Ovarian Steroid Hormone Modulation of the Acute Effects of Cocaine on Luteinizing Hormone and Prolactin Levels in Ovariectomized Rhesus Monkeys

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Revised July 17, 2003: accepted September, 26, 2003

ABSTRACT
Cocaine stimulates significant increases in luteinizing hormone (LH) and decreases prolactin levels in gonadally intact rhesus monkeys, but cocaine did not alter plasma levels of these anterior pituitary hormones in ovariectomized females. These findings suggested that ovarian steroid hormones may contribute to the endocrine effects of acute cocaine administration. To test this hypothesis, the acute effects of cocaine and placebo-cocaine on plasma LH and prolactin levels were examined in five ovariectomized rhesus females during three chronic hormone replacement conditions: 1) estradiol (E2/H9252) treatment (0.0015–0.006 mg/kg/day i.m.), 2) progesterone treatment (0.32 mg/kg/day i.m.), and 3) combinations of progesterone (0.32 mg/kg/day i.m.) and E2/H9252 (0.002 and 0.004 mg/kg/day i.m.). Cocaine (0.8 mg/kg i.v.) did not alter prolactin or LH in ovariectomized monkeys without ovarian steroid replacement. During chronic estradiol treatment, cocaine produced an estradiol dose-dependent decrease in prolactin. Cocaine also decreased prolactin during treatment with progesterone alone and progesterone + E2β (0.004 mg/kg/day i.m.). Cocaine stimulated a significant increase in LH during treatment with progesterone alone, but not during treatment with progesterone + E2β, or three of four estradiol treatment doses. Cocaine pharmacokinetics did not differ as a function of hormone replacement conditions. Together, these data suggest that both E2/H9252 and progesterone modulate cocaine’s effects on prolactin, whereas E2/H9252 alone and in combination with progesterone, do not facilitate LH release in response to cocaine in ovariectomized rhesus females.

There is increasing evidence that ovarian steroid hormones may modulate some biological and behavioral effects of cocaine, but the nature of these interactions is poorly understood (for review, see Mello and Mendelson, 2002). In behavioral studies, estradiol may enhance the reinforcing and locomotor effects of cocaine in rats (Roberts et al., 1989; Lynch et al., 2000; Sell et al., 2000). In endocrine studies, cocaine stimulates a rapid increase in estradiol in female rhesus monkeys during the follicular phase of the menstrual cycle when ovarian steroid hormone levels are low, but not during the luteal phase when ovarian steroid hormone levels are high (Mello et al., 2000). Moreover, there is general agreement that cocaine stimulates release of some anterior pituitary hormones (Mello and Mendelson, 2002). For example, acute cocaine administration stimulates significant increases in luteinizing hormone (LH) and adrenocorticotropin hormone (ACTH) in humans (Mendelson et al., 1992, 2001; Sholar et al., 1998) and in rhesus monkeys (Mello et al., 1990a, 1993; Sarnyai et al., 1996; Broadbear et al., 1999a,b).

The influence of the ovarian steroid hormone milieu on the hormonal effects of cocaine has not been systematically examined. However, the potential importance of gonadal steroid hormones in modulating the effects of cocaine on anterior pituitary hormones is suggested by the observation that cocaine did not stimulate LH or ACTH in ovariectomized rhesus monkeys as it did in gonadally intact male and female rhesus monkeys (Mello et al., 1995; Sarnyai et al., 1995). This finding was surprising, because the ovariectomized females, gonadally intact males, and follicular and luteal phase females were given the same dose of cocaine (0.8 mg/kg i.v.) and were studied under identical conditions (Mello et al., 1990a, 1993; Sarnyai et al., 1996). When synthetic luteinizing-hormone-releasing-hormone or synthetic corticotropin-releasing-factor was administered to the same ovariectomized females, LH and ACTH increased significantly after
luteinizing-hormone-releasing-hormone and corticotropin-releasing-factor, respectively (Mello et al., 1995; Sarnyai et al., 1995). Thus, the absence of LH and ACTH stimulation by cocaine in these ovariectomized females could not be explained by a “ceiling effect” that impaired pituitary responsiveness and prevented further increases in LH and ACTH (Mello et al., 1995; Sarnyai et al., 1995). One major difference between gonadally intact and ovariectomized rhesus females is the basal levels of ovarian steroid and gonadotropin hormones. After ovariectomy, estradiol and progesterone decrease to very low levels, and in the absence of tonic inhibition by ovarian steroid hormones, LH increases to peri-ovulatory levels (Hotchkiss and Knobil, 1994).

In contrast to cocaine-induced stimulation of LH and ACTH, acute administration of cocaine significantly decreases prolactin levels in gonadally intact rhesus monkeys (Mello et al., 1990a, 1994; for review, see Mello and Mendelson, 2002). This effect of cocaine is consistent with its pharmacological actions as an indirect dopamine agonist because prolactin release is under inhibitory dopaminergic control (Ben-Jonathan, 1985; Ben-Jonathan and Hnasko, 2001). Yet in ovariectomized female rhesus monkeys, acute cocaine administration did not decrease prolactin as it did in gonadally intact rhesus males and females studied under identical conditions (Mello et al., 1990b, 1993, 1995). Together, these findings suggested that the ovarian steroid milieu may be an important modulator of cocaine’s effects on anterior pituitary hormones.

The goal of the present study was to test the hypothesis that ovarian steroid hormone levels are one determinant of cocaine’s effects on anterior pituitary hormones. Ovariectomized monkeys were given steroid hormone replacement regimens that were designed to produce physiological levels of estradiol and progesterone. We examined the acute effects of cocaine on prolactin and LH during chronic maintenance on low-, moderate-, and high-dose estradiol replacement. Estradiol replacement was compared with progesterone replacement and with maintenance on estradiol + progesterone combinations. We now report that basal ovarian steroid hormone levels can influence the effect of cocaine on prolactin but have less consistent effects on LH in ovariectomized rhesus females.

Materials and Methods

Subjects

Five adult ovariectomized female rhesus monkeys (Macaca mulatta; 6.0–11.0 kg) lived in individual cages in a room with sexually mature male and female monkeys. Monkeys were maintained on ad libitum food and water, and monkey chow was supplemented with fresh fruit, vegetables, and multiple vitamins each day. A 12-h light/dark cycle (7:00 AM–7:00 PM) was in effect. All females had been ovariectomized for over 1 year and had been adapted to the laboratory for at least 12 months before these studies began. No subject had a history of chronic drug exposure. Each subject was used as her own control across hormone treatment conditions, and successive endocrine study days were separated by at least 6 to 8 weeks.

Animal maintenance and research were conducted in accordance with the guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facility is licensed by the U.S. Department of Agriculture. This protocol was approved by the McLean Hospital Institutional Animal Care and Use Committee. Monkeys had visual, auditory, and olfactory contact with other monkeys throughout the study. Enrichment was provided by toys (puzzle feeders, mirrors, chew toys), food treats, television, and music. The health of the monkeys was monitored periodically by a consultant veterinarian trained in primate medicine.

Ovarian Steroid Hormone Administration

The effects of cocaine on anterior pituitary hormones were studied during chronic saline administration, and during chronic administration of estradiol benzoate (E2β), progesterone and combinations of E2β and progesterone. Successive doses of each hormone were separated by a hormone-free interval to prevent any carryover effects from the previous hormone treatment condition. In preliminary studies, we measured basal levels of 17β-estradiol to verify the hormonal status of each ovariectomized monkey. After ovariectomy, 17β-estradiol levels decreased within 6 to 12 days and fluctuated between 0 and 23 pg/ml. Two monkeys were observed for 145 days after ovariectomy, and samples for 17β-estradiol analysis were collected every 5 or 7 days. The lowest detectable 17β-estradiol values averaged between 12.6 ± 1.3 and 14.1 ± 1.7 pg/ml over the period of observation. On the basis of these data, we defined baseline estradiol levels as less than 20 pg/ml in ovariectomized females. This empirically derived criterion also provided a basis for evaluating the effect of the estradiol replacement regimens on basal levels of 17β-estradiol. The rationale for hormone dose selection and the details of hormone administration are described below.

Estradiol Administration.

Daily intramuscular injections of E2β (0.0015, 0.002, 0.004, and 0.006 mg/kg/day) were given for at least 8 days before each endocrine study day. E2β doses were administered in an ascending order. E2β was administered at 10:30 every morning to unanesthetized monkeys. The daily injection procedure yielded relatively stable and predictable levels of estradiol at the doses studied. Blood samples for estradiol analysis were collected 3 times a week to follow 17β-estradiol levels during E2β replacement. A single sample for estradiol analysis was also collected on each endocrine study day. At the end of each E2β dose condition, samples for estradiol analysis were collected every 5 to 7 days for 6 to 8 weeks. The next estradiol replacement condition did not begin until after basal 17β-estradiol levels returned to baseline levels.

In gonadally intact females, estradiol and LH feedback relationships control levels of each hormone across the menstrual cycle (Hotchkiss and Knobil, 1994). Estradiol inhibits LH except at the peri-ovulatory phase when estradiol stimulates an LH surge (Hotchkiss and Knobil, 1994). After ovariectomy or natural menopause, the loss of estradiol inhibitory feedback results in high levels of LH. In the present study, the lowest doses of E2β were extrapolated from transdermal estradiol doses used to treat menopausal symptoms (0.05–0.10 mg/day for a 50-kg woman is the equivalent of 0.001–0.002 mg/kg/day) (cf. Carr, 1998). These doses were selected to mimic estradiol levels during the follicular phase of the normal menstrual cycle. The highest doses of E2β studied were selected to mimic estradiol levels during the peri-ovulatory phase of the menstrual cycle (Hotchkiss and Knobil, 1994). E2β was administered chronically because acute E2β administration can stimulate an LH surge in ovariectomized monkeys that is comparable to the peri-ovulatory LH surge in normal females (Yamaji et al., 1972; Williams and Hodgen, 1983; Levine et al., 1985; Norman et al., 1986; Mello et al., 1992). Moreover, acute administration of E2β has a biphasic effect on LH release. During the first 12 to 16 h after E2β administration to ovariectomized females, LH decreases below baseline levels and then increases to peak levels within 42 to 56 h (Levine et al., 1985;
Terasawa, 1985). LH levels were used as a physiological index of the chronic effects of E$_2$$\beta$ treatment in the present study, and monkeys were maintained on E$_2$$\beta$ until stable LH and estradiol levels were reached.

**Progesterone Administration.** In preliminary studies, a range of i.m. progesterone doses were evaluated to identify a dose that provided progesterone levels characteristic of the early luteal and mid-luteal phases of a normal ovulatory menstrual cycle in female rhesus monkeys. Ovulation is usually inferred from a luteal phase increase in progesterone to 8.5 ng/ml or above (Filicori et al., 1984). In our previous studies in rhesus monkeys, peak progesterone levels during ovulatory menstrual cycles averaged between 12 and 16 ng/ml (Mello et al., 1997). In ovarioctomized females in the present study, a progesterone dose of 0.32 mg/kg/day administered intramuscularly resulted in progesterone plasma levels that ranged between 9 and 21 pg/ml.

**Administration of Progesterone and Estradiol Combinations.** To study the effect of progesterone and E$_2$$\beta$ in combination, the same dose of progesterone (0.32 mg/kg i.m.) was administered with 0.002 and 0.004 mg/kg/day of E$_2$$\beta$. When administered alone, these two doses of E$_2$$\beta$ resulted in levels of 17$\beta$-estradiol that averaged 114 ± 20 and 186 ± 30 pg/ml, respectively. In combination with 0.32 mg/kg i.m. progesterone, 0.002 and 0.004 mg/kg/day E$_2$$\beta$ produced lower levels of 17$\beta$-estradiol that averaged 94.9 ± 17.2 and 79.6 ± 13.3 pg/ml, respectively. Progesterone levels averaged 13.5 ± 1.15 ng/ml in combination with 0.002 mg/kg estradiol and 13.7 ± 2.4 ng/ml in combination with 0.004 mg/kg/day E$_2$$\beta$. Baseline values of 17$\beta$-estradiol and progesterone in ovarioctomized females after chronic hormone replacement are shown in Table 1.

**Cocaine Administration**

The acute effects of cocaine (0.8 mg/kg i.v.) and placebo-cocaine on basal levels of LH and prolactin were evaluated. This dose of cocaine stimulated a significant increase in LH and a decrease in prolactin levels in gonadally intact rhesus males and females (Mello et al., 1990a,b, 1993). However, as noted earlier, acute administration of 0.8 mg/kg i.v. cocaine under identical conditions did not alter basal levels of anterior pituitary hormones in ovarioctomized females (Mello et al., 1995; Sarnyai et al., 1995). Higher cocaine doses were not studied, because we have found that administration of 1.0 mg/kg i.v. cocaine resulted in hyperactivity and agitated behavior, and because the convulsant dose range for cocaine is 3 to 8 mg/kg i.v. (Matsuzaki et al., 1976; Misra et al., 1977). Cocaine or placebo-cocaine was administered over 10 s into the saphenous vein of the leg opposite the exfusion catheter.

**Frequency and Duration of Blood Sample Collection**

Baseline levels of LH and prolactin were measured at ~10 min before cocaine or placebo-cocaine was administered. After i.v. cocaine or placebo administration, samples for analysis of LH and prolactin were collected at 8, 16, 20, 35, 50, 65, 80, 95, and 110 min. This sampling frequency was based on our previous studies of cocaine's hormonal effects in gonadally intact rhesus monkeys. In those studies, LH increased significantly within 10 to 20 min after 0.8 mg/kg i.v. cocaine and reached peak levels within 30 min (Mello et al., 1990a,b, 1993). Prolactin decreased gradually after 0.8 mg/kg i.v. cocaine and reached a nadir within 40 to 80 min (Mello et al., 1990a). Samples for analysis of plasma cocaine levels were collected at 4-min intervals for the first 20 min after cocaine administration and at 15-min intervals thereafter. The frequency of sample collection for cocaine analysis was based on studies of cocaine pharmacokinetics in which peak cocaine plasma levels were detected within 2 to 4 min after intravenous administration in rhesus monkeys (Sarnyai et al., 1996; Mendelson et al., 1999a; Mello et al., 2002), and within 4 to 8 min in humans (Mendelson et al., 1999b). The duration of sampling was determined in part, by the half-life of cocaine in female rhesus monkeys, which averages between 40 and 60 min after an i.v. bolus injection of 0.8 mg/kg cocaine (Mendelson et al., 1999a). The duration of sampling was limited by the volume of blood that can be safely exsufed from a rhesus monkey. In the present study, between 60 and 62.5 ml of blood was collected over 120 min. The 110-min postcocaine sampling period permitted measurement of hormonal changes during and after the period of maximal cocaine concentrations in plasma.

**Venous Catheterization and Blood Sample Collection Procedures**

Monkeys were anesthetized with ketamine hydrochloride (5–10 mg/kg i.m.), which does not alter pituitary gonadotropins in rhesus monkeys (Ferin et al., 1976; Fuller et al., 1984). A Sur-Flo Intracath containing a 20-gauge needle (i.d. 0.80 × 51 mm) (Terumo Medical Corporation, Elkton, MD) was inserted into the saphenous vein of each leg using aseptic procedures. After removal of the needle stylet, each catheter was joined to heparin-impregnated sterile silicon tubing and secured with sutures. One catheter was used for injection of cocaine solutions, and one catheter was used for withdrawal of blood samples to measure plasma levels of cocaine and anterior pituitary hormones. The monkey was placed in a standard primate chair for at least 90 min before collection of baseline samples. Monkeys were adapted to chair-restraint on several occasions before this study began.

Blood samples for cocaine analysis were collected in tubes containing potassium oxalate and sodium fluoride (2.5 ng/ml) to prevent cocaine hydrolysis by serum esterases (Jatlow and Bailey, 1975). Blood samples for LH and prolactin were collected in heparinized tubes. 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, and solutions were prepared by dissolving cocaine in sterile saline for injection U.S.P. The solution was filter-sterilized using a 0.11-$\mu$m Millipore filter (Millipore Corporation, Bedford, MA). Cocaine (0.8 mg/kg) or an equal volume vehicle control solution was infused into the saphenous vein of the leg opposite the exfusion catheter.

**Estradiol and Progesterone. β-Estradiol 3-benzoate (1,3,5-[10]-estratriene-3,17β-diol) and progesterone were purchased from the Sigma-Aldrich (St. Louis, MO). Estradiol benzoate and progesterone

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**TABLE 1**

<table>
<thead>
<tr>
<th>Basal ovarian steroid hormone levels in ovarioctomized female Rhesus monkeys with and without hormone replacement</th>
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<tr>
<td>No Hormone Replacement</td>
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<tr>
<td>17$\beta$-estradiol (pg/ml)</td>
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<td>Progesterone (ng/ml)</td>
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were suspended in sesame oil and diluted to the appropriate milligram per kilogram dose for individual animals. Aliquots of less than 1 ml were injected intramuscularly at the same time each day. Estradiol and progesterone powder and stock solutions were stored in the dark at room temperature.

**Plasma Hormone and Cocaine Analyses**

Data are reported for the analysis of LH, prolactin, and plasma cocaine levels using the following assay procedures.

**LH Radioimmunoassay.** Plasma LH concentrations were measured in duplicate by a double-antibody radioimmunoassay procedure similar to that described by Midgley (1966) using rhesus pituitary LH reference preparation (NICHID-rhLH, also known as WP-XV-20), prepared by W. Peckham and Recombinant (Genzyme, Cambridge, MA) cynomolgus monkey luteinizing hormone antigen (AFP-6936A) for iodination and anti-recombinant cynomolgus monkey luteinizing hormone serum (rabbit) (AFP342994) obtained through the National Hormone and Pituitary Program (Dr. A. F. Parlow), supported by the National Institute of Child Health and Human Development and the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. Radioiodination was performed using chloramine-T (Greenwood et al., 1963) with sodium iodide-125 purchased from PerkinElmer Life Sciences (Boston, MA). Goat anti-rabbit gammaglobulin was obtained from Calbiochem-Novabiochem (La Jolla, CA). Results are expressed in nanograms per milliliter in terms of the reference preparation. The LH assay sensitivity was 6.9 ng/ml. Intra- and interassay coefficients of variance were 4.5 and 8.4%, respectively. The standard RIA curve is shown in Fig. 1.

**Prolactin Radioimmunoassay.** Plasma prolactin concentrations were measured using a double antibody RIA kit purchased from Pantex, Santa Monica, CA. Results are expressed in nanograms per milliliter in terms of the reference preparation. The PRL assay sensitivity was 2.2 ng/ml. Intra- and interassay coefficients of variance were 9.8 and 12.0%, respectively. The standard RIA curve is shown in Fig. 1.

**Estradiol Radioimmunoassay.** Plasma concentrations of 17β-estradiol were determined in duplicate using a double antibody RIA kit purchased from ICN Biomedicals Inc. (Cosa Mesa, CA). A modification was made to the protocol. Before analysis, the plasma samples were extracted and then reconstituted in zero standard. The estradiol assay sensitivity was 8.2 pg/ml. Intra- and interassay coefficients of variance were 7.2 and 12.7%, respectively.

**Progesterone Radioimmunoassay.** Plasma progesterone concentrations were determined in duplicate using a direct, double-antibody RIA kit purchased from ICN Biomedicals Inc. The progesterone assay sensitivity was 0.12 ng/ml, and the intra- and interassay coefficients of variance were 5.9 and 10.1%, respectively.

**Plasma Cocaine Analysis.** Plasma cocaine levels were measured in duplicate using a solid phase extraction method described by Spec Instructions Manual by Ansys with a Hewlett Packard model 5890 Series II gas chromatograph equipped with a capillary column and a Hewlett Packard 5971 Series mass selective detector (Abusada et al., 1993). The assay sensitivity was 10 ng/ml, and the intra-assay coefficient of variance was 2.2%.

**Pharmacokinetic Analysis.** Estimates of cocaine’s primary kinetic parameters (i.e., peak plasma cocaine concentrations and time to peak plasma concentration) were calculated, and secondary parameters (i.e., area under the curve, initial and terminal phase half-lives) were obtained directly from a nonlinear regression estimation software program based upon 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 (\( t_{1/2} \)) were obtained from the computer-fitted model. A one-compartment model was used, because the duration of sampling was 110 min.

**Statistical Analysis.** The effects of cocaine and its placebo on anterior pituitary hormones were evaluated with analysis of variance (ANOVA) Abacus Concepts, Inc., Berkeley, CA. If ANOVA for repeated measures showed a significant main effect, contrast tests were used to determine which mean values were statistically different from each other within each treatment condition. ANOVA was used to compare treatment groups and the significance of group mean values at each sample period were compared with baseline means using contrast tests for comparison of multiple experimental groups with a single control group. Hyndt-Feldt epsilon adjustments were used in all contrast tests. Probability levels of \( P < 0.05 \) to 0.0001 are reported as statistically significant.
Results

Three studies were conducted to characterize the interactions between ovarian steroid hormones and the acute effects of cocaine on LH and prolactin in ovariectomized rhesus females. In the first study, the dose range over which daily E_2β administration produced physiologically relevant levels of 17β-estradiol was evaluated. E_2β was studied up to doses that significantly decreased LH levels in ovariectomized monkeys. In the second study, the effect of cocaine on anterior pituitary hormones was studied in ovariectomized monkeys without estradiol replacement. These control data provided a basis for comparison with the effects of estradiol replacement. In the third study, the effects of cocaine on prolactin and LH were studied during chronic administration of E_2β alone (0.0015–0.006 mg/kg/day), progesterone alone (0.32 mg/kg/day), and the same dose of progesterone in combination with two doses of E_2β (0.002 and 0.004 mg/kg/day).

Study 1: Effects of Chronic E_2β Administration on Basal Levels of 17β-Estradiol, LH, and Prolactin (Fig. 2)

17β-Estradiol. Basal 17β-estradiol levels averaged 22.7 ± 2.1 pg/ml (data not shown). Figure 2 shows that after E_2β replacement, the average levels of 17β-estradiol increased with increasing doses of E_2β dose (r = 0.799, P = 0.0001). The lowest doses of E_2β (0.001–0.0015 mg/kg/day) did not increase 17β-estradiol levels significantly above baseline levels. Higher E_2β doses (0.004–0.006 mg/kg/day) each produced significant increases in 17β-estradiol levels in comparison to baseline (P < 0.01–0.005). The time required to reach stable estradiol levels at each E_2β dose varied across monkeys and averaged between 3 and 6 days. After maintenance on E_2β was discontinued, estradiol returned to baseline levels within 3 to 6 days.

LH. Before estradiol replacement, baseline LH levels exceeded 100 ng/ml and these levels are characteristic of ovariectomized females in this laboratory (Mello et al., 1995). The relationship between LH and 17β-estradiol during E_2β treatment is shown in Fig. 2. As 17β-estradiol levels increased, there was a corresponding decrease in LH. Average LH levels decreased significantly from 125 ± 17 to 28.2 ± 2 ng/ml during maintenance on 0.001 to 0.004 mg/kg E_2β (P < 0.02–0.0001). However, LH did not decrease further during maintenance on the highest dose of E_2β studied (0.006 mg/kg/day) and averaged 23.6 ± 1.7 ng/ml. During maintenance on estradiol doses of 0.004 and 0.006 mg/kg/day, LH levels were in the normal range for the mid-follicular phase of the menstrual cycle in gonadally intact monkeys (Mello et al., 1997).

Prolactin. Baseline prolactin levels were in the low normal range and averaged between 4 and 10 ng/ml. Average prolactin levels increased as a function of increasing doses of E_2β, but these changes were not significantly different from baseline (data not shown). Prolactin levels remained within the normal range (10.3 ± 2.6–18.3 ± 3.5 ng/ml) during maintenance on 0.001 to 0.004 mg/kg/day E_2β. During treatment with the highest dose of E_2β studied (0.006 mg/kg/day), basal prolactin levels increased to 21.4 ± 2.1 ng/ml, and this level approaches the hyperprolactinemic range defined as daytime levels >25 ng/ml (Martin and Reichlin, 1987).

Study 2: Effects of Cocaine and Saline on Anterior Pituitary Hormones in Ovariectomized Monkeys without Hormone Replacement (Fig. 3)

Basal levels of 17β-estradiol and progesterone on the study day are shown in Table 1. Baseline prolactin and LH levels did not differ significantly before 0.8 mg/kg i.v. cocaine and saline administration in ovariectomized female rhesus monkeys without hormone replacement. Baseline prolactin levels averaged 5.82 ± 1.3 ng/ml before saline administration and 7.17 ± 1.6 ng/ml before cocaine administration. Baseline LH levels averaged 121 ± 17 ng/ml before saline administration and 115 ± 19 ng/ml before cocaine administration. Cocaine reached peak plasma levels of 204 ± 45 ng/ml within 4 min after i.v. administration. Figure 3 shows that neither saline nor cocaine (0.8 mg/kg i.v.) produced significant changes in basal levels of prolactin or LH over the 110-min sampling period.

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Fig. 2. Effects of E_2β on LH (nanograms per milliliter) and 17β estradiol (picograms per milliliter) in ovariectomized rhesus monkeys. Doses of E_2β are shown on the abscissae, and 17β-estradiol (picograms per milliliter) levels (closed circles) and LH (nanograms per milliliter) levels (open circles) are shown on the left and right ordinates. At E_2β doses of 0.001 and 0.002 mg/kg/day, each data point is the average (± S.E.) of three or four monkeys. At E_2β doses of 0.004 and 0.006 mg/kg/day, each data point is the average (± S.E.) of five monkeys.
Fig. 3. Effects of saline or cocaine on prolactin, LH, and plasma cocaine in ovariectomized rhesus monkeys without hormone replacement. Pretreatment baseline levels of prolactin (row 1) and LH (row 2) are shown above baseline (BL) and time (minutes) after saline or cocaine injection is shown on the abscissae. Plasma hormone and cocaine levels (nanograms per milliliter) are shown on the left ordinates. The vertical dotted line above time 0 indicates when saline (open circles) or cocaine (closed circles) was injected. Each data point before and after saline administration is the average (±S.E.) of three monkeys, and each point before and after cocaine (0.8 mg/kg i.v.) is the average (±S.E.) of five monkeys. Plasma cocaine levels (nanograms per milliliter) are shown as open squares in row 3, and each data point is the average of three monkeys because of problems with sample handling.
Study 3: Cocaine Effects on Prolactin and LH during Ovarian Steroid Hormone Replacement

Cocaine Effects on Prolactin during Chronic Administration of E₂β Alone (Fig. 4, row 1). The effects of cocaine on prolactin during daily maintenance on 0.0015 to 0.006 mg/kg/day E₂β are shown in Fig. 4, row 1. These doses of E₂β produced average estradiol levels of 86 ± 37 to 221 ± 37 pg/ml on the study day (Table 1). At low estradiol doses, baseline prolactin levels were in the normal range and averaged 10.34 ± 2.6 to 14.18 ± 3.5 ng/ml. However, at the highest estradiol dose, baseline prolactin levels increased to average 23.4 ± 9.6 ng/ml. During chronic treatment with all doses of E₂β, cocaine administration was followed by a significant decrease in prolactin levels (P < 0.05–0.001). Prolactin decreased significantly below baseline within 8 min after i.v. cocaine administration during maintenance on 0.0015, 0.002, and 0.006 mg/kg/day E₂β. During maintenance on 0.004 mg/kg/day E₂β, prolactin decreased more slowly and fell significantly below baseline levels within 20 to 35 min after cocaine administration. The significant decreases in prolactin from baseline levels were sustained throughout the 110-min postcocaine sampling period (P < 0.05–0.001).

Cocaine Effects on Prolactin during Chronic Administration of Progesterone Alone (Fig. 4, row 2). Before progesterone replacement, basal progesterone levels averaged less than 1.5 ng/ml. Table 1 shows that after chronic treatment with 0.32 mg/kg/day progesterone, basal progesterone levels were characteristic of early luteal phase values measured in female rhesus monkeys during a normal ovulatory menstrual cycle (Mello et al., 1997). On the study day,
baseline prolactin levels averaged 9.05 ± 2.36 ng/ml before cocaine administration. Figure 4 (row 2) shows that prolactin decreased significantly within 16 min after cocaine administration ($P < 0.001$). Prolactin remained significantly below precocaine baseline levels for the remainder of the sampling period ($P < 0.05–0.001$).

**Cocaine Effects on Prolactin during Chronic Administration of E$_2$β and Progesterone Combinations (Fig. 4, row 3).** Table 1 shows basal progesterone and estradiol levels on the study day after chronic treatment with a combination of progesterone and E$_2$β. After chronic treatment with progesterone and 0.002 mg/kg/day E$_2$β, baseline prolactin levels averaged 12.62 ± 6.25 ng/ml, and there were no significant changes in prolactin levels after cocaine administration (Fig. 3, row 3). After chronic treatment with progesterone and 0.004 mg/kg/day E$_2$β, baseline prolactin levels averaged 49 ± 24 ng/ml on the study day. After cocaine administration, prolactin levels decreased and remained below baseline throughout the sample period ($P < 0.001$). There were no statistically significant differences in prolactin values at each time point between the two progesterone + estradiol treatment conditions.

**Cocaine Effects on LH during Chronic Administration of E$_2$β Alone (Fig. 5, row 1).** The effect of cocaine on LH during maintenance on 0.0015 to 0.006 mg/kg/day E$_2$β is shown in Fig. 5 (row 1). Baseline LH levels decreased as doses of E$_2$β increased. LH averaged 88.6 ± 10, 73.4 ± 10, 28.2 ± 2.2, and 23.6 ± 1.7 ng/ml during maintenance on E$_2$β at doses of 0.0015 to 0.006 mg/kg/day. Cocaine stimulated a small but significant increase in LH within 16 min in monkeys maintained on 0.002 mg/kg/day E$_2$β ($P < 0.05$). However, during maintenance on both lower and higher doses of E$_2$β, LH levels did not change significantly from precocaine baseline levels throughout the sampling period after cocaine administration.

**Cocaine Effects on LH during Chronic Administration of Progesterone Alone (Fig. 5, row 2).** On the study day, after daily administration of 0.32 mg/kg progesterone alone, baseline LH levels averaged 99.8 ± 16.27 ng/ml before cocaine administration. Figure 5 (row 2) shows that LH increased significantly within 8 min after cocaine administration and remained elevated for 20 min ($P < 0.05–0.01$).

**Cocaine Effects on LH during Chronic Administration of E$_2$β and Progesterone Combinations (Fig. 5, row 3).** On the study day, baseline LH levels averaged 28.2 ± 7.2 and 26.3 ± 1.2 ng/ml during chronic treatment with 0.32 mg/kg progesterone in combination with 0.002 or 0.004 mg/kg E$_2$β, respectively. Basal E$_2$β and progesterone levels decreased as doses of E$_2$β increased. LH averaged 88.6 ± 10, 73.4 ± 10, 28.2 ± 2.2, and 23.6 ± 1.7 ng/ml during maintenance on E$_2$β at doses of 0.0015 to 0.006 mg/kg/day. Cocaine stimulated a small but significant increase in LH within 16 min in monkeys maintained on 0.002 mg/kg/day E$_2$β ($P < 0.05$). However, during maintenance on both lower and higher doses of E$_2$β, LH levels did not change significantly from precocaine baseline levels throughout the sampling period after cocaine administration.
levels are shown in Table 2. During chronic maintenance on a combination of 0.002 mg/kg estradiol + 0.32 mg/kg progesterone, cocaine stimulated a nonsignificant increase in LH within 8 min, and LH remained elevated for 20 min. There was considerable variability between monkeys. LH increased by 130 to over 150% within 8 to 16 min in two monkeys, but did not change appreciably in two other monkeys. During chronic maintenance on 0.32 mg/kg progesterone in combination with 0.004 mg/kg E2, LH levels increased progressively across the sampling period and averaged 45.8 ± 11.8 ng/ml at 110 min after cocaine administration. However, there were no statistically significant changes in LH after cocaine administration.

Plasma Cocaine Levels during Hormone Replacement (Table 2). Table 2 shows average plasma cocaine levels during chronic treatment with E2β alone (0.002–0.006 mg/kg/day), progesterone alone (0.32 mg/kg/day) alone, and E2β (0.002 mg/kg) + progesterone (0.32 mg/kg/day) in combination. Plasma cocaine levels after treatment with 0.004 mg/kg/day E2β, in combination with 0.32 mg/kg/day progesterone and 0.0015 mg/kg/day E2β alone are not reported because of problems with sample handling. A pharmacokinetic analysis of plasma cocaine levels in these ovariectomized females without hormone replacement and during each hormone condition was performed, and the results are summarized in Table 2. There were no significant differences in any pharmacokinetic parameter as a function of chronic hormone treatment in comparison with control conditions. The time to reach maximum plasma cocaine concentrations (Tmax) was 4 to 5.3 min after intravenous cocaine administration during chronic administration of E2β, progesterone and E2β + progesterone combinations. The peak plasma cocaine concentration (Cmax) averaged between 174 ± 19.3 and 238 ± 33.4 ng/ml, and there were no significant differences across treatment conditions. The average half-life of plasma cocaine (t1/2) was between 36.9 and 55 min and also did not vary significantly as a function of the hormone treatment condition.

Discussion

One major finding of this study is that daily administration of physiologically relevant doses of estradiol or progesterone to ovariectomized rhesus monkeys significantly influenced the effects of cocaine on prolactin. Under control conditions without hormone replacement, cocaine did not alter prolactin; however, during chronic treatment with estradiol or progesterone, cocaine significantly decreased prolactin. Hormone replacement had less consistent effects on cocaine’s interactions with LH. Cocaine did not stimulate LH release in untreated ovariectomized monkeys, and it also did not reliably increase LH release during treatment with ovarian steroid hormones. The only exceptions to this general finding were that cocaine significantly increased plasma LH levels during treatment with an intermediate dose of 0.004 mg/kg/day E2β alone and with 0.32 mg/kg/day progesterone alone. However, these effects were relatively small, and any modulation of cocaine effects on LH by E2β was not monotonically related to the E2β dose. There were no statistically significant differences in plasma cocaine levels or cocaine pharmacokinetics between control conditions and hormone replacement conditions, which suggest that any hormone-associated changes in the endocrine effects of cocaine could not be attributed to changes in the pharmacokinetics of cocaine.

Effects of Ovarian Steroid Hormone replacement on LH and Prolactin Levels. The present study manipulated ovarian steroid hormone levels in ovariectomized rhesus monkeys to test the hypothesis that ovarian steroid hormone levels may influence cocaine’s endocrine effects. In accordance with previous studies, daily treatment with E2β (0.0015–0.006 mg/kg/day) and progesterone (0.32 mg/kg/day) produced physiological plasma levels of estradiol and progesterone. Specifically, doses of 0.0015–0.002 mg/kg/day E2β produced estradiol levels similar to those observed during the follicular and luteal phases of the menstrual cycle, and higher doses of 0.004 to 0.006 mg/kg/day E2β produced estradiol levels similar to those observed during the peri-ovulatory phase. In addition, 0.32 mg/kg/day progesterone produced sustained levels of progesterone similar to those observed during the luteal phase of an ovulatory menstrual cycle (Mello et al., 1997).

These ovarian hormone treatment regimens also produced expected changes in basal LH and prolactin levels. E2β produced a dose-dependent decrease in LH and an increase in prolactin levels. These findings are consistent with the effects of estradiol treatment in postmenopausal women (Carr, 1998) and ovariectomized rats (Ben-Jonathan, 1985; De-Maria et al., 2000). In contrast, the dose of progesterone used in this study (0.32 mg/kg/day) had little effect on either LH or prolactin levels.

Cocaine’s Effects on Prolactin. Prolactin regulation is under inhibitory dopaminergic control, and dopamine is released from three separate groups of hypothalamic neuroendocrine dopaminergic neurons (Ben-Jonathan, 1985; De-Maria et al., 1998, Ben-Jonathan and Hnasko, 2001). Cocaine acts as an indirect dopamine agonist by inhibiting dopamine reuptake by the dopamine transporter (Woolverton and Johnson, 1992; Kuhar, 1993), and dopamine transporters are present in all three groups of hypothalamic neuroendocrine dopaminergic neurons in rats (DeMaria et al., 2000). Thus, a

### Table 2

Pharmacokinetics of cocaine (0.8 mg/kg/i.v.) in ovariectomized female Rhesus monkeys with and without ovarian steroid hormone replacement

<table>
<thead>
<tr>
<th>Pharmacokinetic Measures</th>
<th>No Hormone Replacement</th>
<th>E2β (0.002 mg/kg/day)</th>
<th>E2β (0.004 mg/kg/day)</th>
<th>E2β (0.006 mg/kg/day)</th>
<th>Prog (0.32 mg/kg/day)</th>
<th>E2β (0.002) and Prog (0.32 mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmax (min)</td>
<td>5.3 ± 1.3</td>
<td>5.0 ± 1.0</td>
<td>4.0 ± 0.0</td>
<td>4.0 ± 0.0</td>
<td>5.3 ± 1.3</td>
<td>4.0 ± 0.0</td>
</tr>
<tr>
<td>Cmax (mg/ml)</td>
<td>214.0 ± 44.2</td>
<td>217.2 ± 56.9</td>
<td>189.5 ± 47.5</td>
<td>205.7 ± 10.7</td>
<td>238.0 ± 33.4</td>
<td>174.0 ± 19.3</td>
</tr>
<tr>
<td>T1/2 (min)</td>
<td>44.3 ± 2.1</td>
<td>45.8 ± 11.7</td>
<td>55.5 ± 12.7</td>
<td>40.2 ± 4.4</td>
<td>37.1 ± 8.7</td>
<td>36.9 ± 2.9</td>
</tr>
<tr>
<td>AUC (ng · min/ml)</td>
<td>9914 ± 2058</td>
<td>7786 ± 1594</td>
<td>7922 ± 893</td>
<td>8793 ± 883</td>
<td>9527 ± 953</td>
<td>7344 ± 729</td>
</tr>
</tbody>
</table>

n = 3  n = 4  n = 4  n = 4  n = 3  n = 4
cocaine-induced decrease in prolactin levels would be anticipated on the basis of cocaine’s blockade of dopamine re-uptake. Cocaine and dopamine administration each decreased prolactin levels in gonadally intact rhesus monkeys (Mello et al., 1990a, 1993, 1994), but cocaine did not decrease prolactin in untreated ovariectomized rhesus monkeys in the present study or in our previous study (Mello et al., 1995). A number of factors regulate prolactin synthesis and release (Ben-Jonathan, 1985; Ben-Jonathan and Hnasko, 2001) and the mechanism(s) underlying the influence of ovariectomy on cocaine’s effects on prolactin cannot be determined with certainty from these data. However, the present results are consistent with the possibility that 1) ovariectomy may disrupt dopaminergic inhibition of prolactin release and attenuate the ability of dopamine agonists such as cocaine to modulate prolactin release, and 2) ovarian steroid hormone replacement may reverse the effects of ovariectomy on dopaminergic inhibition of prolactin release and restore the ability of dopamine agonists to modulate prolactin release. In support of this hypothesis, ovariectomy has been reported to decrease the density of striatal D₁ and D₂ receptors under some conditions in rats (Tonnaer et al., 1989; Bossé and Di Paolo, 1995). Moreover, an ovariectomy-induced and persistent decrease in striatal D₂ receptor density was reversed by estradiol treatment (Bossé and Di Paolo, 1995). The effects of ovariectomy and steroid replacement on the hypothalamic neuroendocrine dopamine system in rhesus monkeys remain to be determined.

Chronic estradiol treatment increased basal prolactin levels from normal to near hyperprolactinemic levels as the maintenance dose of E₂/β increased. These data are consistent with reports that estrogen administration increased prolactin levels in postmenopausal women (Yen and Jaffe, 1999) and ovariectomized rats (Ben-Jonathan, 1985; DeMaria et al., 2000). Estrogen-induced increases in prolactin reflect an increase in the amplitude of prolactin pulsatile release with no change in pulse frequency (Veldhuis et al., 1989). Estrogen seems to increase prolactin synthesis and release by several mechanisms, including interference with dopamine D₁ receptor inhibition of prolactin release (Yen and Jaffe, 1999). However, the observed cocaine-induced decreases in prolactin during E₂/β treatment did not seem to be directly related to baseline prolactin levels. Cocaine significantly decreased prolactin when baseline levels were low (10–12 ng/ml) and high (above 20 ng/ml). Similarly, dopamine infusions decreased prolactin significantly from a baseline of 6.2 ng/ml in gonadally intact rhesus females (Mello et al., 1994). During chronic progesterone treatment, basal prolactin levels remained in the normal range.

Interestingly, prolactin levels decreased more rapidly in ovariectomized females during ovarian steroid treatment than in gonadally-intact rhesus monkeys studied under comparable conditions. For example, cocaine-related decreases in prolactin usually occurred within 8 min during E₂/β treatment and within 16 min during progesterone treatment. In gonadally intact rhesus females, significant decreases in prolactin were not detected until 30 to 50 min after cocaine administration (Mello et al., 1990a; Mello et al., 1993) and 60 to 80 min after dopamine infusion (Mello et al., 1994).

Cocaine’s Effects on LH. Cocaine consistently stimulates LH release in gonadally intact rhesus males and females (Mello et al., 1990a, 1993; Mendelson et al., 1999b) and in men and women (Mendelson et al., 2001), but not in ovariectomized rhesus females without ovarian steroid hormone replacement (Mello et al., 1995). In the present study, cocaine stimulated LH during progesterone treatment, but not during treatment with combinations of progesterone and estradiol, or with estradiol alone at doses that were followed by a cocaine-induced decrease in prolactin.

In gonadally intact females, estradiol has an inhibitory effect on LH release throughout the menstrual cycle, except at the periovulatory phase when high estradiol levels are necessary to stimulate the LH surge (Hotchkiss and Knobil, 1994). Estradiol produced the anticipated dose-dependent decrease in basal LH levels in ovariectomized females in the present study. It may be that the inhibitory effects of estradiol on LH prevented the commonly observed stimulation of LH by cocaine (Mello et al., 2001; Mello and Mendelson, 2002). Consistent with this hypothesis, when the inhibitory effect of estradiol on LH was absent during treatment with progesterone alone, cocaine stimulated LH release within 8 min. This time course was similar to that observed in gonadally intact, luteal phase rhesus females after 0.8 mg/kg cocaine (10 min) (Mello et al., 1993) and in luteal phase human females after 0.4 mg/kg i.v. cocaine (12 min) (Mello et al., 2001). Cocaine stimulation of LH release during chronic progesterone treatment also is consistent with reports that acute progesterone administration can stimulate an LH surge in ovariectomized monkeys (Terasawa, 1985).

Basal LH levels did not seem to influence cocaine’s effects, because cocaine did not stimulate LH when basal LH levels were low (23–28 ng/ml) or high (73–88 ng/ml). The time course of LH stimulation during progesterone treatment was most similar to that previously observed in mid-luteal females, insofar as LH increased significantly within 10 to 20 min (Mello et al., 1993). However, LH remained elevated for 50 min in mid-luteal females but returned to baseline within 30 min in these ovariectomized females. In follicular phase females, the onset of significant LH increases occurred more slowly, within 20 min, but remained above baseline for 40 to 50 min (Mello et al., 1990a).

Cocaine Interactions with Ovarian Steroid Hormones. Taken together, these findings are consistent with the hypothesis that ovarian steroid hormones may modulate cocaine’s effects on anterior pituitary hormones (Mello et al., 2000; Mello and Mendelson, 2002). Moreover, it seems that ovarian steroid hormone replacement restored cocaine’s ability to decrease prolactin levels more effectively than it restored cocaine’s ability to increase LH levels. These findings are also of interest in view of the considerable evidence that ovarian steroid hormones may influence some of cocaine’s behavioral effects (for review, see Mello and Mendelson, 2002). For example, high levels of estradiol before estrus were associated with higher levels of cocaine-induced locomotor effects than at other stages of the estrous cycle in rats, and ovariectomy decreased cocaine-induced locomotion (Sell et al., 2000). In ovariectomized rats, replacement of estradiol, and estradiol and progesterone in combination, increased cocaine-induced locomotor activity significantly above levels measured during replacement of progesterone alone or no hormone replacement (Sell et al., 2000; Kuhn et al., 2001). In cocaine self-administration studies, the reinforcing effects of cocaine were greatest when estradiol levels were high (Roberts et al., 1989; Lynch et al., 2000). Information about the
influence of ovariectomy on cocaine’s abuse-related effects is less consistent. In rats trained to self-administer cocaine, neither ovariectomy nor estradiol replacement significantly altered cocaine self-administration dose-effect curves (S. B. Caine, C. A. Bowen, G. Yu, D. Zuzga, S. S. Negus, and N. K. Mello, submitted). Rats learned to self-administer cocaine after ovariectomy (S. B. Caine, C. A. Bowen, G. Yu, D. Zuzga, S. S. Negus, and N. K. Mello, submitted), and administration of estradiol tended to decrease cocaine self-administration (Grimm and See, 1996). The extent to which these interactions between cocaine and ovarian steroid hormones may have implications for some cocaine-related behavioral effects remains to be determined (for review, see Mello and Mendelson, 2002).

Acknowledgments

We thank Yonghong Cheng for excellent technical assistance in analyzing plasma cocaine levels. We are grateful to the Behavioral Science Laboratory staff for expert technical assistance and to Elizabeth Hall for veterinary consultation. We thank Bruce Stephen for analyzing plasma cocaine levels. We are grateful to the Behavioral Pharmacologist. 

References


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