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The Effects of Cocaine on Gonadal Steroid Hormones and LH in Male and Female Rhesus Monkeys

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Cocaine stimulates significant increases in estradiol, testosterone (T), and luteinizing hormone (LH) in rhesus monkeys, but the temporal interactions between the gonadal steroid hormones and LH have not been determined. The effects of i.v. cocaine (0.8 mg/kg) or saline placebo administration on estradiol, T, and LH were compared in follicular phase female and male rhesus monkeys. Samples for hormone analysis were collected at 2-min intervals for 20 min, then at 10-min intervals for 50 min. Peak plasma cocaine levels were detected at 4 min and pharmacokinetic analyses showed no significant gender differences. Baseline hormone levels were equivalent before saline and cocaine administration, and saline did not alter LH or estradiol levels. In females, when baseline estradiol levels were low (<100 pg/ml), LH increased significantly within 8 min after cocaine administration ($P<0.05$), but when baseline estradiol levels were high (>100 pg/ml), LH levels did not change significantly after cocaine administration. Estradiol and T increased significantly after LH, within 16 min after cocaine administration ($P<0.01$–0.001). In males, significant LH increases were detected at 16 min after cocaine administration ($P<0.05$–0.001), but estradiol and T did not change significantly. Thus, cocaine may stimulate significant increases in estradiol and T in females but not in males. These rapid hormonal changes may contribute to cocaine's abuse-related effects, as well as to disruptions of the menstrual cycle during chronic cocaine administration.

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INTRODUCTION

There is considerable evidence that cocaine stimulates the hypothalamic–pituitary–adrenal (HPA) axis (for a review, see Mello and Mendelson, 2002). Moreover, the rapid increases in plasma adrenocorticotropin hormone (ACTH) and cortisol levels appear to be important modulators of cocaine's abuse-related effects (for a review, see Goeders, 1997, 2002a, b; Mello and Mendelson, 2002). There has been less attention to the effects of cocaine on the hypothalamic–pituitary–gonadal (HPG) axis, and relatively little is known about the interactions between cocaine and the gonadal steroid hormones that modulate and are modulated by LH release (Mello and Mendelson, 2002). Cocaine consistently stimulates luteinizing hormone (LH) release in men and women (Mendelson *et al*, 2002) and in male and female rhesus monkeys (Mello *et al*, 1990a, b; 1993). Cocaine also increased plasma estradiol levels significantly in female rhesus monkeys during the follicular phase of the menstrual cycle, but cocaine had no effect on estradiol levels during the luteal phase or after human chorionic gonadotropin (hCG) administration (Mello *et al*, 2000). Cocaine administration did not alter progesterone levels during either the follicular or the luteal phase of the menstrual cycle (Mello *et al*, 2000).

This finding was of interest because cocaine-induced increases in estradiol levels during the follicular phase could contribute to the abnormalities of the menstrual cycle observed during chronic cocaine administration to non-human primates (Mello *et al*, 1997; Potter *et al*, 1998, 1999). For example, high levels of estradiol during the follicular phase may suppress release of follicle-stimulating hormone (FSH) and disrupt folliculogenesis, which, in turn, may lead to anovulation and luteal phase dysfunction (Zeleznik, 1981; Dierschke *et al*, 1985, 1987). Moreover, high estradiol levels during the early luteal phase could result in a short luteal phase and compromise fertility (Hutchison *et al*, 1987). Since estradiol can stimulate an LH surge in ovariectomized monkeys (Terasawa, 1985; Mello *et al*, 1992), and in gonadally intact females during the follicular phase of the menstrual cycle (Yamaji *et al*, 1971; Ordog *et al*, 1998), we hypothesized that cocaine-induced increases in estradiol levels might account, in part, for the increase in LH release that has been consistently observed (Mello *et al*, 2000). Consistent with this notion, an antecedent increase in estradiol levels is essential for the periovulatory LH surge in gonadally intact females at mid-cycle (Hotchkiss and

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Knobil, 1994). However, during the rest of the menstrual cycle, regulation of LH and estradiol release is controlled through reciprocal negative feedback mechanisms (Bardin, 1986; Karsch, 1987; Hotchkiss and Knobil, 1994). In addition, after ovariectomy or natural menopause, estradiol, progesterone, and testosterone (T) levels decrease significantly, and LH increases to sustained high levels in the absence of gonadal steroid negative feedback (Karsch, 1987; Hotchkiss and Knobil, 1994; Shifren *et al*, 2000). In males, the regulatory interactions between LH and T release are similar, and estradiol and T also suppress LH release (Veldhuis and Dufau, 1987). Thus, an alternative hypothesis is that increases in estradiol levels could attenuate rather than facilitate a cocaine-induced increase in LH release.

The contribution of the ovarian steroid hormone milieu to modulation of cocaine's neuroendocrine effects is illustrated by the finding that cocaine did not stimulate LH or ACTH release in ovariectomized rhesus monkeys as it did in gonadally intact females studied under the same conditions (Mello *et al*, 1995; Sarnyai *et al*, 1995). The possible contribution of ovarian steroid hormones, rather than pituitary dysfunction, to those findings in ovariectomized monkeys, was suggested by the fact that synthetic luteinizing-hormone releasing-hormone (LHRH) and synthetic corticotropin releasing factor (CRF) each stimulated significant increases in LH and ACTH in the same monkeys (Mello *et al*, 1995; Sarnyai *et al*, 1995). Cocaine also did not decrease prolactin in ovariectomized monkeys (Mello *et al*, 2004) as it did in gonadally intact monkeys (Mello *et al*, 1990a, 1994). However, when ovariectomized monkeys were chronically treated with estradiol or progesterone, cocaine significantly decreased prolactin, consistent with its actions as an indirect dopamine agonist (Mello *et al*, 2004).

One goal of this study was to examine the time course and sequence of cocaine-induced changes in LH and gonadal steroid hormones using rapid sampling procedures. Most studies of the endocrine effects of acute cocaine administration in rhesus monkeys have collected integrated samples or bolus blood samples at 10- or 15-min intervals (Mello and Mendelson, 2002). This slow rate of sample collection prevents detection of rapid changes in hormone levels, and limits analysis of the temporal relation between hormone increases after acute cocaine administration. In the present study, samples for analysis of LH and gonadal steroid hormones were collected at 2-min intervals for the first 20 min after i.v. cocaine or placebo–cocaine administration. We selected this sample collection frequency because this procedure provided good resolution of temporal changes in LH and plasma cocaine in men and women (Mendelson *et al*, 2001).

The importance of using a rapid sample collection procedure to characterize cocaine's endocrine effects is suggested by the fact that plasma cocaine levels increase rapidly after i.v. administration, and peak levels are detected within 4 to 5 min in venous blood (Evans *et al*, 1996; Mendelson *et al*, 1999a). Similarly, plasma levels of the anterior pituitary hormones LH and ACTH increase significantly within 4–6 min after cocaine administration and are significantly correlated with increases in plasma cocaine levels (Mendelson *et al*, 2001, 2002). The possible importance of these rapid hormonal changes for the abuse-related effects of cocaine is suggested by several recent reports. In clinical studies, cocaine-stimulated increases in ACTH, measured at 2-min intervals, were temporally correlated with ratings of subjective 'high' in men (Mendelson *et al*, 2002). In preclinical studies, stimulation of the HPA axis appears to be essential for acquisition and maintenance of cocaine self-administration in rats (Goeders, 1997, 2002a, b). Moreover, administration of a corticotropin-releasing hormone (CRH) antagonist produced a dose-dependent decrease in cocaine self-administration in rats (Goeders and Guerin, 2000).

A second goal of this study was to evaluate the influence of basal estradiol levels on cocaine's acute hormonal effects by comparing gonadally intact females with baseline estradiol levels above and below 100 pg/ml. In an earlier study conducted in rhesus females during the luteal phase of the menstrual cycle, cocaine stimulated a significant increase in LH, but estradiol levels were not measured (Mello *et al*, 1993). In ovariectomized females, chronic estradiol treatment (0.0015–0.006 mg/kg/day, i.m.) produced 17-β estradiol levels of 86–221 pg/ml, and reduced baseline LH levels dose dependently (Mello *et al*, 2004). Under these estradiol treatment conditions, cocaine did not stimulate LH release (Mello *et al*, 2004). This is the first systematic examination of the extent to which variations in estradiol levels during the follicular phase may affect LH release after cocaine administration.

A third goal was to compare the effects of acute cocaine administration on LH, estradiol, and T in both male and female rhesus monkeys to determine if there are significant gender differences in the time course or relative magnitude of any hormonal changes. Gender differences in the behavioral effects of cocaine have been reported, and the biological basis for these differences remains to be determined (for a review, see Lynch *et al*, 2002, Mello and Mendelson, 2002). Although differences in gonadal steroid hormone profiles are one obvious basis for gender differences in responses to cocaine, very little is known about the acute effects of cocaine on estradiol and T in male and female rhesus monkeys.

MATERIALS AND METHODS

Subjects

A total of 12 adult female rhesus monkeys and seven adult male rhesus males (*Macaca mulatta*) (4.8–7.5 kg) lived in individual cages and were maintained on *ad libitum* food and water. Monkeys were fed twice each day at 0900 and 1700 Lab Diet Jumbo Monkey Biscuits (PMI Foods, Inc., St Louis, MO) and were supplemented with fresh fruit, vegetables, and multiple vitamins each day. Monkeys had visual, auditory, and olfactory contact with other monkeys. A variety of toys were available, and auditory and visual enrichment were provided. A 12-h light–dark cycle (lights on from 0700–1900) was in effect throughout the study. Each monkey was adapted to placement in a standard primate restraining chair on several occasions before these studies began. Successive studies of the acute effects of cocaine or placebo on LH and gonadal steroid hormones were separated by at least one menstrual cycle in females and 4–6 weeks in males.

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, NIH. This protocol was approved by the McLean Hospital Institutional Animal Care and Use Committee. The facility is licensed by the US Department of Agriculture. The health of the monkeys was periodically monitored by consultant veterinarians trained in primate medicine.

Menstrual Cycle Monitoring

Menstrual cycle regularity was monitored with vaginal smears to determine the onset and duration of vaginal bleeding. All endocrine study days in females were scheduled during the mid- to late-follicular phase of the menstrual cycle, 8–12 days after the onset of menstruation. The contribution of basal estradiol values to the effects of cocaine on LH and the gonadal steroid hormones was examined by analyzing data separately for groups of females with baseline estradiol levels below 100 pg/ml and above 100 pg/ml. Final data are reported for six cocaine and seven placebo-treated subjects in the low baseline estradiol group and for five cocaine and three placebo-treated subjects in the high baseline estradiol group.

Experimental Conditions

The effects of acute administration of cocaine and placebo–cocaine on the interactions between LH and gonadal steroid hormones were studied. The acute effects of placebo–cocaine and cocaine (0.8 mg/kg, i.v.) on LH, estradiol, and T were evaluated in male and female rhesus monkeys. On each study day, basal levels of LH, estradiol, and T were measured 10 min before placebo–cocaine or cocaine was administered. Following i.v. placebo or cocaine administration, 10 samples were collected at 2-min intervals for 20 min, then five samples were collected at 10-min intervals for the remainder of the 70 min sampling period. In previous studies, peak levels of cocaine in plasma were detected within 2–4 min after intravenous administration in female rhesus monkeys, and the half-life of cocaine was 56–61 min (Mendelson *et al*, 1999a). Accordingly, samples for analysis of plasma cocaine levels were collected at 4, 8, 12, 16, 20, 30, 50, and 70 min after cocaine administration in the present study.

Cocaine Dose Selection

The cocaine dose was selected on the basis of our previous studies in which 0.8 mg/kg of cocaine stimulated LH in both male and female rhesus monkeys (Mello *et al*, 1990a, 1993) and estradiol in female rhesus monkeys (Mello *et al*, 2000). Higher cocaine doses were not studied because we have found that 1.0 mg/kg, i.v. cocaine produced hyperactivity and agitated behavior, and because the convulsant dose range for cocaine is 3–8 mg/kg, i.v. (Matsuzaki *et al*, 1976). Lower cocaine doses (0.4 mg/kg) did not reliably stimulate LH in preliminary studies. Placebo–cocaine was a vehicle control consisting of sterile saline for injection. Cocaine and placebo–cocaine treatments were given in an irregular order, counter-balanced across subjects.

Acute Venous Catheter Implantation and Blood Sample Collection

Monkeys were anesthetized with ketamine hydrochloride (5–10 mg/kg, i.m.). A Sur-Flo Intercath containing a 20-gauge needle (I.D. 0.80×51 mm, Terumo Medical Corporation, Elkton, MD) was inserted into the saphenous vein using aseptic techniques. After removal of the needle stylet, the catheter was joined to heparin-impregnated sterile silicon tubing and secured with sutures. A second catheter for intravenous infusion of saline control or cocaine solutions was implanted in the opposite leg. Each monkey was placed in a standard primate restraint chair for 2 h before sample collection began to reduce any possible stress associated with the catheter implantation procedure and to ensure that the sedative effects of ketamine had dissipated. Placebo–cocaine or cocaine was administered as an i.v. bolus over 1 min into the saphenous vein of the leg opposite the exfusion catheter. After cocaine or saline infusion, a 0.9% NaCl solution was infused at a rate of 2 ml/h. Blood samples for LH, estradiol, and T analysis were collected in heparinized tubes. 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). Samples were centrifuged, and aliquots of plasma were drawn and stored at -70°C until analysis.

Cocaine Preparation

Cocaine hydrochloride was obtained from the National Institute on Drug Abuse (Rockville, MD) and solutions were prepared by dissolving cocaine in sterile saline for injection U.S.P. The solution was filter-sterilized using a 0.11 μm Millipore filter (Bedford, MA).

Plasma Hormone and Cocaine Analyses

Data are reported for the analysis of LH, estradiol, T, and cocaine in plasma. Details of these assay procedures follow:

LH assay. 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 (NICHD-rhLH, also known as WP-XV-20) prepared by Dr W Peckham, Recombinant (Genzyme) Cynomolgus Monkey Luteinizing Hormone Antigen (AFP-6936A) for iodination and Anti-Recombinant Cynomolgus Monkey Luteinizing Hormone Serum (Rabbit) (AFP342994) obtained through the NIDDK's National Hormone and Peptide Program and Dr AF Parlow (Harbor-UCLA Medical Center). Radioiodination was performed using chloromine-T (Greenwood *et al*, 1963) with sodium iodide-125 purchased from Perkin–Elmer Life Sciences (Billerica, MA). Goat antirabbit gammaglobulin was obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA). Results were expressed in nanograms per milliliter in terms of the reference preparation. The assay sensitivity was 4.7 ng/ml and the intra- and interassay CVs were 4.4 and 8.6%, respectively.

Estradiol assay. Plasma concentrations of 17β-estradiol were determined in duplicate using a direct double-antibody RIA kit purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). The following modification was made to the protocol: prior to analysis, the plasma samples were extracted, then reconstituted in zero standard. The assay sensitivity was 4.9 pg/ml and the intra- and interassay CVs were 9.9 and 11.2%, respectively.

T assay. Plasma T concentrations were measured in duplicate using a direct, double-antibody RIA kit purchased from ICN Biomedicals, Inc. (Costa Mesa, CA). The assay sensitivity was 1.6 ng/dl and the intra- and interassay CVs were 7.0 and 10.4%, respectively.

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

Statistical Analyses

The effects of placebo–cocaine and cocaine on plasma levels of LH (ng/ml), estradiol (pg/ml), and T (ng/dl) were evaluated with analysis of variance (ANOVA) for repeated measures (Prism, GraphPad Software, San Diego, CA). Within each of the three experimental groups, a one-factor ANOVA was used to compare mean values at each sample period with baseline means using contrast tests. Paired *t*-tests were used to evaluate baseline hormone levels before placebo–cocaine and cocaine administration within each group and between groups. Probability levels of $P<0.05$ are reported as statistically significant. Since baseline hormone values differed significantly between males and females, as well as between females with basal estradiol levels above and below 100 pg/ml, data are displayed as percent change from baseline to facilitate comparisons of the effects of placebo and cocaine administration on LH, estradiol, and T.

Pharmacokinetic Analyses

Estimates of cocaine's primary kinetic parameters (ie peak plasma cocaine concentrations and time to peak plasma concentration) in males and females with high and low baseline estradiol levels were obtained 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.

RESULTS

Plasma Cocaine Levels in Female and Male Rhesus Monkeys (Figure 1)

Figure 1 shows plasma cocaine levels for a group of six females with low baseline estradiol levels (<100 pg/ml) and five females with high baseline estradiol levels (>100 pg/ml) (rows 1 and 2). Peak plasma cocaine levels were detected at 4 min after i.v. cocaine administration. Peak plasma cocaine levels did not differ significantly between the low and high baseline estradiol females and averaged 225 ± 31 and 211 ± 13 ng/ml, respectively. Analysis of plasma cocaine pharmacokinetics showed that there were no statistically significant differences between the females with low and high baseline estradiol levels. Specifically, in the low baseline estradiol group, average peak cocaine levels occurred at 5.3 ± 0.8 min (T_{max}) postinjection with a half life ($T_{1/2}$) of 39.4 ± 1.5 min. In the high baseline estradiol group, average peak cocaine levels occurred at 4.8 ± 0.8 min (T_{max}) postinjection with a half life ($T_{1/2}$) of 44.6 ± 1.5 min.

Plasma cocaine levels for a group of five males are shown in the third row of Figure 1. Peak plasma cocaine levels averaged 274 ± 60 ng/ml at 4 min after i.v. cocaine administration. Pharmacokinetic analysis showed that average peak cocaine levels occurred at 4.8 ± 0.8 min (T_{max}), and the cocaine half-life ($T_{1/2}$) was 34.9 ± 3.7 min. These pharmacokinetic parameters for cocaine in males did not differ significantly from those in the female monkeys with low or high baseline estradiol levels.

Effects of Cocaine on LH, Estradiol, and T in Rhesus Females with Low Baseline Estradiol Levels (<100 Pg/Ml) (Figure 2)

Baseline levels of LH, estradiol, and T. There were no significant differences in baseline levels of LH, estradiol, or T before placebo and cocaine administration in females with basal estradiol levels below 100 pg/ml. Basal levels of LH averaged 28 ± 3.7 ng/ml before placebo–cocaine administration and 27 ± 2.8 ng/ml before cocaine administration. Basal estradiol levels averaged 45.9 ± 2.5 pg/ml before placebo–cocaine administration and 57.8 ± 9.3 pg/ml before cocaine administration. Basal T levels averaged 10.2 ± 2.6 ng/dl before placebo–cocaine administration and 11.5 ± 5.5 ng/dl before cocaine administration.

LH, estradiol, and T levels after placebo and cocaine administration. Figure 2 shows average LH, estradiol, and T levels for these females after cocaine or placebo administration. After placebo–cocaine administration, LH and estradiol levels did not increase significantly in comparison to baseline. T levels increased significantly at 60 and 70 min after placebo administration ($P<0.05$). After cocaine administration, LH and estradiol levels each increased significantly above baseline ($P<0.05$–0.001), but LH increased more rapidly than estradiol. LH increased significantly within 8 min, and peak LH levels were detected at 10 min after cocaine administration. Peak LH levels averaged 40% above baseline. LH remained significantly above baseline for 22 min, then gradually returned to baseline levels.

Estradiol increased abruptly by 50% at 16 min after cocaine administration ($P<0.001$). Estradiol remained significantly above baseline for 14 min, between 16 and 30 min post cocaine, and then declined at 40 min after cocaine administration. An additional estradiol peak was detected at 50 min post cocaine ($P<0.05$). At the end of the

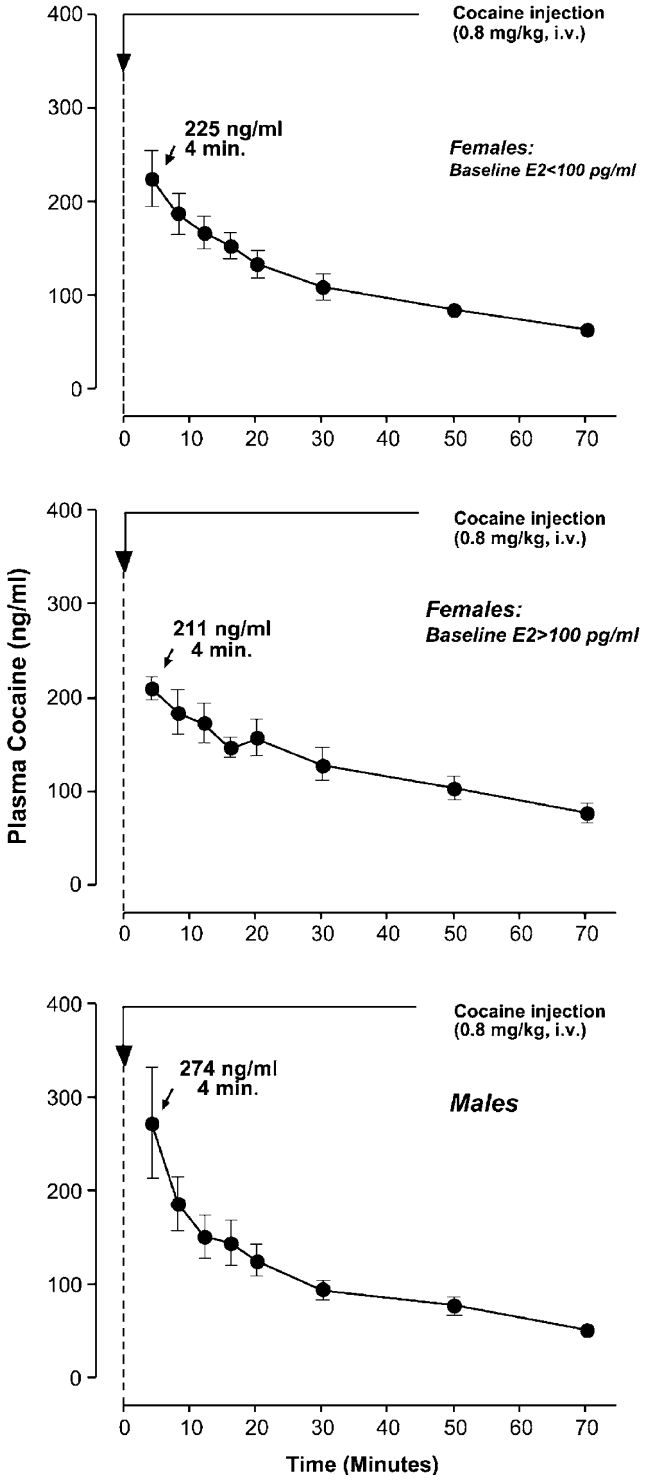

Figure 1 Cocaine plasma levels: cocaine (0.8 mg/kg, i.v.) was administered over 1 min as indicated at the vertical dotted line. The abscissa shows consecutive samples collected at 4, 8, 12, 16, 20, 30, 50, and 70 min after cocaine administration. The left ordinate shows levels of cocaine in plasma (ng/ml). Data shown in the top panel is the average (±SEM) of plasma cocaine levels in six female monkeys with low baseline estradiol levels, and data shown in the middle panel is the average (±SEM) of plasma cocaine levels in five females with high baseline estradiol levels. Each data point in bottom panel is the average (±SEM) plasma cocaine levels in five male rhesus monkeys.

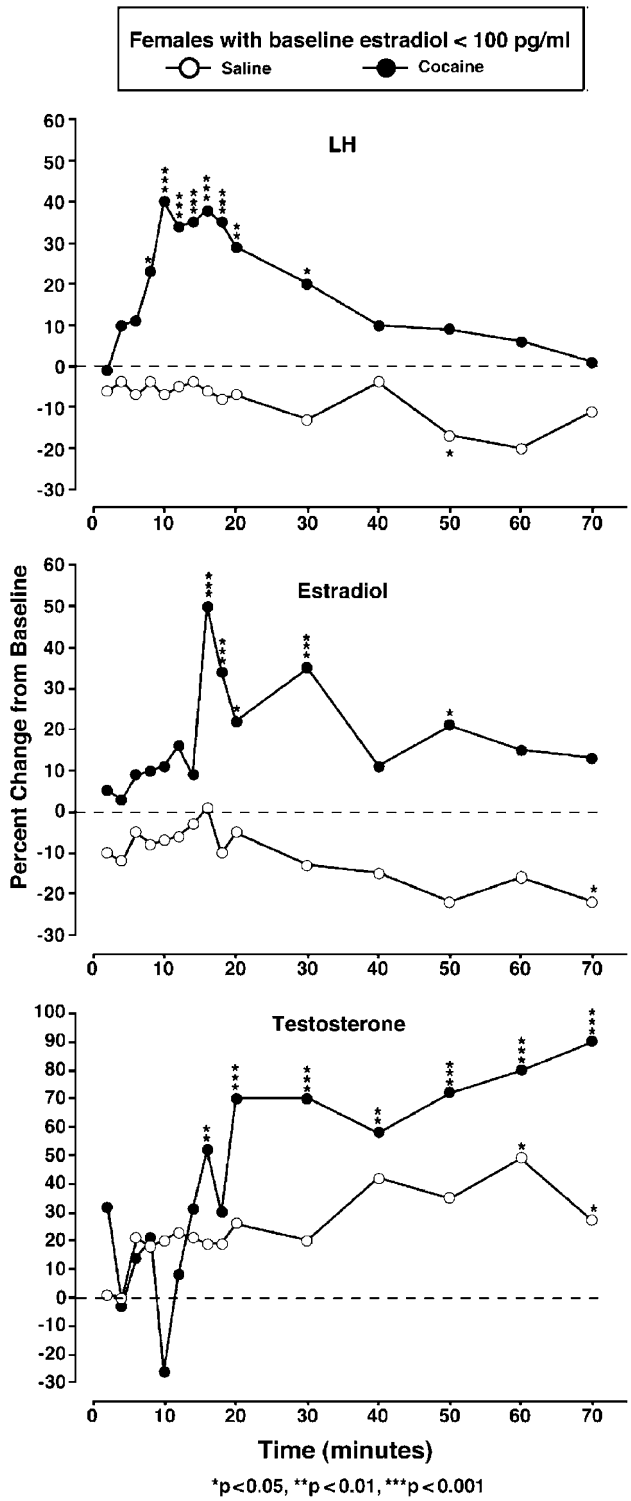

Figure 2 The effects of placebo and cocaine on LH, estradiol, and T in follicular phase female rhesus monkeys with low baseline estradiol levels (<100 pg/ml). The abscissae show consecutive samples collected at 2-min intervals for 20 min after intravenous placebo or cocaine administration, and at 10-min intervals thereafter. Placebo conditions are shown as open circles, and cocaine conditions are shown as solid circles. The left ordinate shows the percent change from baseline for LH, estradiol, and T. Each data point is based on the average (±SEM) of six rhesus females after cocaine administration and seven rhesus females after placebo administration. Statistically significant changes from the pre-placebo or pre-cocaine baseline are indicated by asterisks (* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$).

70 min sampling period, estradiol remained 13% above baseline.

Testosterone also increased significantly within 16 min after cocaine administration ($P<0.01$) and continued to increase as LH and estradiol levels declined. The T level remained significantly above baseline for 54 min ($P<0.05$–0.001). At the end of the sampling period, T averaged 90% above precocaine baseline levels.

Effects of Cocaine on LH, Estradiol, and T in Rhesus Females with High Baseline Estradiol Levels (>100 pg/ml) (Figure 3)

Baseline levels of LH, estradiol, and T. Baseline estradiol levels above 100 pg/ml defined the high baseline estradiol group. Basal estradiol levels were significantly higher in these females ($P<0.01$–0.001) than in the low baseline estradiol females described earlier in Figure 2. However, baseline LH and T levels were equivalent in the two groups of females. Within the high baseline estradiol females, there were no significant differences in LH, estradiol, or T before placebo and cocaine administration. Basal LH levels averaged 22.2 ± 1.6 ng/ml before placebo–cocaine administration and 26.5 ± 3.3 ng/ml before cocaine administration. Basal estradiol levels averaged 158.1 ± 30.7 pg/ml before placebo–cocaine administration and 136.5 ± 25.4 before cocaine administration. Basal T levels averaged 15.5 ± 1.3 ng/dl before placebo–cocaine administration and 13.1 ± 3.8 ng/dl before cocaine administration.

LH, estradiol, and T levels after placebo and cocaine administration. Figure 3 shows the effects of cocaine or placebo administration in females with high baseline estradiol levels. In contrast to females with low baseline estradiol levels, LH levels did not change significantly from baseline after cocaine administration. LH and estradiol did not change significantly after placebo administration. After cocaine administration, estradiol levels initially decreased then increased significantly above baseline at 8 min and again at 50 min ($P<0.05$). Testosterone increased significantly above baseline at 4 min after placebo injection and again at 40, 60, and 70 min ($P<0.05$–0.001). After cocaine administration, the first T peak was detected at 4 min ($P<0.05$), then T decreased below baseline, and increased again at 8 min. Peak increases in T averaged 37% above baseline at 20 min after cocaine administration. The T levels remained significantly elevated between 8 and 70 min after cocaine injection ($P<0.05$–0.001).

Effects of Cocaine on LH, Estradiol, and T Levels in Rhesus Males (Figure 4)

Baseline levels of LH, T, and estradiol. There were no significant differences in baseline levels of LH, T, and estradiol before placebo and cocaine administration. Basal LH levels averaged 17.8 ± 0.9 ng/ml before placebo–cocaine administration and 19.6 ± 1.2 ng/ml before cocaine administration. These basal LH levels did not differ significantly from basal LH levels in the females shown in Figures 2 and 3. Estradiol levels in males were significantly lower than basal estradiol levels in the females shown in Figures 2 and 3 ($P<0.01$–0.001). Basal levels of estradiol averaged

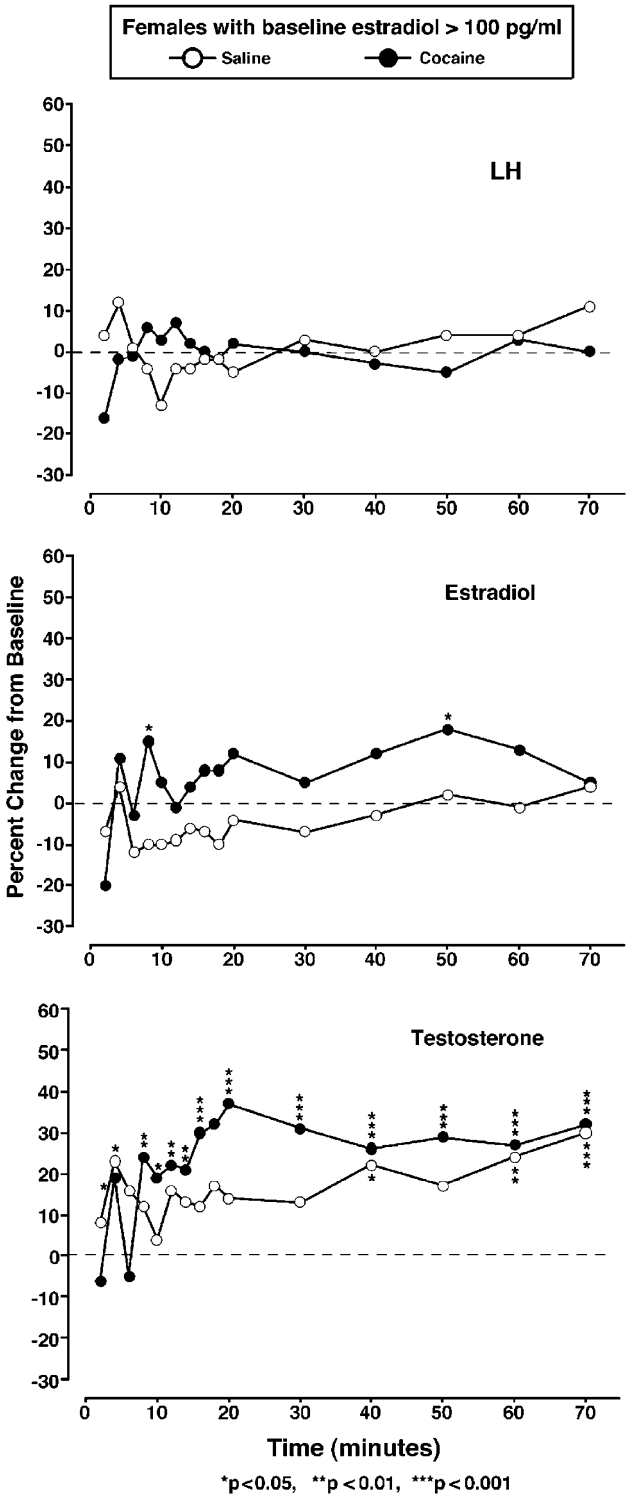

Figure 3 The effects of placebo and cocaine on LH, estradiol, and T in follicular phase female rhesus monkeys with high baseline estradiol levels (>100 pg/ml). The abscissae show consecutive samples collected at 2-min intervals for 20 min after intravenous placebo and cocaine administration, and at 10-min intervals thereafter. Placebo conditions are shown as open circles, and cocaine conditions are shown as solid circles. The left ordinate shows the percent change from baseline for LH, estradiol, and T. Each point is based on the average (±SEM) data from five rhesus females after cocaine administration and three rhesus females after placebo administration. Statistically significant changes from the pre-placebo or pre-cocaine baseline are indicated by asterisks (* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$).

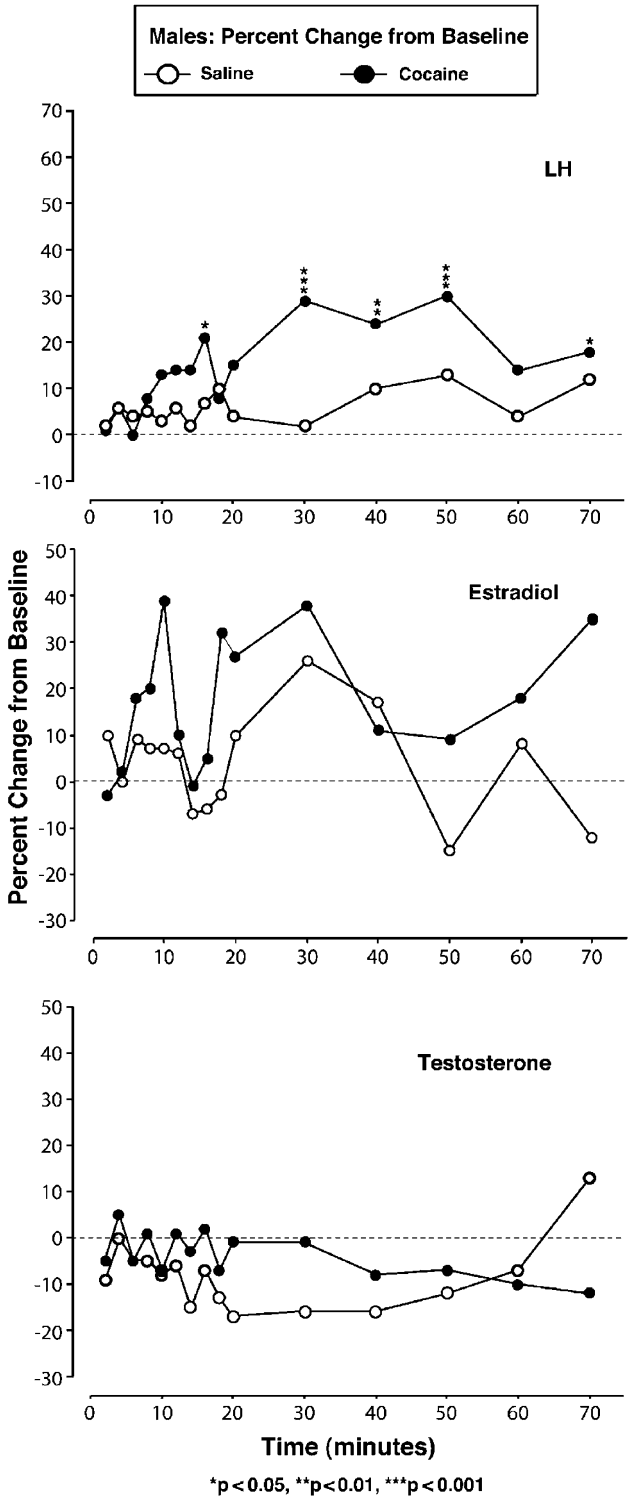

Figure 4 The effects of placebo and cocaine on LH, estradiol, and T in male rhesus monkeys. The abscissae show consecutive samples collected at 2-min intervals for 20-min after intravenous placebo and cocaine administration, and at 10-min intervals thereafter. Placebo conditions are shown as open circles, and cocaine conditions are shown as solid circles. The left ordinate shows the percent change from baseline for LH, estradiol, and T. Each data point is based on the average (±SEM) of five rhesus males after cocaine administration and seven rhesus males after placebo administration. Statistically significant changes from the pre-placebo or pre-cocaine baseline are indicated by asterisks (* = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$).

13.4±4.3 pg/ml before placebo–cocaine administration and 7.7±1.8 pg/ml before cocaine administration. Basal T levels were significantly higher in males than in females ($P<0.001$). Basal T levels in males averaged 114.4± 18.0 ng/dl before placebo–cocaine administration and 111.4± 18.7 ng/dl before cocaine administration.

LH, estradiol, and T levels after placebo and cocaine administration. Figure 4 shows plasma levels of LH, estradiol, and T after administration of placebo–cocaine or 0.8 mg/kg cocaine. After placebo–cocaine administration, LH, T, and estradiol did not change significantly from baseline. After cocaine administration, LH increased significantly within 16 min ($P<0.05$), and peak LH levels were detected between 30 and 50 min ($P<0.01$–0.001). LH remained significantly above baseline at the end of the 70 min sampling period. Estradiol levels were extremely variable after both placebo–cocaine and cocaine administration, and there were no statistically significant changes from baseline in either condition. The T levels also did not change significantly after cocaine or placebo administration.

DISCUSSION

The goal of this study was to characterize the acute effects of cocaine on LH and the gonadal steroid hormones in male and female rhesus monkeys. The influence of gender and baseline levels of gonadal steroid hormones on the sequence, time course, and relative magnitude of cocaine's hormonal effects were examined. Consistent with previous reports in rats and in humans, the pharmacokinetics of cocaine did not differ in males and females (Bowman *et al*, 1999; Mendelson *et al*, 1999b). However, both gender and baseline levels of gonadal steroid hormones appeared to be significant determinants of the acute endocrine effects of cocaine. The major findings were as follows: (1) cocaine administration was followed by a rapid increase in plasma LH levels in males, and in female rhesus monkeys with low baseline estradiol levels, but not in females with high basal estradiol levels; (2) LH increased more rapidly than either estradiol or T in females with low baseline estradiol levels, and this suggested that after cocaine administration, LH stimulated subsequent increases in the gonadal steroid hormones rather than the converse; and (3) rapid onset and sustained elevations in T occurred in both groups of females but not in males. The relation of these findings to previous studies and the possible biological significance of these cocaine-induced changes in hypothalamic–pituitary–gonadal hormones are discussed below.

Cocaine's Effects on Estradiol

The rapid sample collection procedure revealed that estradiol increased abruptly to 50% above baseline within 16 min after cocaine administration in females with low baseline estradiol levels and remained significantly above baseline for 14 min. To put this rapid estradiol increase in perspective, it takes about 61.3 h for estradiol levels to double before the ovulatory LH surge (Yen, 1999). Thus, the cocaine-induced increases in plasma estradiol levels are

smaller in magnitude and much shorter in duration than the increments in estradiol that precede the pre-ovulatory LH surge during the normal ovulatory menstrual cycle. In follicular phase women, an estradiol pulse is released about every 92 min, and each pulse lasts for 88 min (Licinio *et al*, 1998). Although differences in assay procedures and sensitivity preclude exact quantitative comparisons, the height of these wide, slow estradiol pulses averages about 55 pg/ml in women (Licinio *et al*, 1998), whereas in rhesus females after cocaine administration, the peak level of estradiol averaged 87 pg/ml. The magnitude of the peak increase in estradiol was similar in the low estradiol baseline group (29 pg/ml) and the high estradiol baseline group (21 pg/ml). This is less than the average estradiol levels measured at 16 min after i.v. administration of 0.0001 mg/kg estradiol in β-cyclodextrin to rhesus females (52 pg/ml) (NK Mello, unpublished observations).

The source of the rapid increase in estradiol after cocaine administration is unclear. In follicular phase women, estradiol production and secretion rates are estimated at 0.9 and 0.8 mg/day, respectively (O'Malley and Strott, 1999). Most estradiol (95%) is secreted from the dominant ovarian follicle, and an estimated 5% of estradiol is derived from peripheral conversion of estrone (O'Malley and Strott, 1999). In men, more than 60% of estradiol is derived peripherally from T. Despite the different pathways for estradiol production, rapid onset bursts of estradiol after cocaine administration occurred in both males and females.

Cocaine consistently stimulates the HPA axis, and it is possible that cocaine may increase the small amounts of estrogens normally produced by the adrenal cortex (O'Malley and Strott, 1999; Mello and Mendelson, 2002). After administration of 0.4 mg/kg cocaine to men, significant increases in ACTH were detected within 8.7 min, and a secondary increase in cortisol occurred within 36 min (Mendelson *et al*, 2002). However, the possible relationship between cocaine's stimulation of ACTH and cortisol, and the cocaine-induced burst of estradiol measured in the present study, cannot be determined from these data.

The time course of the cocaine-induced increase in estradiol was similar to that in our previous study in follicular phase females, in which bolus blood samples were collected every 15 min (Mello *et al*, 2000). In that study, estradiol increased significantly above baseline within 15 min after 0.8 mg/kg i.v. cocaine administration, and remained significantly above baseline for 45 min. In the present study, the first significant increase in estradiol was detected at 16 min after cocaine, and subsequent estradiol peaks were measured for 50 min after cocaine administration.

The two groups of females were classified on the basis of estradiol levels on the study day rather than on time-based estimates of cycle phase. All baseline estradiol levels were in the physiological range, but the significant increases in estradiol detected after cocaine administration in females with baseline estradiol levels above 100 pg/ml were less than 20%. This may reflect a ceiling effect because baseline estradiol levels in the high estradiol females were 50 pg/ml higher than the peak estradiol levels in the low baseline estradiol females after cocaine administration. It is likely that the high baseline estradiol females were approaching the periovulatory phase of the menstrual cycle at the time of the study.

Cocaine's Effects on T

Cocaine did not alter T in rhesus males, and this finding is consistent with findings in human males where cocaine (0.4 mg/kg i.v.) also did not change T levels (Mendelson *et al*, 2003). In contrast to males, cocaine increased T significantly in both groups of females. However, it is unlikely that this apparent gender difference was biologically significant. Baseline T levels were very low in the females and ranged between 9.5 and 15 ng/dl. After cocaine, the maximum increases in T averaged between 5 and 10 ng/dl or 3–6 times the sensitivity of the T assay. Moreover, some significant increases in T were also detected after saline administration. In females with low baseline estradiol levels, the sustained increases in T paralleled the decreases in LH and estradiol, consistent with established negative feedback regulation of LH and T. However, this pattern was not evident in females with high baseline estradiol levels or in males.

Cocaine's Effects on LH

Cocaine's stimulation of LH release has been consistently reported in both clinical and preclinical studies (for a review, see Mello and Mendelson, 2002). Deconvolution analysis indicated that cocaine's stimulation of LH release probably reflected a burst of hypothalamic LHRH in rhesus monkeys (Mello and Mendelson, 2002). Moreover, the half-life of LH was not significantly different after cocaine and placebo administration, which suggests that dispositional factors did not account for cocaine-induced LH release. Interestingly, cocaine also enhanced synthetic LHRH stimulation of LH release in early follicular phase rhesus females (Mello *et al*, 1990b). The time course of increases in LH after cocaine administration in this study cannot be directly compared with our previous results, because relatively infrequent integrated plasma samples were collected in those studies (Mello *et al*, 1990a, b, 1993).

LH increased more slowly in rhesus males than in females, and remained significantly elevated above baseline for longer (54 *vs* 22 min). The time course of cocaine's effects on LH in male and female rhesus monkeys in the present study is concordant with a clinical study in which similar rapid sampling procedures were used (Mendelson *et al*, 2001). In follicular phase women, LH levels began to increase within 4 min after 0.4 mg/kg i.v. cocaine administration, and peak levels were detected within 16 min. In men, LH increased significantly within 8 min after the same dose of cocaine, and reached peak levels within 20 min. Moreover, LH also remained significantly elevated for a longer time in men (32 min) than in follicular and luteal phase women (4 and 8 min) (Mendelson *et al*, 2001). Thus, the gender difference in the onset and duration of LH stimulation observed in these rhesus monkeys is consistent with differences in cocaine's effects on LH in men and women (Mendelson *et al*, 2001).

LH and Gonadal Steroid Interactions

Data obtained in the present study suggest that the increase in LH after cocaine administration is not stimulated by estradiol as we originally postulated (Mello *et al*, 2000).

Rather the peak increase in LH levels was detected 6 min before a significant increase in estradiol levels. These data suggest that the cocaine-induced increase in LH release may have stimulated a subsequent increase in estradiol release in rhesus females. It is likely that the observed sequence of LH release followed by estradiol release was a direct effect of cocaine rather than a reflection of spontaneous pulsatile release patterns of LH and estradiol. In follicular phase women, fluctuations in LH and estradiol levels showed no significant crosscorrelation (Licinio *et al*, 1998).

The cocaine-induced sequence of increases in LH before increases in estradiol is exactly the opposite of the sequence of estradiol–LH interactions at the periovulatory phase of the menstrual cycle. At that time, an antecedent increase in estradiol stimulates the LH surge necessary for ovulation (Hotchkiss and Knobil, 1994). However, the magnitude of the preovulatory estradiol surge (>150 pg/ml) is far greater than the average peak estradiol levels measured in the present study (87 pg/ml). Moreover, the preovulatory elevations in estradiol must be sustained for at least 36 h to effectively trigger the ovulatory surge in LH in rhesus females (Hotchkiss and Knobil, 1994). Similarly, chronic estradiol exposure is required to amplify LHRH stimulation of LH secretory burst duration (Quyyumi *et al*, 1993).

Only a single dose of cocaine was administered in this study, and the effects of repeated cocaine injections on LH, estradiol, and T are unknown. In human cocaine abusers, repeated 'binge' cocaine administration is the most common use pattern (Ward *et al*, 1997). If repeated cocaine administration continued to stimulate comparable increases in LH and estradiol, this could contribute to the menstrual cycle disruptions seen during chronic cocaine exposure in rhesus monkeys (Mello *et al*, 1997; Potter *et al*, 1998, 1999). For example, continuous exposure to estradiol for only 24 h on day 6 of the menstrual cycle resulted in atresia of the dominant ovarian follicle in rhesus monkeys (Dierschke *et al*, 1985). Repeated 24-h exposures to estradiol at 10-day intervals also resulted in recurrent atresia of the dominant ovarian follicle (Dierschke *et al*, 1987). Atresia of the dominant ovarian follicle usually results in anovulation with no increase in estradiol and LH at mid-cycle. Anovulatory cycles were often observed in rhesus monkeys during chronic cocaine self-administration (Mello *et al*, 1997) and daily noncontingent cocaine administration (Chen *et al*, 1998; Potter *et al*, 1998, 1999).

The importance of the HPA axis in the initiation and maintenance of cocaine self-administration has been well established in rodents (Goeders, 1997, 2002a, b; Goeders and Guerin, 2000) and suggested by clinical studies (Mendelson *et al*, 2002). It is interesting to speculate that the HPG axis may also contribute to cocaine's reinforcing effects. In humans, LH, estradiol, and T appear to modulate feelings of well-being and sexuality (Klaiber *et al*, 1997; Rubinow *et al*, 1998; Pope and Brower, 2000; Shifren *et al*, 2000; Mello and Mendelson, 2002). In rodents, estradiol appears to enhance both the acute locomotor and abuse-related effects of cocaine under some conditions (Roberts *et al*, 1989; Lynch *et al*, 2000; Sell *et al*, 2000; Kuhn *et al*, 2001; Lynch *et al*, 2001, 2002). However, under other conditions, neither gonadectomy nor gonadal steroid replacement altered the shape or position of cocaine self-administration dose–effect curves in rats (Caine *et al*, 2004). The generality of these findings in rodents to humans and non-human primates remains to be determined.

The relatively rapid perturbation of the HPG axis by cocaine observed in the present study is consistent with the idea that these hormonal changes may influence the abuse-related effects of cocaine. *In vitro* studies have consistently shown that physiological concentrations of estradiol stimulate dopamine synthesis/release in striatal tissue (Becker, 1990; McDermott, 1993; Pasqualini *et al*, 1995, 1996). Moreover in rats, high levels of estradiol at estrus were associated with significantly higher levels of amphetamine-stimulated striatal dopamine release than at diestrus (Becker and Cha, 1989). Thus, it is possible that a rapid increase in estradiol may stimulate dopamine release and augment cocaine-induced increases in extracellular dopamine levels. However, we were unable to locate any microdialysis studies of the time course of estradiol-induced increases in dopamine in non-human primates. One context for comparison is the time course of cocaine-induced increases in extracellular dopamine. However, the dopamine peak after i.v. cocaine administration varied with the frequency of dialysate sample collection. For example, in vervet or rhesus monkeys, peak levels of dopamine were detected at 4 min when dialysate samples were collected at 2 min, and at 20 min when dialysate samples were collected at 20-min intervals (Iyer *et al*, 1995; Bradberry, 2000; Bradberry *et al*, 2000). Similarly, in microdialysis studies in rats, increases in striatal dopamine release were detected within 20 min, and peaked at 40 min after i.p. amphetamine administration (Becker and Cha, 1989). The functional significance of sustained increases in estradiol within 16 min after i.v. cocaine administration remains to be determined. However, there is increasing evidence that estradiol has rapid, nongenomic effects that may precede its traditional genomic steroid actions and influence behavior as well as neuroendocrine and reproductive functions (Wong *et al*, 1996; Moore and Evans, 1999; Falkenstein *et al*, 2000). Behavioral studies to assess the influence of gonadal steroid hormones on the reinforcing and discriminative stimulus effects of cocaine in non-human primates are underway in this laboratory.

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