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OVARIAN STEROID HORMONE MODULATION OF THE ACUTE EFFECTS OF COCAINE ON LUTEINIZING HORMONE AND PROLACTIN LEVELS IN OVARIECTOMIZED RHESUS MONKEYS

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Abbreviations

ACTH	Adrenocorticotropin hormone
CRF	Corticotropin-releasing-factor
$E_2\beta$	Estradiol benzoate
LH	Luteinizing hormone
LHRH	Luteinizing-hormone-releasing-hormone

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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 ($E_2\beta$) 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 $E_2\beta$ (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 + $E_2\beta$ (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 + $E_2\beta$, or three of four estradiol treatment doses. Cocaine pharmacokinetics did not differ as a function of hormone replacement conditions. Taken together, these data suggest that both $E_2\beta$ and progesterone modulate cocaine's effects on prolactin, whereas $E_2\beta$ alone and in combination with progesterone, do not facilitate LH release in response to cocaine in ovariectomized rhesus females.

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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 (see for review (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; Sholar et al., 1998; Mendelson et al., 2001) and in rhesus monkeys (Mello et al., 1990a; Mello et al., 1993; Sarnyai et al., 1996; Broadbear et al., 1999a; Broadbear et al., 1999b).

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; Mello et al., 1993; Sarnyai et al., 1996). When synthetic luteinizing-hormone-releasing-hormone (LHRH) or synthetic corticotropin-releasing-factor (CRF) was administered to the same ovariectomized females, LH and ACTH increased significantly after LHRH and CRF, 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 responsivity 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; Mello et al., 1994); see for review (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; Mello et al., 1993; Mello et al., 1995). Taken 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.

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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-hr light-dark cycle (7:00 a.m. to 7:00 p.m.) was in effect. All females had been ovariectomized for over one 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 6-8 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 ($E_2\beta$), progesterone and combinations of $E_2\beta$ and progesterone. Successive doses of each hormone were separated by a hormone-free interval

to prevent any carry-over 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 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 $E_2\beta$ (0.0015, 0.002, 0.004 and 0.006 mg/kg/day) were given for at least eight days before each endocrine study day. $E_2\beta$ doses were administered in an ascending order. $E_2\beta$ 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 three times a week to follow 17β-estradiol levels during $E_2\beta$replacement. A single sample for estradiol analysis was also collected on each endocrine study day. At the end of each $E_2\beta$ 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 periovulatory 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 $E_2\beta$ 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 $E_2\beta$ studied were selected to mimic estradiol levels during the peri-ovulatory phase of the menstrual cycle (Hotchkiss and Knobil, 1994). $E_2\beta$ was administered chronically because *acute* $E_2\beta$ 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 $E_2\beta$ has a biphasic effect on LH release. During the first 12 to 16 hours after $E_2\beta$ administration to ovariectomized females, LH decreases below baseline levels, then increases to peak levels within 42 to 56 hours (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 ovariectomized 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 17β 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 17β 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 17β estradiol and progesterone in ovariectomized females after chronic hormone replacement are shown in **Table 1**.

Cocaine Administration

The acute effects of cocaine (0.8 mg/kg, iv) 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; Mello et al., 1990b; Mello et al., 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 ovariectomized 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 were administered over 10 sec 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. Following 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; Mello et al., 1990b; Mello et al., 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 0.80 mg/kg cocaine (Mendelson et al., 1999a). The duration of sampling was limited by the volume of blood that can be safely exfused from a rhesus monkey. In the present study, between 60 and 62.5 ml of blood was collected over 120 min. The 110-min post-cocaine 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 x 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 analysis 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 (NIDA) and solutions were prepared by dissolving cocaine in sterile saline for injection U.S.P. The solution was filter-sterilized using a 0.11 □g Millipore filter (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 in a single bolus injection over 10 sec.

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Estradiol and Progesterone. β-Estradiol 3-Benzoate (1, 3, 5 [10] -Estratriene-3, 17β-diol) and progesterone were purchased from the Sigma Chemical Company, St. Louis, MO. Estradiol benzoate and progesterone were suspended in sesame oil and diluted to the appropriate mg/kg 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 (Midgley, 1966) using rhesus pituitary LH reference preparation (NICHD-rhLH, also known as WP-XV-20), prepared by W. Peckham and Recombinant (Genzyme) 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, supported by the National Institute of Child Health and Human Development and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases and Dr. A.F. Parlow. Radioiodination was performed using chloramine-T (Greenwood et al., 1963) with sodium iodide-125 purchased from PerkinElmer Life Sciences, Inc. (Boston, MA). Goat antirabbit gammaglobulin was obtained from Cal Biochem-Nova Biochem Corp. (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 Figure 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 Figure 1.

Estradiol radioimmunoassay. Plasma concentrations of 17β-estradiol (E_2) 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. Prior to analysis, the plasma samples were extracted, 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., Costa Mesa, CA. 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 non

linear 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.

Figure 1 about here

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. Hyundt-Feldt epsilon adjustments were used in all contrast tests. Probability levels of $P < 0.05$ to 0.0001 are reported as statistically significant.

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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 $E_2\beta$ administration produced physiologically relevant levels of 17β-estradiol was evaluated. $E_2\beta$ 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 $E_2\beta$ 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 $E_2\beta$ (0.002 and 0.004 mg/kg/day).

Study 1: Effects of Chronic $E_2\beta$ Administration on Basal Levels of 17βEstradiol, LH and Prolactin (Figure 2)

17β-Estradiol: Basal 17β-estradiol levels averaged 22.7 ± 2.1 pg/ml (data not shown). **Figure 2** shows that after $E_2\beta$ replacement, the average levels of 17β-estradiol increased with increasing doses of $E_2\beta$ dose ($r= 0.799$; $P = 0.0001$). The lowest doses of $E_2\beta$ (0.001 - 0.0015 mg/kg/day) did not increase 17β-estradiol levels significantly above baseline levels. Higher $E_2\beta$ doses (0.004 - 0.006 mg/kg/day) each produced significant increases in 17β-estradiol levels in comparison to baseline ($P < 0.01$ to 0.005). The time required to reach stable estradiol levels at each $E_2\beta$ dose varied across monkeys and averaged between 3 and 6 days. After maintenance on $E_2\beta$ 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 $E_2\beta$ treatment is shown in **Figure 2**. As 17β-estradiol levels increased, there was a corresponding decrease in LH. Average LH levels decreased significantly from 125 ± 17 ng/ml to 28.2 ± 2 ng/ml during maintenance on 0.001 to 0.004 mg/kg $E_2\beta$P□□! However, LH did not decrease further during maintenance on the highest dose of $E_2\beta$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) .

Figure 2 about here

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 $E_2\beta$, 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 $E_2\beta$. During treatment with the highest dose of $E_2\beta$ 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).

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Study 2: Effects of Cocaine and Saline on Anterior Pituitary Hormones in Ovariectomized Monkeys Without Hormone Replacement (Figure 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.

Figure 3 and Table 1 about here

Study 3: Cocaine Effects on Prolactin and LH During Ovarian Steroid Hormone Replacement

Cocaine Effects on Prolactin During Chronic Administration of $E_2\beta$ Alone (Figure 4, Row 1): The effects of cocaine on prolactin during daily maintenance on 0.0015-0.006 mg/kg/day $E_2\beta$are shown in Figure 4, row 1These doses of $E_2\beta$produced average estradiol levels of 86 ± 37 pg/ml 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 ng/ml 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 $E_2\beta$ 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 $E_2\beta$. During maintenance on 0.004 mg/kg/day $E_2\beta$, 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 post-cocaine sampling period ($P < 0.05$ - 0.001).

Cocaine Effects on Prolactin During Chronic Administration of Progesterone Alone (Figure 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 pre-cocaine baseline levels for the remainder of the sampling period ($P < 0.05$-0.001).

Cocaine Effects on Prolactin During Chronic Administration of $E_2\beta$ and Progesterone Combinations (Figure 4, Row 3): Table 1 shows basal progesterone and estradiol levels on the study day after chronic treatment with a combination of progesterone and $E_2\beta$. After chronic treatment with progesterone and 0.002 mg/kg/day $E_2\beta$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 $E_2\beta$, 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.

Figure 4 about here

Cocaine Effects on LH during ChronicAdministration of $E_2\beta$ Alone (Figure 5, Row 1): The effect of cocaine on LH during maintenance on 0.0015-0.006 mg/kg/day $E_2\beta$is shown in **Figure 5** (row 1). Baseline LH levels decreased as doses of $E_2\beta$ increased. LH averaged 88.6 ± 10 ng/ml, 73.4 ± 10 ng/ml, 28.2 ± 2.2 ng/ml and 23.6 ± 1.7 ng/ml during maintenance on $E_2\beta$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 $E_2\beta$($P < 0.05$). However, during maintenance on both lower and higher doses of $E_2\beta$, LH levels did not change significantly from pre-cocaine baseline levels throughout the sampling period after cocaine administration.

Cocaine Effects on LH During Chronic Administration of Progesterone Alone (Figure 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 $E_2\beta$ and Progesterone Combinations (Figure 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 $E_2\beta$, respectively. Basal

$E_2\beta$ and progesterone 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 non-significant 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 percent 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 $E_2\beta$, 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.

Figure 5 about here

Plasma Cocaine Levels During Hormone Replacement (Table 2)

Table 2 shows average plasma cocaine levels during chronic treatment with $E_2\beta$ alone (0.002 - 0.006 mg/kg/day), progesterone alone (0.32 mg/kg/day) alone, and $E_2\beta$ (0.002 mg/kg) + progesterone (0.32 mg/kg/day) in combination. Plasma cocaine levels after treatment with 0.004 mg/kg/day $E_2\beta$,in combination with a 0.32 mg/kg/day progesterone and 0.0015 mg/kg/day $E_2\beta$ 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 to control conditions. The time to reach maximum plasma cocaine concentrations (T_{max}) was 4 to 5.3 min after intravenous cocaine administration during chronic administration of $E_2\beta$, progesterone and $E_2\beta$ + progesterone

combinations. The peak plasma cocaine concentration (C_{max}) averaged between 174 ± 19.3 ng/ml and 238 ± 33.4 ng/ml and there were no significant differences across treatment conditions. The average half-life of plasma cocaine ($T_{1/2}$) was between 36.9 and 55 min, and also did not vary significantly as a function of the hormone treatment condition.

Table 2 about here

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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 $E_2\beta$ 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 $E_2\beta$ was not monotonically related to the $E_2\beta$ dose. There were no statistically significant differences in plasma cocaine levels or cocaine pharmacokinetics between control conditions and hormone replacement conditions, which suggests 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 $E_2\beta$ (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 $E_2\beta$ produced

estradiol levels similar to those observed during the follicular and luteal phases of the menstrual cycle, and higher doses of 0.004-0.006 mg/kg/day $E_2\beta$ 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. $E_2\beta$ produced a dose-dependent decrease in LH and an increase in prolactin levels. These findings are consistent with the effects of estradiol treatment in post-menopausal women (Carr, 1998) and ovariectomized rats (Ben-Jonathan, 1985; DeMaria 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; DeMaria 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 cocaine-induced decrease in prolactin levels would be anticipated on the basis of cocaine's blockade of dopamine reuptake. Cocaine and dopamine administration each decreased prolactin levels in gonadally intact rhesus monkeys (Mello et al., 1990a; Mello et al., 1993; Mello et al., 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 (a) ovariectomy may disrupt dopaminergic inhibition of prolactin release and attenuate the ability of dopamine agonists like cocaine to modulate prolactin release, and (b) 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 D_1 and D_2 receptors under some conditions in rats (Tonnaer et al., 1989; Bossé and Di Paolo, 1995). Moreover, an ovariectomy-induced and persistent decrease in striatal D_2 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 $E_2\beta$ increased. These data are consistent with reports that estrogen administration increased prolactin levels in post-menopausal women (Yen and Jaffe, 1999) and ovariectomized rats (Ben-Jonathan, 1985; DeMaria et al., 2000). Estrogen-related increases in prolactin reflect an increase in the amplitude of prolactin pulsatile release with no change in pulse frequency (Veldhuis et al., 1989). Estrogen appears to increase prolactin synthesis and release by several mechanisms, including interference with dopamine D_2 receptor inhibition of prolactin release (Yen and Jaffe, 1999). However, the observed cocaine-induced decreases in prolactin during $E_2\beta$ treatment did not appear to be directly related

to baseline prolactin levels. Cocaine significantly decreased prolactin when baseline levels were low (10 to 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 $E_2\beta$ 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; Mello et al., 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 (Mendelson 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) (Mendelson 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 appear to influence cocaine's effects, because cocaine did not stimulate LH when basal LH levels were low (23 to 28 ng/ml) or high (73 to 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 appears 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 (see for review (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 (Caine et al., 2003 submitted). Rats learned to self-administer cocaine after ovariectomy (Caine et al., 2003 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 (see for review (Mello and Mendelson, 2002).

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. Standard RIA curves for prolactin (top) and LH (bottom). Each standard curve was generated by plotting the percent B/Bo versus the log of the concentration of PRL or LH. Percent B/Bo represents the binding of the standards relative to the zero calibrator. Each data point for PRL is the average of 12 determinations. Each data point for LH is the average of 21 determinations ($\bar{x}$ ± S.E.).

Figure 2. The effects of estradiol ($E_2\beta$) on LH (ng/ml) and 17βestradiol (pg/ml) in ovariectomized rhesus monkeys. Doses of $E_2\beta$ are shown on the abscissae and 17βestradiol (pg/ml) levels (closed circles) and LH (ng/ml) levels (open circles) are shown on the left and right ordinates. At $E_2\beta$ doses of 0.001 and 0.002 mg/kg/day, each data point is the average (± S.E.) of 3 or 4 monkeys. At $E_2\beta$ doses of 0.004 and 0.006 mg/kg/day, each data point is the average (± S.E.) of 5 monkeys.

Figure 3. The effects of saline or cocaine on prolactin, LH and plasma cocaine in ovariectomized rhesus monkeys without hormone replacement. Pre-treatment baseline levels of prolactin (row 1) and LH (row 2) are shown above BL and time (min) after saline or cocaine injection is shown on the abscissae. Plasma hormone and cocaine levels (ng/ml) are shown on the left ordinates. The vertical dotted line above time O 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 3 monkeys and each point before and after cocaine (0.8 mg/kg, i.v.) is the average (± S.E.) of 5 monkeys. Plasma cocaine levels (ng/ml) are shown as open squares in row 3 and each data point is the average of 3 monkeys because of problems with sample handling.

Figure 4. The effects of cocaine on prolactin levels in ovariectomized rhesus monkeys during ovarian steroid hormone replacement. Time (min) after administration of 0.8 mg/kg, i.v. cocaine is shown on the abscissae. BL is the pre-cocaine baseline prolactin level. Prolactin levels (ng/ml) are shown on the left ordinate. Row 1 shows prolactin levels before and after cocaine administration during daily maintenance on $E_2\beta$ (0.0015-0.006 mg/kg, i.m.). Row 2 shows prolactin levels before and after cocaine administration during daily administration of progesterone (0.32 mg/kg, i.m.). Row 3 shows prolactin levels during daily administration of progesterone with $E_2\beta$ (0.002 or 0.004 mg/kg, i.m.). Each data point in row 1 is the average (± S.E.) of 4 or 5 monkeys. Each data point in rows 2 and 3 is the average (± S.E.) of 4 monkeys. Y symbols, daggers, asterisks and diamonds shown above the $E_2\beta$ doses indicate the points at each dose that differ significantly from the pre-cocaine baseline (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

Figure 5. The effects of cocaine on LH levels in ovariectomized rhesus monkeys during ovarian steroid hormone replacement. Time (min) after administration of 0.8 mg/kg, i.v. cocaine is shown on the abscissae. BL is the pre-cocaine baseline LH level. LH levels (ng/ml) are shown on the left ordinate. Row 1 shows prolactin levels before and after cocaine administration during daily maintenance on $E_2\beta$ (0.0015-0.006 mg/kg, i.m.). Row 2 shows LH levels before and after cocaine administration during daily administration of progesterone (0.32 mg/kg, i.m.). Row 3 shows LH levels during daily administration of progesterone (0.32 mg/kg, i.m.) in combination with $E_2\beta$ (0.002 or 0.004 mg/kg, i.m.). Each data point in row 1 is the average (± S.E.) of 4 or 5 monkeys. Each data point in rows 2 and 3 is the average (± S.E.) of 4 monkeys. Daggers (0.02 mg/kg/day) and asterisks indicate points that differ significantly from the pre-cocaine baseline (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$).

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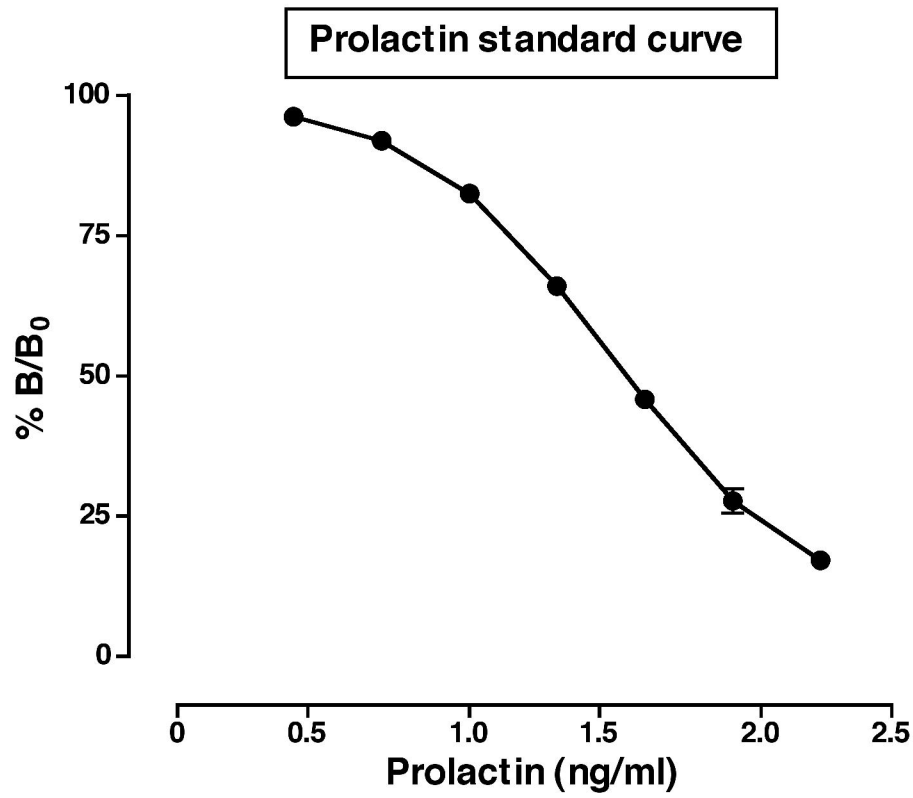
Figure 1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2.5
Prolactin (ng/ml)

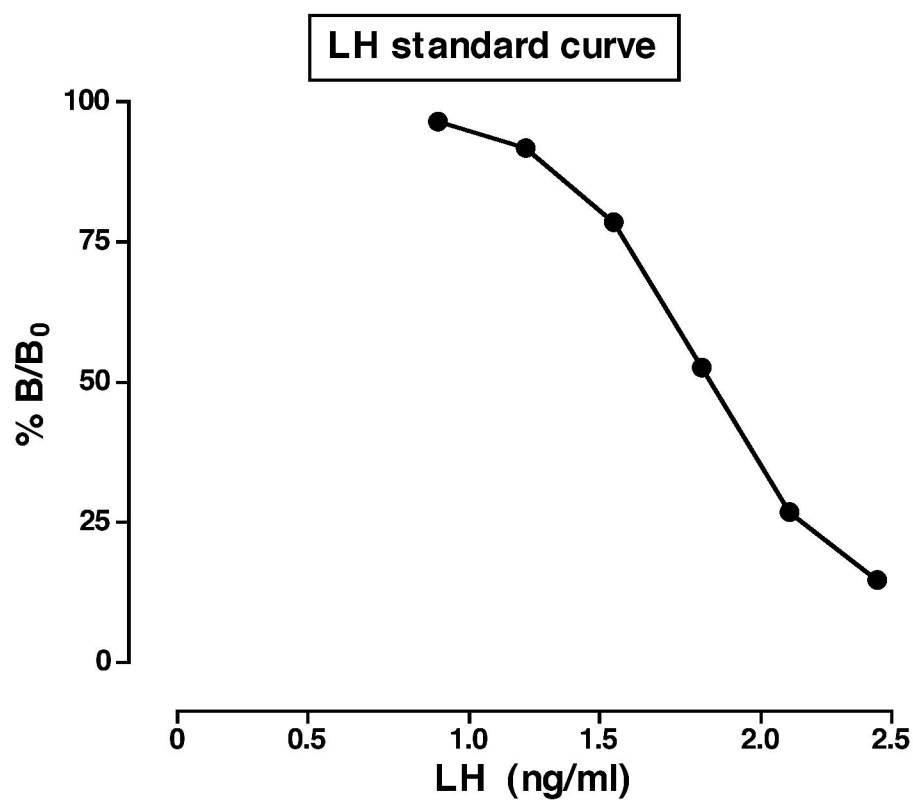
LH standard curve
100
75
50
25
0
% B/B0
0
0.5
1.0
1.5
2.0
2.5
LH (ng/ml)

Figure 2

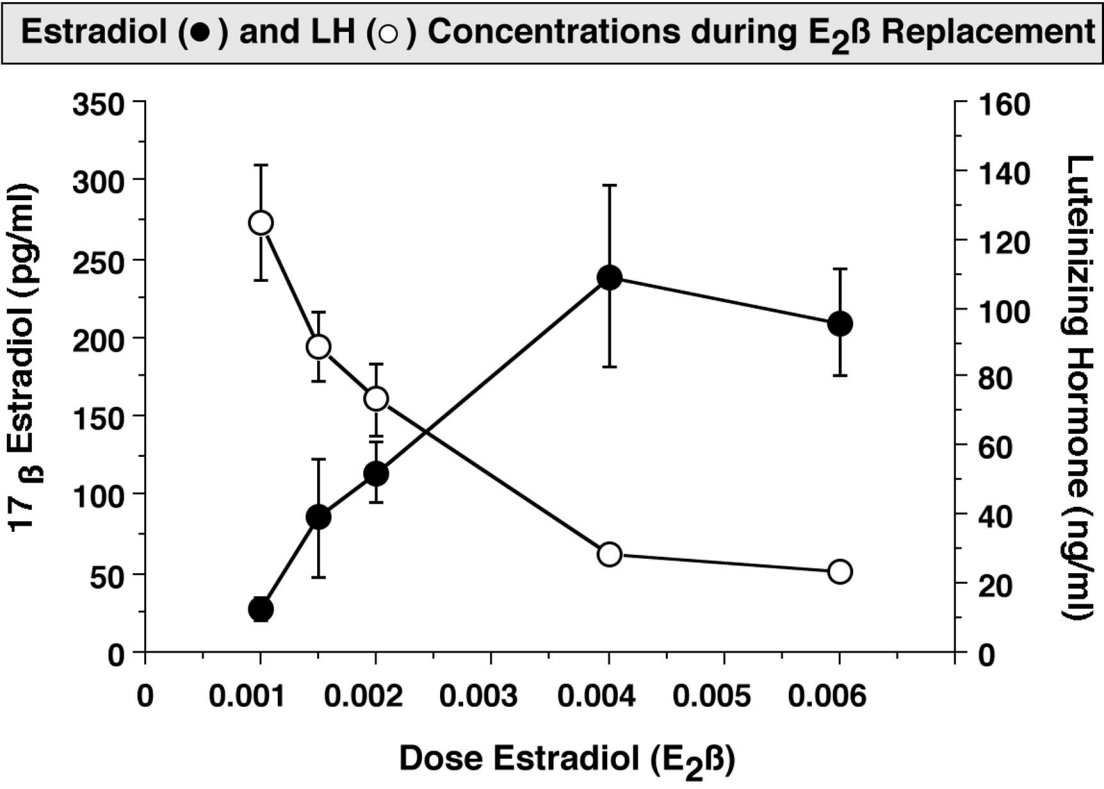
Estradiol (●) and LH (○) Concentrations during $E_2ß$ Replacement
17 ß Estradiol (pg/ml)
Luteinizing Hormone (ng/ml)
350
300
250
200
150
100
50
0
160
140
120
100
80
60
40
20
0
0
0.001
0.002
0.003
0.004
0.005
0.006
Dose Estradiol ($E_2ß$)

Figure 3

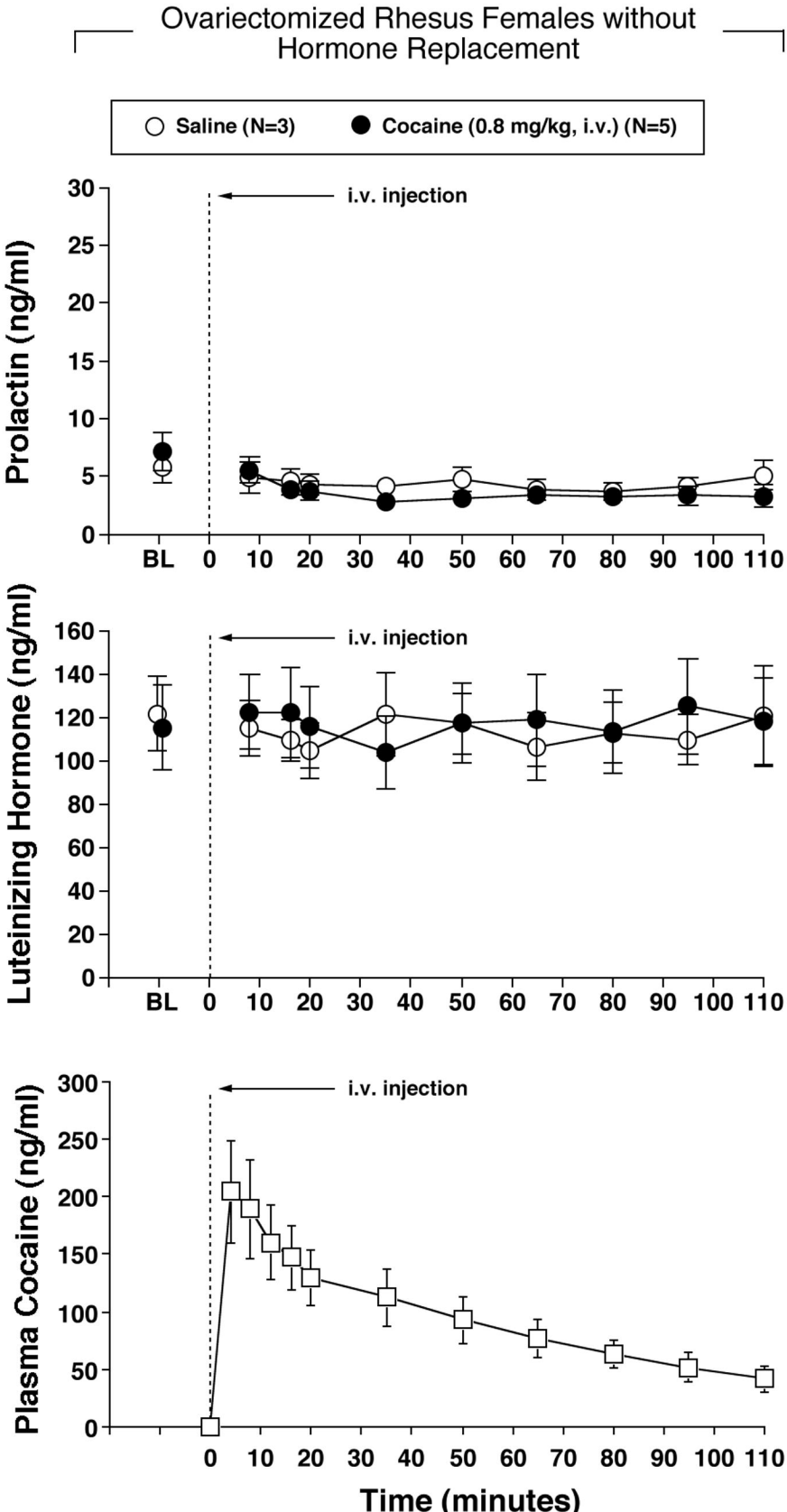
Ovariectomized Rhesus Females without Hormone Replacement
Saline (N=3)
Cocaine (0.8 mg/kg, i.v.) (N=5)
i.v. injection
Prolactin (ng/ml)
Luteinizing Hormone (ng/ml)
Plasma Cocaine (ng/ml)
BL
0
10
20
30
40
50
60
70
80
90
100
110
Time (minutes)

Figure 4

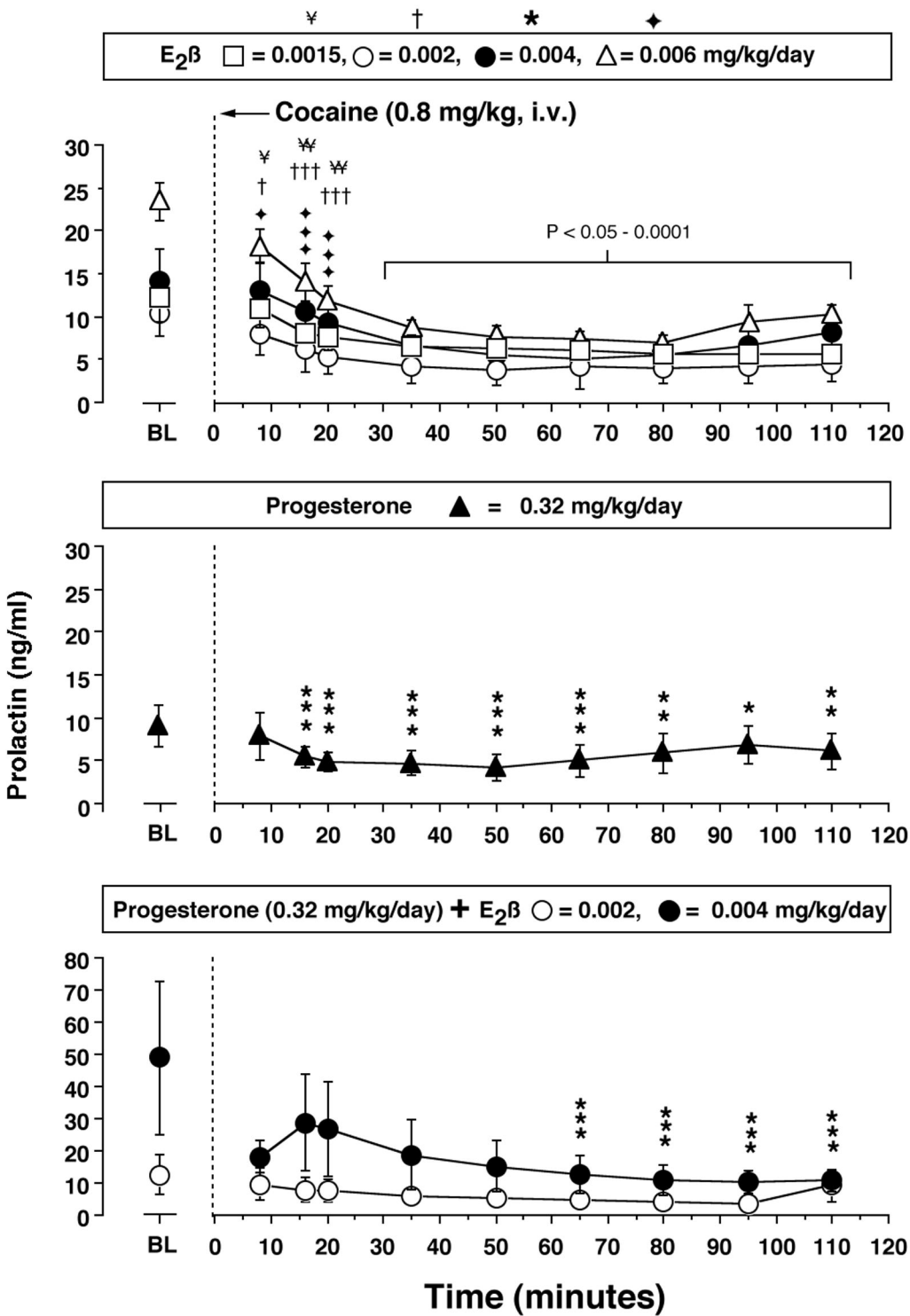
Prolactin in OVX Monkeys
During Ovarian Steroid Hormone Replacement
¥ † * ✦
E2ß □ = 0.0015, ○ = 0.002, ● = 0.004, △ = 0.006 mg/kg/day
Cocaine (0.8 mg/kg, i.v.)
P < 0.05 - 0.0001
Progesterone ▲ = 0.32 mg/kg/day
Progesterone (0.32 mg/kg/day) + E2ß ○ = 0.002, ● = 0.004 mg/kg/day
Prolactin (ng/ml)
BL
Time (minutes)

Figure 5

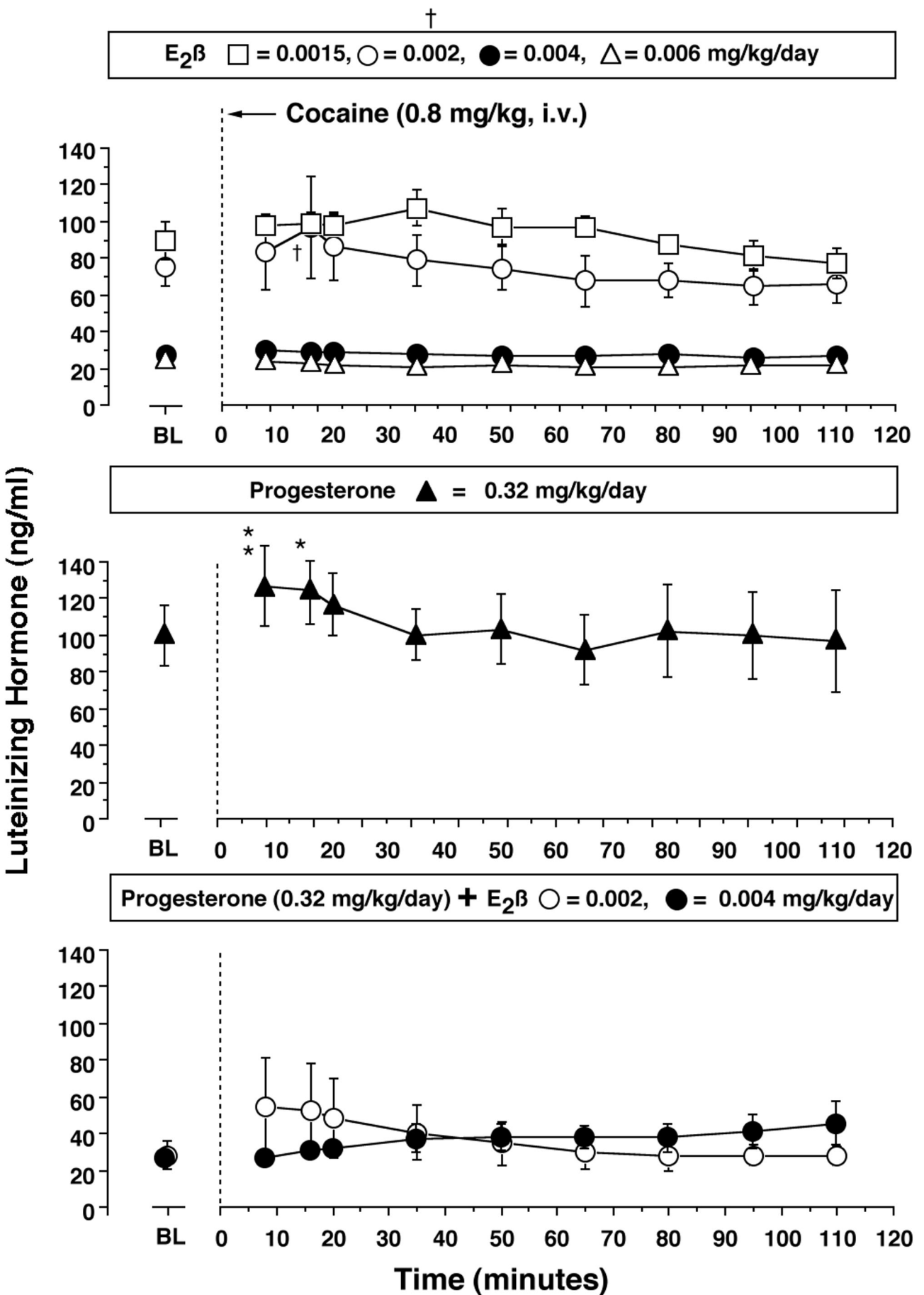
Luteinizing Hormone in OVX Monkeys
During Ovarian Steroid Hormone Replacement
†
E2ß □ = 0.0015, ○ = 0.002, ● = 0.004, △ = 0.006 mg/kg/day
Cocaine (0.8 mg/kg, i.v.)
140
120
100
80
60
40
20
0
BL
0 10 20 30 40 50 60 70 80 90 100 110 120
Progesterone ▲ = 0.32 mg/kg/day
* *
*
Luteinizing Hormone (ng/ml)
Progesterone (0.32 mg/kg/day) + E2ß ○ = 0.002, ● = 0.004 mg/kg/day
Time (minutes)

TABLE 1

Basal Ovarian Steroid Hormone Levels in Ovariectomized Female Rhesus Monkeys With and Without Hormone Replacement								
	No Hormone Replacement	**E_2ß (0.0015 mg/kg/day)**	**E_2ß (0.002 mg/kg/day)**	**E_2ß (0.004 mg/kg/day)**	**E_2ß (0.006 mg/kg/day)**	**Prog (0.32 mg/kg/day)**	**E_2ß (0.002) & Prog (0.32) mg/kg/day**	**E_2ß (0.004) & Prog (0.32) mg/kg/day**
17ß estradiol (pg/ml)	17.68 ± 2.18 22.74 ± 2.15	86 ± 37	114 ± 20	186 ± 30	221 ± 37	13.37 ± 1.26	94.9 ± 17.25	79.6 ± 13.3
Progesterone (ng/ml)	< 1.5	< 1.5	< 1.5	< 1.5	< 1.5	9.97 ± 1.5	13.55 ± 1.15	13.70 ± 2.4
	N=3	N=3	N=4	N=4	N=4	N=3	N=4	N=3

TABLE 2

Pharmacokinetics of Cocaine (0.8 mg/kg/i.v.) in Ovariectomized Female Rhesus Monkeys With and Without Ovarian Steroid Hormone Replacement

Pharmacokinetic Measures	No Hormone Replacement	E_2ß (0.002 mg/kg/day)	E_2ß (0.004 mg/kg/day)	E_2ß (0.006 mg/kg/day)	Prog (0.32 mg/kg/day)	E_2ß (0.002) & Prog (0.32) mg/kg/day
T_{max} (min)	5.3 ± 1.3	5.0 ± 1.0	4.0 ± 0	4.0 ± 0	5.3 ± 1.3	4.0 ± 0
C_{max} (ng/ml)	214.0 ± 44.2	217.2 ± 56.9	189.5 ± 47.5	205.7 ± 10.7	238.0 ± 33.4	174.0 ± 19.3
$T_{1/2}$ (min)	44.3 ± 2.1	45.8 ± 11.7	55.5 ± 12.7	40.2 ± 4.4	37.1 ± 8.7	36.9 ± 2.9
AUC (ng•min/ml)	9914 ± 2058	7786 ± 1594	7922 ± 893	8793 ± 883	9527 ± 953	7344 ± 729
	N=3	N=4	N=4	N=4	N=3	N=4