter in a single 1-min bolus injection.

**hCG.** hCG (Profasi) was purchased from Sigma Chemical Co. as sterile lyophilized powder and reconstituted with bacteriostatic water for i.m. injection.

**Plasma Hormone and Cocaine Analyses**

Data are reported for the analysis of E2, progesterone, and levels of hCG and cocaine in plasma. Details of the assay procedures follow:

- **E2.** Plasma concentration of 17β-E2 was determined in duplicate using a direct, double-antibody RIA kit purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). The following modification was made to the protocol: before analysis, the plasma samples were extracted and then reconstituted in zero standard. The assay sensitivity was 8.7 pg/ml, and the intra-assay and inter-assay coefficients of variation were 5.5 and 10.7%, respectively.

- **hCG.** Plasma hCG was determined in duplicate using a direct, double-antibody RIA kit purchased from ICN Biomedicals, Inc. The assay sensitivity was 0.20 ng/ml, and the intra-assay and inter-assay coefficients of variation were 6.6 and 8.7%, respectively.

**Progesterone.** Plasma progesterone was determined in duplicate using a direct, double-antibody RIA kit purchased from ICN Biomedicals. The assay sensitivity was 0.13 ng/ml, and the intra-assay and inter-assay coefficients of variation were 6.6 and 8.7%, respectively.

**Plasma Cocaine.** Levels of cocaine in plasma were measured in duplicate using gas chromatographic procedures with a nitrogen detector (Jacob et al., 1987). Assay sensitivity was 1.8 ng/ml. The intra-assay coefficient of variation was 3.1%.

**Statistical Analyses.** The effects of placebo-cocaine, cocaine, and hCG on ovarian steroid hormones were evaluated with ANOVA for repeated measures (Super ANOVA; Abacus Concepts, Inc., Berkeley, CA, 1989). ANOVA for repeated measures was used to compare group mean values at each sample period with baseline mean values using contrast tests. If ANOVA showed a significant main effect, contrast tests were used to determine which points were statistically different from each other. Paired t tests were used to evaluate baseline hormone levels before placebo-cocaine and cocaine administration. Correlational analyses were used to evaluate possible relationships between hormone baseline levels and the effects of cocaine. Probability levels of P < .05 or above are reported as statistically significant. Individual data are displayed as percentage change from baseline to facilitate comparisons of the effects of placebo and cocaine administration on E2.

**Pharmacokinetic Analyses.** Estimates of the primary kinetic parameters of cocaine (i.e., peak plasma cocaine concentrations and time to peak plasma concentration) were obtained from a nonlinear regression estimation software program based on the Manual of Pharmacologic Calculations with Computer Programs using PHARM/PCS Version 4.2 (MicroComputer Specialist MCS, Philadelphia, PA). Plasma drug concentrations were fitted to a single-dose, one-compartment model with bolus input, first order output, and elimination. Plasma concentrations were weighted by the reciprocal of the predicted concentrations. Estimates of the elimination half-life (t1/2) were obtained from the computer-fitted model.

**Results**

**Experiment I: Effects of Cocaine on Ovarian Steroid Hormones in Mid-Follicular Phase Rhesus Females (n = 6)**

**Baseline Levels of E2 and Progesterone.** There were no significant differences in baseline levels of E2 or progesterone before placebo and cocaine administration. Basal levels of E2 averaged 168 ± 44 pg/ml before placebo-cocaine administration and 139 ± 37 before cocaine administration. Basal progesterone levels averaged 0.24 ± 0.04 ng/ml before placebo-cocaine administration and 0.69 ± 0.30 ng/ml before cocaine administration. These basal hormone levels are consistent with expected levels for the mid-follicular phase in female rhesus monkeys studied in this laboratory.

**E2 and Progesterone Levels after Placebo and Cocaine Administration.** Figure 1 shows average E2 and progesterone levels for six mid-follicular phase females. After placebo-cocaine administration, E2 and progesterone levels did not change significantly from baseline. After cocaine administration, E2 levels increased significantly
Cocaine effects on ovarian steroid hormones in mid-follicular phase female rhesus monkeys

![Graph showing estradiol and progesterone levels](image)

**Fig. 1.** The effects of placebo and cocaine on estradiol and progesterone in mid-follicular phase female rhesus monkeys: the abscissas show consecutive samples collected at 15-min intervals before and after i.v. placebo and cocaine administration. Cocaine (0.8 mg/kg) or placebo was administered over 1 min as indicated at the vertical dotted line. Placebo conditions are shown as ○ and cocaine conditions are shown as ●. The left ordinate shows estradiol levels (pg/ml), and the right ordinate shows progesterone levels (ng/ml). Each data point is based on the average ± S.E. estradiol or progesterone level in five or six rhesus monkeys. Statistically significant changes from the pre-placebo or pre-cocaine baseline (BL) are indicated by asterisks (*P < .05, **P < .003, ***P < .001).

above baseline within 15 min (P < .001). The average increase in E2 levels was 28% above baseline at 15 min after cocaine administration. E2 remained significantly elevated above baseline levels for 45 min (P < .05–0.01). Progesterone levels did not change significantly from baseline after cocaine administration. Further analysis did not reveal a significant correlation between pre-cocaine baseline E2 levels and cocaine-induced increases in E2.

**Plasma Cocaine Levels.** Figure 2 shows plasma cocaine levels for the group of six mid-follicular phase monkeys (top). Peak plasma cocaine levels for the group averaged 160 ± 22 ng/ml at 4 min after i.v. cocaine administration. In two individuals, peak plasma cocaine levels were measured at 10 and 20 min post-injection, respectively. Analysis of plasma cocaine pharmacokinetics showed an average peak level (Cmax) of 176 ± 20 ng/ml at 7.6 ± 2.6 min (Tmax) post-injection.

**E2 Levels in Individual Monkeys.** Figure 3 shows the percentage change from baseline in E2 levels in three individual monkeys after cocaine or placebo administration. In monkey 25F, E2 levels increased 56% above baseline within 15 min after cocaine administration. Peak plasma cocaine levels of 166 ng/ml were measured 5 min before the E2 peak. There were no significant changes in E2 levels after placebo administration. In monkey 154F, E2 levels increased 45% above baseline at 45 min after cocaine administration. Cocaine plasma levels peaked at 195 ng/ml within 20 min after cocaine administration. There were no significant changes in E2 levels after placebo administration. In monkey 91B102, E2 levels increased 47% above baseline within 15 min after cocaine administration. Cocaine plasma levels peaked at 177 ng/ml at 10 min after cocaine administration. Placebo administration was followed by a small increase in E2 of 17% within 15 min, which gradually decreased over the remainder of the sampling period.

**Experiment II: Effects of Cocaine on Ovarian Steroid Hormones in Mid-Luteal Phase Rhesus Females (n = 4 to 6)**

**Baseline Levels of E2 and Progesterone.** There were no significant differences in baseline levels of E2 or progesterone before placebo and cocaine administration. Basal levels of E2 averaged 113 ± 8.36 pg/ml before placebo-cocaine administration and 84 ± 26 pg/ml before cocaine administration. Basal progesterone levels averaged 7.8 ± 0.87 ng/ml before placebo-cocaine administration and 6.4 ± 0.81 ng/ml before cocaine administration. These luteal phase basal progesterone levels were significantly higher than basal levels measured during the follicular phase (P < .0001). Progesterone levels measured on the day before the study were always higher than baseline progesterone levels measured on the day of the study. For
example, in the placebo group, basal progesterone levels averaged 10.9 ± 1.0 ng/ml on the day before the study. In the cocaine group, basal progesterone levels averaged 11.7 ± 1.5 ng/ml on the day before the study. These data suggest that these studies were conducted on the descending limb of the luteal phase progesterone curve when E2 levels are decreasing.

**E2 and Progesterone Levels after Placebo and Cocaine Administration.** Group data for four mid-luteal phase females are shown in Fig. 4. E2 levels did not change significantly from baseline after placebo-cocaine or cocaine administration. Progesterone levels also did not change significantly from baseline after placebo-cocaine or cocaine administration.

**Plasma Cocaine Levels.** Figure 2 shows plasma cocaine levels for the group of four mid-luteal phase monkeys (bottom). Peak plasma cocaine levels for the group averaged 170 ± 22.6 ng/ml at 4 min after i.v. cocaine administration. These peak plasma cocaine levels did not differ significantly from those measured in follicular phase females. Analysis of plasma cocaine pharmacokinetics showed an average peak level (Cmax) of 219 ± 27 ng/ml at 9.5 ± 3.7 min (Tmax) post-injection. There were no statistically significant differences between mid-luteal and mid-follicular phase females for these pharmacokinetic measures.

**Experiment III: Effects of Cocaine on Ovarian Steroid Hormones in Mid-Luteal Phase Females after hCG Administration (n = 6)**

**Plasma hCG Levels.** Figure 5 shows plasma hCG levels before and after hCG administration (row 1). Before hCG administration, basal levels of hCG averaged 0.61 ± 0.08, 1.66 ± 1.04, and 4.12 ± 2.6 ng/ml in the placebo and low- and high-dose cocaine groups (Sample 1). hCG levels increased significantly (P < .0001) within 2 h after hCG administration (Sample 2). Four hours after hCG administration (Sample 3) and immediately before i.v. administration of placebo-cocaine and 0.4 and 0.8 mg/kg cocaine, plasma hCG levels averaged 29.4 ± 7.6, 33.1 ± 11.7, and 38.3 ± 8.9 ng/ml, respectively. There were no significant differences in hCG before placebo or cocaine administration.
Cocaine Effects on Acute hCG Stimulated Ovarian Hormones

Fig. 5. The effects of hCG and cocaine on estradiol and progesterone in mid-luteal phase female rhesus monkeys (n = 6): the abscissas show consecutive samples collected before and after hCG and cocaine administration. The left ordinates show hCG (ng/ml), E2 (pg/ml), and progesterone (ng/ml) levels. Plasma hCG (500 IU i.m.) was administered immediately after collection of the baseline sample (sample 1) at 9:30 AM as indicated at the dotted vertical line. Samples 2 and 3 were collected at 11:30 AM and 1:30 PM, and then cocaine (0.4 (●) or 0.8 (▲) mg/kg, i.v.) or placebo (○) was administered at 1:45 PM as indicated by the vertical solid line. Sample 4 was collected at 2:00 PM, and the remaining 12 samples (samples 4–16) were collected at 15-min intervals for 180 min. ○, placebo-cocaine condition. ●, 0.4 mg/kg cocaine. ▲, 0.8 mg/kg cocaine. Each data point is based on the average ± S.E. level in six rhesus monkeys. The first sample that was significantly different from baseline is indicated by an asterisk (*, P < .001). All subsequent points were significantly above baseline throughout the sample collection period.

There were no significant differences in progesterone levels immediately before placebo or low- or high-dose cocaine administration. Basal progesterone levels averaged 12.10 ± 2.2, 10.6 ± 2.1, and 10.2 ± 1.0 ng/ml before the administration of hCG (Sample 1). Progesterone increased significantly above baseline levels within 2 h after hCG administration (Sample 2) (P < .0001). Four hours after hCG administration, progesterone levels averaged 16.2 ± 2, 13.7 ± 1.9, and 15.2 ± 2.5 ng/ml (Sample 3).

E2 and Progesterone Levels after hCG, Placebo, and Cocaine Administration. As shown in Fig. 5, E2 levels tended to increase after placebo and after low- and high-dose cocaine administration (Samples 4–16). There were no significant differences in E2 levels between placebo and cocaine treatment conditions. Progesterone levels remained significantly above baseline throughout the sampling period (P < .004–0.001). Progesterone levels did not change significantly after placebo or cocaine administration, and there were no significant differences between placebo and cocaine treatment conditions.

tion, and hCG levels did not change significantly from pre-placebo and pre-cocaine levels throughout the 180-min sampling period.

Baseline Levels of E2 and Progesterone before and after hCG Administration. Figure 5 shows plasma levels of E2 and progesterone before and after the administration of hCG and placebo-cocaine or 0.4 or 0.8 mg/kg cocaine (rows 2 and 3). Before hCG administration, basal levels of E2 averaged 85.6 ± 15.5, 67.4 ± 12, and 66.4 ± 10.7 pg/ml in the placebo and low- and high-dose cocaine groups (Sample 1). E2 increased significantly above baseline (P < .004) within 2 h after hCG administration (Sample 2). Four hours after hCG administration (Sample 3), E2 increased to an average of 164.8 ± 16, 134.6 ± 22.9, and 144 ± 22.4 pg/ml under placebo and low- and high-dose cocaine conditions, respectively.

There were no significant differences in E2 levels before the administration of placebo and low- and high-dose cocaine. E2 levels remained significantly above baseline throughout the sampling period (P < .0001).
Cocaine Effects on Ovarian Steroid Hormones

Discussion

Cocaine administration was followed by a significant increase in basal E2 levels during the mid-follicular phase but not during the mid-luteal phase of the menstrual cycle in female rhesus monkeys. Progesterone levels were not affected by i.v. cocaine administration in either phase of the menstrual cycle. This is the first report of a cocaine-stimulated increase in E2 in female rhesus monkeys. In addition, this is the first evidence that the effects of cocaine on an ovarian steroid hormone are menstrual cycle phase-dependent. It is unlikely that the observed increases in E2 are due to factors other than cocaine administration because there were no changes in E2 levels after placebo administration to follicular phase females. Basal E2 levels did not differ significantly before cocaine and placebo administration, and cocaine pharmacokinetics did not differ between menstrual cycle phases. The possible biological significance of this cocaine-induced E2 increase and the implications for cocaine-related menstrual cycle abnormalities are discussed later. The temporal concordance between the stimulation by cocaine of an anterior pituitary hormone, LH, and the ovarian steroid hormone, E2, is described. Some limitations of this experiment that may influence interpretation of these data are also discussed.

Stimulation by Cocaine of E2 in Mid-Follicular Phase Female Rhesus Monkeys. The time course of the cocaine-stimulated increase in E2 was comparable to that previously reported for cocaine-stimulated increases in LH in rhesus males and females (Mello et al., 1990, 1993). In the current study, peak levels of E2 were measured 15 min after 0.8 mg/kg i.v. cocaine administration, 5 min after peak levels of plasma cocaine were detected. We previously found that LH increased significantly within 10 to 20 min after 0.8 mg/kg i.v. cocaine administration, and peak LH levels were detected within 20 to 30 min (Mello et al., 1990, 1993). E2 levels remained significantly above baseline for 45 min after cocaine administration, and in our previous studies, LH also remained above baseline levels for 40 to 50 min (Mello et al., 1990, 1993). However, in contrast to the present study, there were no menstrual cycle phase differences in the effects of cocaine on LH (Mello et al., 1990, 1993). As noted earlier, robust increases in LH levels after cocaine administration have been consistently observed in humans, but the effects of cocaine on E2 have not been studied (see Mello and Mendelson, 1997, for review).

The increase in E2 in these follicular phase females averaged 48.8 pg/ml and exceeded 70 pg/ml in some individuals. The source of this relatively rapid increase in E2 is unclear. Normally, 95% of E2 is secreted directly from the ovary, primarily from the dominant follicle (O'Malley and Strott, 1999). During the periovulatory phase of the menstrual cycle, there is a dramatic increase in E2 that precedes the ovulatory LH surge (Hotchkiss and Knobil, 1994). However, it is unlikely that these monkeys were periovulatory on days 8 and 9 of their menstrual cycles. Moreover, E2 returned to baseline levels before the end of the sampling period. Pre-cocaine baseline E2 levels did not correlate significantly with the magnitude of the E2 increase in individual monkeys. However, the first blood sample was collected at 15 min after cocaine administration, so it is possible that higher E2 levels occurred earlier, when plasma cocaine levels were ascending.

The possible biological significance of the cocaine-stimulated increases in E2 is suggested by previous reports that E2 administration disrupts folliculogenesis in rhesus monkeys (Zeleznik, 1981; Dierschke et al., 1985, 1987). A small increase in E2 (from 60 to 90 pg/ml) on days 3 to 6 of the menstrual cycle reduced FSH levels and lengthened the follicular phase (Zeleznik, 1981). Similarly, in ovariectomized monkeys, the administration of as little as 18 ± 2 pg/ml E2 resulted in a gradual, significant decrease in FSH from 320 ± 8 to 20 ± 5 ng/ml over 7 days (Bassett and Zeleznik, 1990). Although differences in assay procedures and sensitivity complicate comparisons between laboratories, the average increase in E2 observed here is within the range shown to have significant effects on FSH in female rhesus monkeys.

In human cocaine abusers, repeated “binge” cocaine administration is the most common use pattern (Ward et al., 1997). Only a single dose of cocaine was administered in this study, and the effects of repeated cocaine injections on E2 are unknown. However, if repeated cocaine administration continued to stimulate comparable increases in E2, this could contribute to the menstrual cycle disruptions seen during chronic cocaine exposure (Mello et al., 1997; Potter et al., 1999, 1998). For example, continuous exposure to E2 for only 24 h on day 6 of the menstrual cycle resulted in atresia of the dominant ovarian follicle in rhesus monkeys (Dierschke et al., 1985). Repeated 24-h exposures to E2 at 10-day intervals resulted in recurrent atresia of the dominant ovarian follicle, but normal ovulatory menstrual cycles occurred when E2 treatments were separated by 14 days (Dierschke et al., 1987). Atresia of the dominant ovarian follicle usually results in an anovulatory cycle, and E2 and LH do not increase at mid-cycle. Anovulatory cycles were often observed during chronic cocaine self-administration (Mello et al., 1997) or chronic cocaine administration (Chen et al., 1998; Potter et al., 1998, 1999). For example, seven of eight rhesus females were anovulatory when high doses of cocaine (4 mg/kg) were administered as an i.v. bolus on days 2 to 15 of the follicular phase (Chen et al., 1998). Seven of eight saline-treated controls had normal ovulatory menstrual cycles, and E2 levels measured between cycle days 6 and 15 were significantly higher than those in the cocaine-treated females (Chen et al., 1998). Several procedural differences, including a 5-fold difference in cocaine dose (4 versus 0.8 mg/kg), limit comparisons between the present study and previous studies of subchronic cocaine administration.

Effects of Cocaine on E2 in Mid-Luteal Phase Female Rhesus Monkeys. Cocaine did not stimulate E2 during the mid-luteal phase of the menstrual cycle under basal or hCG-stimulated conditions. The most likely explanation is that the relatively high levels of progesterone during the mid-luteal phase limited the E2 response to cocaine. Basal progesterone levels were significantly higher during the mid-luteal phase than during the mid-follicular phase, and it is well established that progesterone limits the physiological effects of estrogens under certain conditions (Clark and Mani, 1994). For example, progesterone administration consistently blocked estrogen-induced LH surges in rhesus monkeys (Dierschke et al., 1973; Wildt et al., 1981; VanVugt et al., 1992), an effect that is somewhat analogous to the inability of cocaine to
stimulate E_2 in mid-luteal phase monkeys observed in the present study. Menstrual cycle phase differences in the cerebral vasoconstrictive effects of cocaine have been reported (Kaufman et al., 1998). The constrictive effects of cocaine on the cerebral vasculature were less during the follicular phase than during the luteal phase of the menstrual cycle in women, and these results were interpreted as indicative of the vascular protective effects of follicular phase estrogen, unopposed by progesterone.

The different effects of cocaine on E_2 during the mid-follicular and the mid-luteal phases cannot be attributed to differences in peak plasma cocaine levels or to differences in the half-life of cocaine in plasma. An examination of cocaine pharmacokinetics in humans showed that these pharmacokinetic parameters did not differ significantly as a function of menstrual cycle phase (Mendelson et al., 1999b). In that study, no gender or menstrual cycle phase differences in cocaine pharmacokinetics were detected in men and women matched for body mass index (Mendelson et al., 1999b).

**Interactions of Cocaine with Progesterone in Mid-Follicular and the Mid-Luteal Phase Female Rhesus Monkeys.** In view of the significant stimulation of E_2 by cocaine, the lack of any significant effect on progesterone was rather surprising. Cocaine did not increase progesterone during the mid-follicular phase when progesterone levels were low. During the mid-luteal phase, when basal progesterone levels were high, cocaine administration was not followed by an increase or a decrease in progesterone levels. It is possible that the high progesterone levels after hCG stimulation (16.8 ± 2.7 pg/ml) were near the physiological maximum, but this explanation could not account for the lack of change in basal mid-luteal progesterone levels (~7 pg/ml). However, production and secretion rates of progesterone are much higher during the luteal phase than the follicular phase in women, although metabolic clearance rates are equivalent (O’Malley and Strott, 1999). Thus, it may be more difficult to perturbate progesterone during the luteal phase when basal progesterone levels are high.

It is very difficult to accurately predict peak levels of progesterone during the mid-luteal phase of the menstrual cycle. In this study, basal progesterone levels were always higher on the day before the study was conducted. This suggests that both progesterone and E_2 were declining on the study day. Because the pulsatile release of both progesterone and E_2 is relatively slow during the mid-luteal phase in humans (8.5–8.9 pulses/24 h) (Rossmanith et al., 1990), a 15-min sample collection frequency should have been frequent enough to detect cocaine-related hormonal changes. Moreover, the duration of sampling (300 min after cocaine) should have been sufficient to detect any decrease in progesterone. If progesterone is not affected by cocaine during the mid-luteal phase of the menstrual cycle, the luteal phase abnormalities observed during chronic cocaine self-administration (Mello et al., 1997) probably reflect disruptions that occurred during follicle development.

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2000

Cocaine Effects on Ovarian Steroid Hormones

1145

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