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Effects of Progesterone and Testosterone on Cocaine Self-Administration and Cocaine Discrimination by Female Rhesus Monkeys

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The neuroactive steroid hormone progesterone attenuates cocaine's abuse-related effects in women and in rodents under some conditions, but the effects of testosterone are unknown. We compared the acute effects of progesterone (0.1, 0.2, and 0.3 mg/kg, intramuscularly (i.m.)), testosterone (0.001, 0.003, and 0.01 mg/kg, i.m.), and placebo on cocaine self-administration and cocaine discrimination dose–effect curves in female rhesus monkeys. Cocaine self-administration (0.03 mg/kg per inj.) was maintained on a fixed ratio 30 schedule of reinforcement, and monkeys had unlimited access to cocaine for 2 h each day. Cocaine doses were administered in an irregular order during each dose–effect curve determination, and the same dose order was used in each subject in all treatment conditions. Blood samples for hormone analysis were collected at the end of each test session. Banana-flavored food pellets (1 g) were also available in three 1-h daily sessions. In drug discrimination studies, the effects of pretreatment with progesterone (0.032–0.32 mg/kg, i.m.) and testosterone (0.001–0.01 mg/kg, i.m.) on the discriminative stimulus effects of cocaine (0.18 mg/kg, i.m.) were examined. Progesterone and testosterone did not alter cocaine discrimination, and did not substitute for cocaine. In contrast, progesterone and testosterone each significantly decreased cocaine self-administration, and produced a downward and rightward shift in the cocaine self-administration dose–effect curve. These findings are concordant with clinical reports that progesterone administration may decrease ratings of positive subjective effects of cocaine in women, and suggest the possible value of neuroactive steroid hormones for the treatment of cocaine abuse and reduction of risk for relapse.

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INTRODUCTION

Neuroactive steroid hormones (progesterone, testosterone, and estradiol) may have excitatory and inhibitory effects in the brain (Rupprecht and Holsboer, 1999a, b; Zinder and Dar, 1999; Rupprecht, 2003). There is increasing evidence that the neuroactive steroid hormones may attenuate anxiety and depression (Su *et al*, 1993; Pope and Brower, 2000; Pope *et al*, 2000; Rupprecht, 2003; Kanayama *et al*, 2007; Rupprecht *et al*, 2009), and these conditions often are associated with cocaine abuse and propensity for relapse after cessation of use (Gold, 1997; Mello and Mendelson, 2010). An emerging literature suggests that the neuroactive steroid hormones may enhance or diminish cocaine's reinforcing effects (see for a review, Lynch *et al*, 2002; Mello and Mendelson, 2002, 2009; Carroll *et al*, 2004; Evans, 2007; Anker and Carroll, 2010; Evans and Foltin, 2010). The mechanisms underlying hormone effects on the abuse-related effects of cocaine are not clear. The neuroactive steroid hormones are positive allosteric modulators of $GABA_A$ receptors (Reddy, 2003; Eser *et al*, 2006; Schumacher *et al*, 2007), and like abused drugs, can alter dopamine synthesis and release under some conditions (Pasqualini *et al*, 1995, 1996; Thilbin *et al*, 1999; Becker *et al*, 2001; Cabrera *et al*, 2002).

Most behavioral research has focused on the interactions between cocaine and estradiol, but there has been recent interest in the effects of progesterone. Estradiol enhanced cocaine self-administration in gonadally intact rodents (Roberts *et al*, 1989; Hecht *et al*, 1999; Lynch *et al*, 2002) and in ovariectomized rodents (Lynch *et al*, 2001; Hu *et al*, 2004; Jackson *et al*, 2006). However, other studies report that estradiol did not enhance cocaine self-administration in rodents (Caine *et al*, 2004; Lynch and Taylor, 2005) or in

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female rhesus monkeys (Mello *et al*, 2008). Although species and procedural variables contribute to these discrepant findings, the unit dose of cocaine used to maintain self-administration appears to be one critical variable (Mello *et al*, 2008). At cocaine doses that maintained robust self-administration, it has been difficult to detect any effect of estradiol (Caine *et al*, 2004; Lynch and Taylor, 2005; Mello *et al*, 2008).

In clinical laboratory studies, estradiol also did not consistently enhance the subjective and reinforcing effects of cocaine or amphetamine. Often no differences in the subjective effects of cocaine or amphetamine at different phases of the menstrual cycle were detected (Lukas *et al*, 1996; Justice and de Wit, 1999, 2000a, 2000b; Mendelson *et al*, 1999; Munro *et al*, 2006; Collins *et al*, 2007). However, in studies where subjective responses to cocaine or amphetamine did vary by menstrual cycle phase, the positive subjective effects usually were greater during follicular phase, when estradiol and progesterone levels are low, than during the luteal phase, when both estradiol and progesterone levels are high (Justice and de Wit, 1999; Sofuoglu *et al*, 1999; White *et al*, 2002; Evans *et al*, 2002). These data suggested that progesterone might attenuate positive subjective responses to cocaine. When physiological doses of progesterone were administered to women during the follicular phase of the menstrual cycle, the positive subjective effects of cocaine were decreased (Sofuoglu *et al*, 2002; Evans and Foltin, 2006; see for a review, Evans, 2007; Evans and Foltin, 2010).

Preclinical studies in rodents are consistent with these clinical reports of progesterone–cocaine interactions. In normally cycling rats, cocaine-seeking was lowest during proestrus when progesterone levels were highest (Feltenstein and See, 2007). In ovariectomized rats, progesterone inhibited estradiol enhancement of acquisition of cocaine self-administration (Jackson *et al*, 2006). Both progesterone and its major metabolite, allopregnanolone, blocked reinstatement of cocaine self-administration (Anker *et al*, 2007, 2009), as well as escalation of cocaine-maintained responding (Larson *et al*, 2007; see for a review, Anker and Carroll, 2010).

Relatively little is known about testosterone–cocaine interactions and preclinical data have been inconsistent. Testosterone was reported to enhance (Martinez-Sanchis *et al*, 2002; Minerly *et al*, 2010) and to prevent cocaine-induced locomotor activity (Long *et al*, 1994). Testosterone was reinforcing in rodents under some conditions (see for a review, Wood, 2004). Testosterone delayed and reduced cocaine-related sensitization in rats (Chen *et al*, 2003).

Testosterone and progesterone share a common biosynthetic pathway and both are essential for normal reproductive function (Rupprecht, 2003; Strauss and Barbieri, 2004). However, clinical studies indicate that these hormones have different effects on mood. Progesterone is often associated with dysphoric mood, fatigue, and sedation in normally cycling and post-menopausal women (Freeman *et al*, 1992; Schechter, 1999; Andreen *et al*, 2006). Although testosterone does not have acute intoxicating effects (Fingerhood *et al*, 1997; Kanayama *et al*, 2009), during chronic treatment, it has mood-enhancing effects in oophorectomized women (Shifren *et al*, 2000) and in men with and without a history of steroid abuse (Kanayama *et al*, 2007, 2009). These clinical data suggest that if testosterone and progesterone had similar effects on the abuse-related effects of cocaine, testosterone's mood-enhancing effects might make it more acceptable to patients.

This is the first study to compare the acute effects of testosterone and progesterone on cocaine self-administration and cocaine discrimination in female rhesus monkeys. This non-human primate model of drug abuse is useful for evaluation of candidate treatment medications, and shows good concordance with clinical trials (Mello, 2005; Mello and Negus, 1996). Cocaine self-administration and drug discrimination dose–effect curves were determined after progesterone, testosterone, and placebo treatment. The reinforcing and discriminative stimulus effects of testosterone and progesterone were also evaluated. Finally, the extent to which progesterone and testosterone shared discriminative stimulus properties with cocaine, and altered cocaine discrimination was examined. These studies indicate that both testosterone and progesterone significantly reduced cocaine self-administration without altering concurrent food-maintained responding. These data suggest that further exploration of neuroactive steroid hormones for the treatment of cocaine abuse and medical management of withdrawal and risk for relapse may be warranted.

METHODS

Subjects

Studies were conducted in 13 female rhesus monkeys (*Macaca mulatta*) (4–6 kg). Five drug-naïve monkeys were studied in cocaine self-administration procedures, one was ovariectomized and four were gonadally intact. Five monkeys with a history of cocaine self-administration were studied in cocaine discrimination procedures. Three other monkeys were used to assess the time course of progesterone and testosterone after intramuscular (i.m.) injection. Monkeys were maintained on a diet of multiple vitamins, fresh fruit, and Lab Diet Jumbo Monkey biscuits (PMI Feeds, St Louis, MO). Water was continuously available. In cocaine self-administration studies, monkeys also worked at an operant task for 1 g banana-flavored pellets (Precision Primate Pellets Formula L/I Banana Flavor, PJ Noyes, Lancaster, NH) during three daily sessions of food availability (see below). In drug discrimination studies, each monkey was maintained on a diet of 8–15 monkey biscuits and one piece of fresh fruit per day. During the week, all food was delivered after the experimental session, whereas on weekends, food was delivered between 0900 hours and noon. A 12-h light–dark cycle was in effect (lights on from 0700hours to 1700 hours).

Animal maintenance and research were conducted in accordance with the guidelines provided by the NIH Committee on Laboratory Animal Resources (ILAR-NRC, 1996). The facility was licensed by the United States Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. Monkeys had visual, auditory, and olfactory contact with other monkeys throughout the study. Environmental enrichment was provided by operant food and drug self-administration procedures, foraging toys and music, and

nature videotapes (Line, 1987). The health of the monkeys was periodically monitored by consulting veterinarians.

Time Course of Progesterone and Testosterone in Cyclodextrin

We measured the time course of plasma levels of progesterone (0.1, 0.2, and 0.3 mg/kg, i.m.) and testosterone (0.001, 0.0032, and 0.01 mg/kg, i.m.) to determine if hormone levels remained elevated for the duration of the cocaine self-administration and cocaine discrimination sessions described below. Samples for analyses were collected at 5-min intervals for 120 min in three awake gonadally intact female monkeys using procedures described previously (Mello *et al*, 1993, 2000, 2008).

Cocaine Self-Administration Procedures

Surgical procedures. Surgical implantation of intravenous catheters for cocaine self-administration was performed under aseptic conditions and isoflurane (1–2% in oxygen) anesthesia. A double-lumen Silicone rubber catheter (inside diameter 0.028 in; outside diameter 0.088 in; Saint Gobain Performance Plastics, Beaverton, MI) was implanted in the internal jugular or femoral vein and exited in the mid-scapular region. After surgery, Procaine Penicillin G (300 000 U/kg per day, i.m.) was given for 5 days, and analgesic doses of buprenorphine (0.032 mg/kg, i.m.) were administered twice daily for 3 days. The intravenous catheter was protected by a custom-fitted nylon vest connected to a flexible stainless-steel cable and fluid swivel (Lomir Biomedical, Malone, NY) that permitted the monkeys to move freely. Ketamine (5 mg/kg, intravenously (i.v.)) was administered through the catheter lumen to determine if the catheter was patent as indicated by a loss of muscle tone within 10 s.

Apparatus and behavioral procedures. Each monkey lived in a well-ventilated stainless-steel chamber ($66 \times 76 \times 94\ cm^3$) equipped with a custom-designed operant panel ($28 \times 28\ cm^2$). Colored stimulus lights projected on the center response key signaled the availability of food, a 1 g banana-flavored pellet (red), or the maintenance dose of cocaine (green) (0.10 mg/kg, i.v.). Behavioral sessions were conducted daily, 7 days a week. Schedules of reinforcement (fixed ratio 30 (FR 30)) and data collection were controlled by microprocessors and software purchased from Med Associates (Georgia, VT).

Cocaine self-administration procedures were identical to those described previously in studies of the effects of estradiol on the abuse-related effects of cocaine (Mello *et al*, 2008). Each 2-h cocaine self-administration session consisted of four 20-min response periods, each separated by a 10-min time-out period. A priming injection of the unit dose of cocaine or saline available for self-administration occurred at the beginning of each of the four components. During the post-injection time-outs (60 s) and time-outs between session components, the response key was yellow. The number of cocaine injections was limited to 20 in each 20-min component of the session, or a total of 80 injections over the 2-h session. In addition to the daily cocaine self-administration session, there were three daily 1-h food sessions. Food was available on an FR 30 schedule of reinforcement, and the number of pellets was limited to 33 in each 1-h session. Cocaine sessions began at 1100 hours and food sessions began at 1600, 2000, and 0800 hours, and occurred every day, 7 days a week.

Cocaine dose–effect curve determinations. During training, cocaine availability was alternated with saline for 2 or 3 days or until saline reliably maintained low levels of responding. After cocaine self-administration was stable on an FR 30 schedule, cocaine dose–effect curves were determined by substituting saline or cocaine (0.001–0.30 mg/kg per inj.) for the cocaine-training dose (0.10 mg/kg per inj.). A different sequence of cocaine doses was available for each monkey, and dose–effect curves were determined in the same order for a given monkey under each hormone treatment condition. During test sessions, an unlimited number of cocaine or saline injections were available to avoid a ceiling effect. Usually two test sessions were run each week on Tuesdays and Fridays. The same behavioral procedure was used to determine if progesterone (0.00001–0.01 mg/kg per inj., i.v.) or testosterone (0.0001–0.01 mg/kg per inj., i.v.) maintained responding leading to its self-administration in cocaine-trained monkeys.

Hormone and saline treatment procedures. In drug self-administration studies, after baseline cocaine dose–effect curves were determined during saline treatment, progesterone (0.1, 0.2, and 0.3 mg/kg, i.m.) or testosterone (0.001, 0.0032, and 0.01 mg/kg, i.m.) was administered i.m. 30 min before the cocaine self-administration session began. After each test session, activity/sedation was rated on a simple scale (Butelman *et al*, 1999) by the same trained observer, then monkeys were briefly anesthetized with a low dose of ketamine (3 mg/kg, i.m.) and blood samples (1.2 ml in a heparinized tube) were collected to determine hormone levels following the cocaine dose–effect curve determinations. Samples were centrifuged, and plasma was stored at $-70\ ^{\circ}F$ until analysis.

Cocaine Discrimination

Apparatus and behavioral procedures. Each monkey was housed individually in a stainless-steel chamber ($56 \times 71 \times 69\ cm^3$) equipped with a computer-controlled operant panel and food pellet dispenser. Discrimination training and testing procedures were identical to those described previously (Mello *et al*, 2007). Training sessions consisted of 1–5 cycles, and each cycle consisted of a 15-min time-out period, followed by a 5-min response period. During the time-out, all stimulus lights were off, and responding had no scheduled consequences. During the response period, the right and left response keys were transilluminated red or green, and monkeys could earn up to 10 food pellets under an FR 30 schedule. On training days, monkeys received saline or 0.18 mg/kg cocaine i.m. at the beginning of each cycle. Following saline administration, responding on only the green, saline-appropriate key produced food, and following 0.18 mg/kg cocaine administration, responding on only the red, drug-appropriate key produced food. Responses on the inappropriate key reset the FR requirement on the appropriate key. If the training

dose of cocaine was administered, it was administered only during the last cycle. The principal dependent variables were (a) percent injection-appropriate responses before delivery of the first reinforcer, (b) percent injection-appropriate responses for the entire cycle, and (c) response rate in responses per s. Training was complete when the following criteria were met during each cycle for 7 of 8 consecutive training sessions: (1) $\geqslant$80% injection-appropriate responding before the first reinforcer; (2) $\geqslant$90% injection-appropriate responding for the entire cycle; and (3) $\geqslant$0.5 responses per s during saline training cycles.

Test compounds were studied using a substitution or pretreatment protocol. For *substitution tests*, increasing doses of hormone in a cyclodextrin vehicle were administered at the beginning of each successive cycle, and each successive dose increased the total cumulative dose by 0.5 log units. Dose–effect curves for each drug were determined twice with overlapping dose ranges offset by 0.25 log units.

In *pretreatment tests*, saline or testosterone or progesterone in cyclodextrin was administered 30 min before determination of a cumulative cocaine discrimination dose–effect curve. A second procedure was designed to assess the slower-onset, longer-acting effects of hormones by suspending each hormone in sesame oil over 3 days. On day 0, if animals met the performance criteria described above, then a single dose of vehicle or hormone in sesame oil was administered within 1 h after the session. On day 1, approximately 22 h after the first sesame oil + hormone treatment, a cumulative cocaine dose–effect curve was determined. A second identical dose of the sesame oil + hormone was administered after the test session. On day 2, approximately 22 h after the second treatment, another cumulative cocaine dose–effect curve was determined. A control cocaine dose–effect curve was determined before each test with vehicle or hormone.

Data analysis. A test drug was considered to substitute for cocaine if it produced $\geqslant$90% cocaine-appropriate responding. Cocaine ED_{50} values were calculated by log–linear interpolation as the dose of cocaine that produced 50% cocaine-appropriate responding. In pretreatment studies, a drug was considered to alter the cocaine discrimination dose–effect curve if the 95% confidence limits of the control ED_{50} did not overlap with the 95% confidence limits of the test ED_{50}.

Gonadal Steroid Hormone Assay Procedures

Progesterone. Plasma concentrations of progesterone were determined in duplicate using a direct, double-antibody radioimmunoassay (RIA) (MP Biomedicals, LLC, Solon, OH). The assay sensitivity was 0.12 ng/ml and the intra- and inter-assay confidence intervals were 8.3% and 10.2%, respectively.

Testosterone. Plasma concentrations of testosterone were determined in duplicate using a direct, double-antibody RIA (MP Biomedicals). The assay sensitivity was 1.3 ng/dl and the intra- and inter-assay confidence intervals were 9.5% and 10.5%, respectively.

Estradiol. Plasma concentrations of estradiol were determined in duplicate by a direct, double-antibody RIA (MP Biomedicals). A modification was made to the protocol: before assay, the plasma samples were extracted, and then reconstituted in zero standard. The assay sensitivity was 4.8 pg/ml and the intra- and interassay confidence intervals were 11.5% and 12.0%, respectively.

Drugs. Cocaine HCl was obtained from the National Institute on Drug Abuse (NIH, Bethesda, MD) and was dissolved in sterile saline. Progesterone and testosterone propionate were purchased from Sigma Chemical (St Louis, MO) and were dissolved in 40% γ-cyclodextrin (Wacker Chemie, AG, Munich, Germany) or in sesame oil (Sigma Chemical). All drug solutions used for intravenous administration were filter-sterilized using a 0.22 μm Millipore filter and stored in pyrogen-free vials. Doses were calculated using the drug forms given above.

To mimic the rapid onset, non-genomic actions of the neuroactive steroid hormones (Wong *et al*, 1996; Moore and Evans, 1999; Falkenstein *et al*, 2000; Vasudevan and Pfaff, 2007), progesterone and testosterone were prepared in a cyclodextrin vehicle for cocaine self-administration studies. In drug discrimination studies, hormone treatment in cyclodextrin and in sesame oil was compared to determine if these vehicles produced different effects.

RESULTS

Time Course of Progesterone and Testosterone after I.M. Administration

The time course of plasma levels of progesterone (0.1–0.3 mg/kg, i.m.) and testosterone (0.001–0.01 mg/kg, i.m.) in cyclodextrin after acute administration are shown in Figure 1. Each hormone produced significant dose-dependent increases in plasma levels within 5 or 10 min after i.m. administration. Peak levels were detected within 40 min. Both progesterone and testosterone levels remained significantly above baseline throughout the 120 min sampling period ($P<0.05$), except at the lowest dose.

Cocaine Self-Administration Dose–Effect Curves During Progesterone and Testosterone Treatment

Progesterone and testosterone had very similar effects on cocaine self-administration dose–effect curves. Figure 2 shows data for groups of 4 or 5 female monkeys. Data obtained in the ovariectomized animal were comparable to intact animals, so these data were included in the group analyses. During saline control treatment, a unit dose of 0.01 mg/kg cocaine was at the peak of the dose–effect curve and monkeys earned an average of 43.6 ± 2.9 cocaine injections. When saline was available for self-administration, monkeys earned an average of 9.2 ± 3–10 ± 3.3 injections during progesterone treatment and 4.6 ± 1.9–7.0 ± 2.7 injections during testosterone treatment and 13.2 ± 4.3 injections during saline treatment. Administration of progesterone produced a dose-dependent decrease in cocaine self-administration ($P<0.001$, $F=7.10$). Decreases in self-administration of 0.01 mg/kg per inj. cocaine were significantly different from control levels after

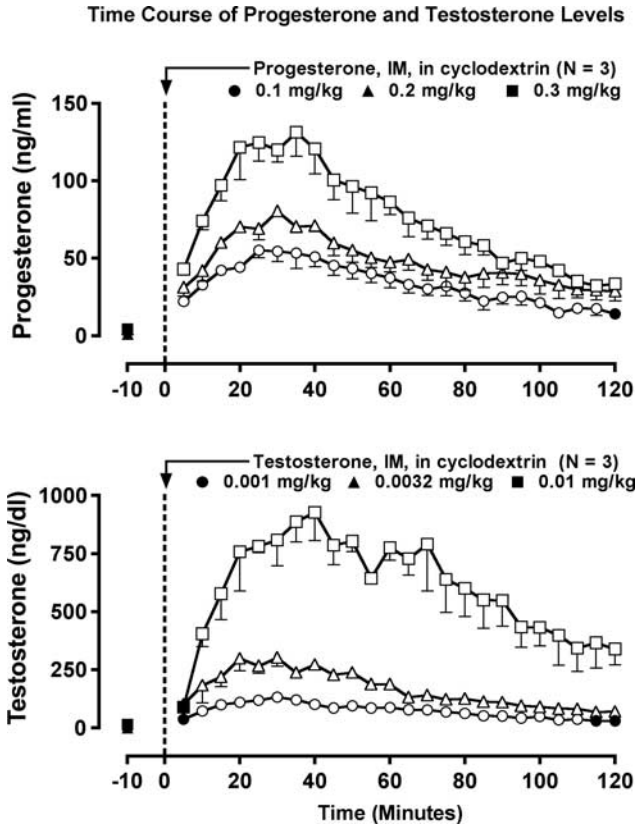

Figure 1 Time course of plasma levels of progesterone and testosterone in a cyclodextrin vehicle after intramuscular (i.m.) injection. Progesterone: the top panel shows the time course of 0.10 (circles), 0.20 (triangles), or 0.30 (squares) mg/kg progesterone, i.m. Testosterone: the bottom panel shows the time course of 0.001 (circles), 0.0032 (triangles), and 0.01 (squares) mg/kg testosterone, i.m.. The time of injection is indicated by the vertical dotted line at time zero. Abscissae: time after injection. Ordinates: plasma levels of progesterone (ng/ml) or testosterone (ng/dl). One-way analysis of variance (ANOVA) for repeated measures indicated a significant main effect of hormone injection on plasma levels at all doses of progesterone ($P<0.0001$, $F=21.6$, 14.3, and 27.2 at 0.10, 0.20, and 0.30 mg/kg, and all doses of testosterone ($P<0.0001$, $F=15.4$, 10.3, and 13.3 at the 0.001, 0.0032, and 0.01 ng/dl). Open symbols represent time points at which hormone levels were significantly higher than baseline (Dunnett's post-test, $P<0.01$–0.05).

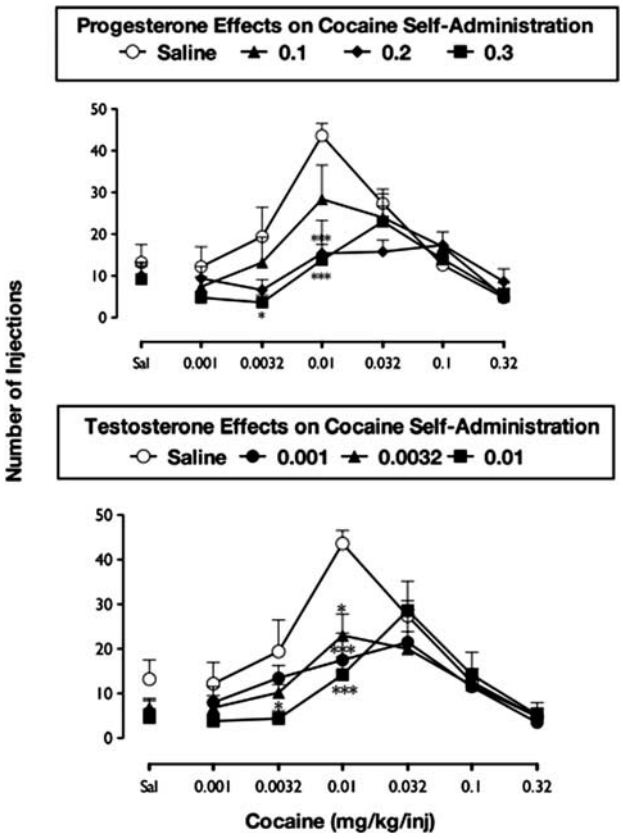

Figure 2 The effects of saline, progesterone, or testosterone on the cocaine self-administration dose–effect curve in a group of four or five female rhesus monkeys. The unit dose of cocaine available for self-administration (mg/kg per inj., i.v.) is shown on the abscissae. The number of cocaine or saline injections is shown on the left ordinate. The number of injections when only saline was available is shown above saline at the left of the graph. The symbol for saline or the pretreatment dose of progesterone or testosterone is shown above each row. Two-way analysis of variance (ANOVA) for repeated measures indicated a significant main effect of progesterone ($P<0.001$, $F=7.10$) and testosterone ($P<0.001$, $F=7.52$) on cocaine self-administration. Dunnett's post-test indicated points that were significantly different from the saline control treatment (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

treatment with 0.2 and 0.3 mg/kg progesterone ($P<0.001$). The highest dose of progesterone shifted the peak of the cocaine self-administration dose–effect curve down and $\frac{1}{2}$ log unit to the right.

Administration of testosterone also produced a dose-dependent decrease in cocaine self-administration ($P<0.001$). Decreases in self-administration of 0.01 mg/kg per inj. cocaine were significantly different from control levels after treatment with 0.001, 0.0032, and 0.01 mg/kg testosterone ($P<0.05$, <0.001). The highest dose of testosterone shifted the peak of the cocaine self-administration dose–effect curve downwards and $\frac{1}{2}$ log unit to the right.

Food-Maintained Responding During Progesterone and Testosterone Treatment

Food-maintained responding did not vary as a function of increasing cocaine doses during saline control treatment (Figure 3). Food-maintained responding also did not change significantly during treatment with increasing doses of progesterone and testosterone. These data suggest that the effect of these neuroactive steroid hormones were selective for cocaine and were not associated with a general disruption of operant responding.

Plasma Levels of Progesterone, Testosterone, and Estradiol after Test Sessions

Baseline hormone levels during saline treatment were at follicular phase levels and averaged 3.74 ± 1.52 ng/ml for progesterone and 1.40 ± 0.41 ng/dl for testosterone. Figure 4 shows average levels of progesterone (ng/ml), testosterone (ng/dl), and estradiol (pg/ml) measured at the end of the test session, approximately 2 h and 45 min after hormone administration. There was a dose-related increase in average progesterone and testosterone levels, but estradiol

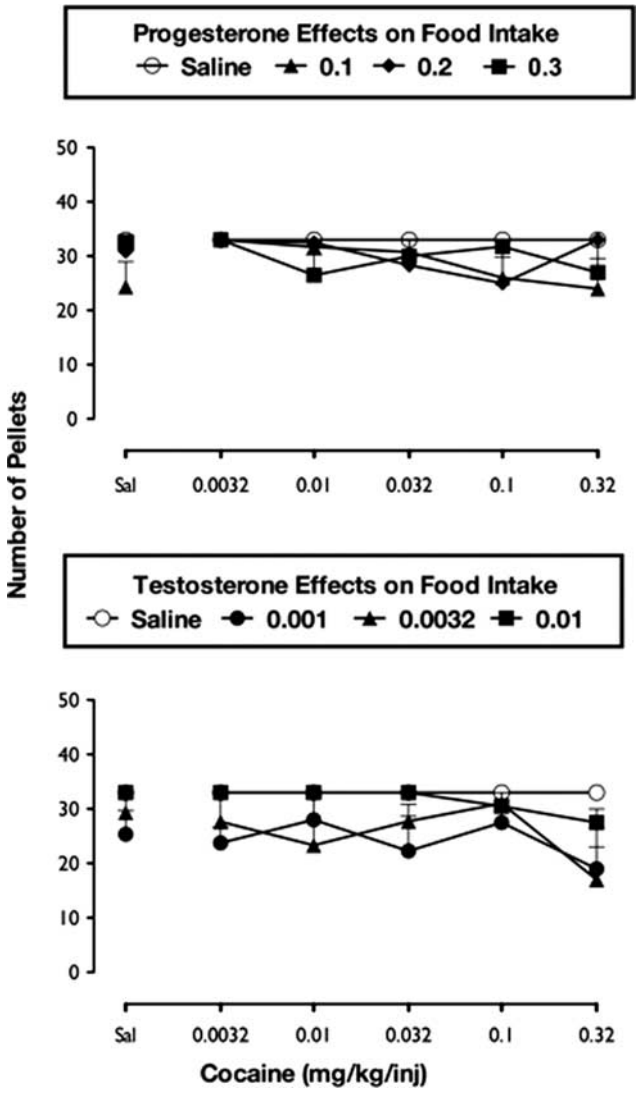

Figure 3 The effects of saline, progesterone, or testosterone on food-maintained responding across the cocaine self-administration dose–effect curve. The unit dose of cocaine available for self-administration (mg/kg per inj., i.v.) is shown on the abscissae. The number of banana-flavored food pellets (1 g) taken during the first 1 h food session after saline or hormone administration is shown on the left ordinate. The number of food pellets taken when only saline was available for self-administration is shown above saline at the left of the graph. The symbol for saline or the pretreatment dose of progesterone or testosterone is shown above each row. Two-way analysis of variance (ANOVA) for repeated measures indicated no significant main effect of progesterone ($P > 0.05$, $F = 3.529$) or testosterone ($P > 0.05$, $F = 3.835$) on food-maintained responding.

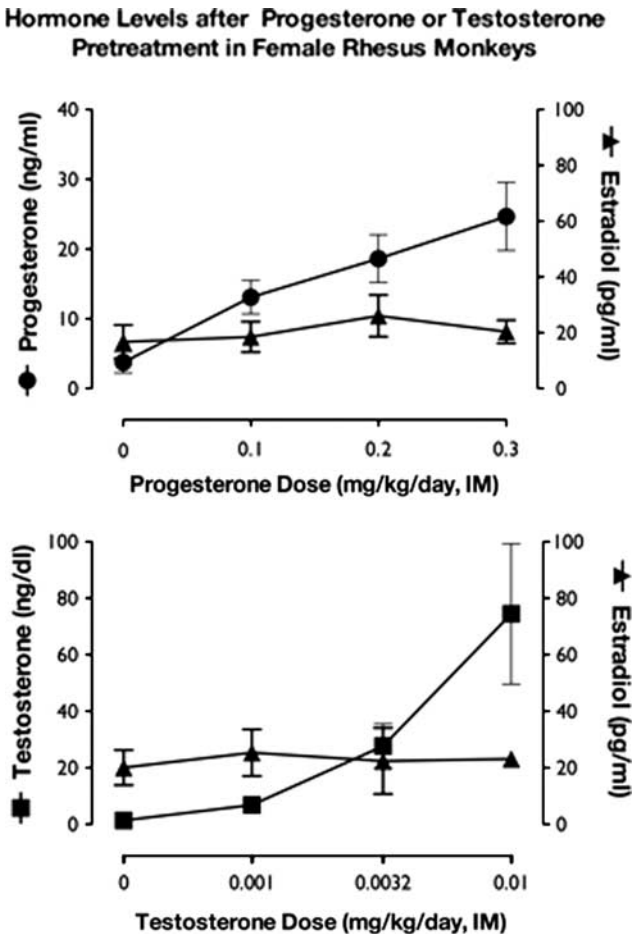

Figure 4 Average plasma hormone levels after progesterone or testosterone pretreatment in female rhesus monkeys. Abscissae: dose of progesterone or testosterone (mg/kg, intramuscular (i.m.)). Left ordinate: plasma levels of progesterone (ng/ml) or testosterone (ng/dl). Right ordinate: Plasma levels of estradiol (pg/ml). Each data point is the average of 3–5 rhesus females.

remained at follicular phase levels (Mello *et al*, 1997) and did not change significantly from baseline. Progesterone levels were within the range measured during the early and late luteal phase in female rhesus monkeys (Mello *et al*, 1997). Testosterone levels were lower than basal levels measured in male rhesus monkeys (Mello *et al*, 2000).

Progesterone or Testosterone Self-Administration

Three monkeys were tested to determine if progesterone or testosterone would maintain responding leading to its self-administration. Neither progesterone (0.00001–0.01 mg/kg per inj., i.v.) nor testosterone (0.0001–0.01 mg/kg per inj., i.v.) maintained responding above saline self-administration levels. Fewer than 13 injections per 2 h session were consistently observed at the doses of progesterone and testosterone studied (data not shown).

Cocaine Discrimination: Progesterone or Testosterone Substitution Studies

Three monkeys were tested in substitution studies with progesterone or testosterone in a cyclodextrin vehicle (Table 1). On training days throughout the course of these studies, monkeys responded almost exclusively on the saline-appropriate key during saline training cycles (mean % saline-appropriate responding ± SEM = 99.99 ± 0.01%) and almost exclusively on the cocaine-appropriate key during cocaine training cycles (mean % cocaine-appropriate responding ± SEM = 99.94 ± 0.06%). Mean response rates ± SEM were 1.88 ± 0.32 responses per second during saline training cycles and 2.11 ± 0.15 responses per second during cocaine training cycles. Thus, there was a tendency

for the training dose of cocaine (0.18 mg/kg) to increase response rates relative to saline treatment.

Cocaine produced a dose-dependent increase in cocaine-appropriate responding, and the control ED_{50} value is shown in Table 1. Neither progesterone (0.032–0.32 mg/kg, i.m.) nor testosterone (0.0001–0.01 mg/kg, i.m.) in cyclodextrin produced greater than 1% cocaine-appropriate responding (data not shown). Progesterone and testosterone also had little effect on response rates across the dose ranges tested. ED_{50} values for cocaine on days 1 and 2 following pretreatment with the oil vehicle and hormones dissolved in oil are shown in Table 2. Neither hormone significantly altered the discriminative stimulus effects of cocaine on either the first or second day of hormone treatment.

Progesterone or Testosterone Effects on Cocaine Discrimination Dose–Effect Curves

As noted above, cocaine increased response rates slightly at the training dose of 0.18 mg/kg, and a higher dose of 0.56 mg/kg tended to decrease response rates (decreases were observed in two of three monkeys). Pretreatment with progesterone or testosterone in cyclodextrin had little effect on the overall pattern of cocaine's rate-altering effects (Figure 5). In one exception to this general finding, the highest dose of cocaine (0.56 mg/kg) did not decrease response rates in any monkey after pretreatment with the highest progesterone dose (0.32 mg/kg).

Table 1 ED_{50} Values in mg/kg (95% CL) for Cocaine Administered Alone or 30 min after Pretreatment with Progesterone or Testosterone in Cyclodextrin Vehicle

Treatment	ED_{50} (95% CL)
Cocaine alone	0.074 (0.039–0.14)
+0.032 Progesterone	0.068 (0.032–0.14)
+0.10 Progesterone	0.068 (0.032–0.14)
+0.32 Progesterone	0.088 (0.031–0.25)
+0.001 Testosterone	0.084 (0.059–0.12)
+0.0032 Testosterone	0.065 (0.031–0.14)
+0.01 Testosterone	0.078 (0.032–0.10)

Figure 6 shows the effects of pretreatment with progesterone and testosterone dissolved in sesame oil on cocaine discrimination dose–effect curves. Neither hormone significantly altered the discriminative stimulus effects of cocaine. The lower panel of Figure 6 shows the rate-altering effects of cocaine administered alone or after pretreatment with gonadal steroid hormones. Again the highest dose of progesterone (0.32 mg/kg) decreased the rate-suppressing effects of the highest dose of cocaine (0.56 mg/kg).

DISCUSSION

This is the first study of the acute effects of progesterone and testosterone administration on cocaine's reinforcing and discriminative stimulus effects in female rhesus monkeys. The major findings are: (1) both progesterone

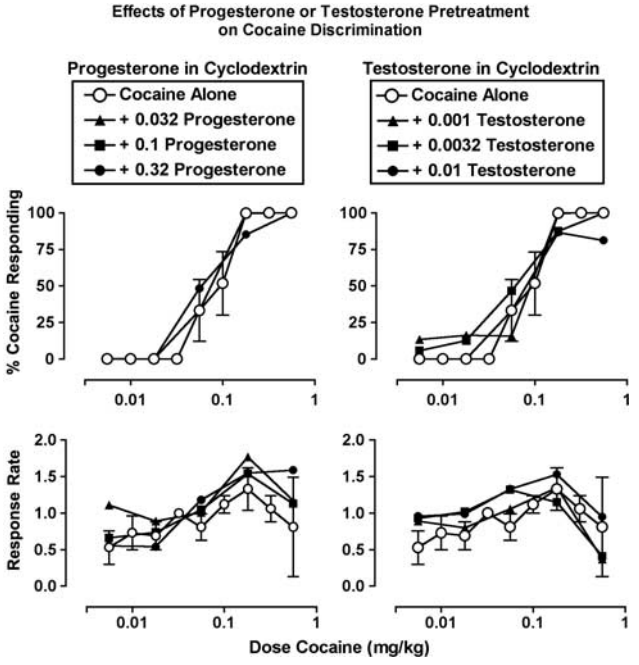

Figure 5 Effects of progesterone or testosterone in a cyclodextrin vehicle on the discriminative stimulus and rate-altering effects of cocaine. Abscissae: Dose cocaine in mg/kg. Ordinates: Percent cocaine appropriate responding (top) or response rate in responses per s (bottom). Each point shows mean data ± SEM in three monkeys.

Table 2 ED_{50} Values in mg/kg (95% CL) for Cocaine Administered Alone or after 1 or 2 Days of Treatment with Sesame Oil Vehicle or Progesterone or Testosterone in Sesame Oil Vehicle

Treatment	Control	Day 1	Day 2
Sesame oil vehicle	0.075 (0.043–0.13)	0.057 (0.031–0.11)	0.057 (0.030–0.11)
+0.032 Progesterone	0.050 (0.029–0.086)	0.037 (0.019–0.074)	0.066 (0.029–0.15)
+0.10 Progesterone	0.10 (0.10–0.10)	0.057 (0.030–0.11)	0.032 (0.006–0.16)
+0.32 Progesterone	0.057 (0.030–0.11)	0.057 (0.030–0.11)	0.10 (0.040–0.25)
+0.001 Testosterone	0.044 (0.026–0.076)	0.053 (0.025–0.11)	0.049 (0.027–0.090)
+0.0032 Testosterone	0.061 (0.035–0.11)	0.10 (0.098–0.11)	0.074 (0.043–0.13)
+0.01 Testosterone	0.048 (0.028–0.082)	0.055 (0.030–0.1)	0.058 (0.032–0.11)

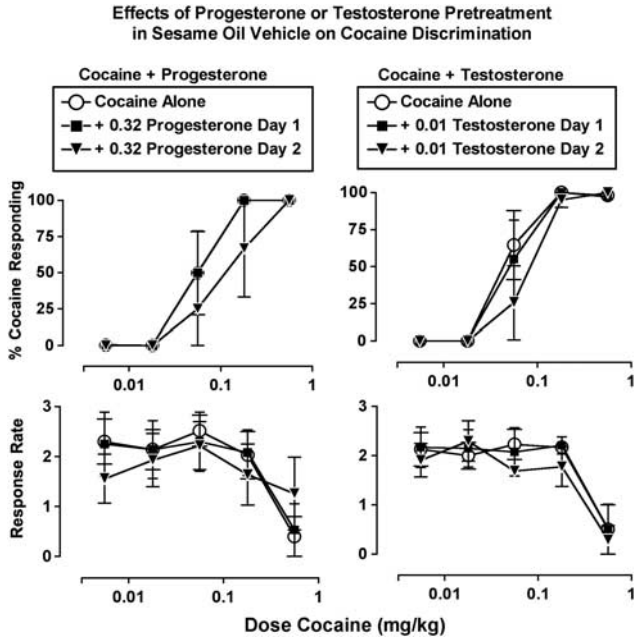

Figure 6 Effects of progesterone or testosterone in a sesame oil vehicle on the discriminative stimulus and rate-altering effects of cocaine. Abscissae: Dose cocaine in mg/kg. Ordinates: Percent cocaine appropriate responding (top) or response rate in responses per s (bottom). Each point shows mean data ± SEM in four monkeys.

and testosterone produced a significant dose-dependent decrease in cocaine self-administration and shifted the cocaine dose–effect curve downwards and to the right; (2) progesterone and testosterone did not alter food-maintained responding, so the effects were selective for cocaine self-administration and did not reflect a disruption of operant responding; (3) progesterone and testosterone did not substitute for cocaine, and did not maintain responding leading to hormone self-administration; (4) progesterone and testosterone also did not substitute for cocaine in a drug discrimination procedure; and (5) progesterone and testosterone pretreatment did not significantly alter the cocaine discrimination dose–effect curve. The relation of these findings to the clinical and preclinical literature, and some possible implications for medication-based treatment of cocaine abuse and risk for relapse, are discussed below.

Interactions Between Cocaine and Neuroactive Steroid Hormones

Progesterone and the abuse-related effects of cocaine. These data in female rhesus monkeys are concordant with clinical laboratory studies of the effects of progesterone on positive subjective responses to cocaine (Sofuoglu *et al*, 1999, 2002; Evans and Foltin, 2006; Evans, 2007). In one illustrative study, women with a current history of cocaine abuse were given up to six doses of smoked cocaine (6, 12, or 25 mg) during their normal follicular and luteal phases (Evans and Foltin, 2006). During a subsequent follicular phase, the same women were given a single oral dose of progesterone that produced plasma levels comparable to their natural luteal phase (Evans and Foltin, 2006). Under these conditions, progesterone-attenuated positive subjective responses to cocaine in comparison to the normal follicular phase when progesterone levels were low (Evans and Foltin, 2006). Similar effects of progesterone were reported in women after a single dose of smoked cocaine (0.4 mg/kg) and i.v. cocaine (0.3 mg/kg) (Sofuoglu *et al*, 2002, 2004). In addition, both cue and stress-induced cocaine craving and anxiety were lower during the luteal phase when progesterone levels were high, than during the follicular phase (Sinha *et al*, 2007). These findings may have implications for treatment because cocaine craving, associated with stress and concomitant increases in hypothalamic-pituitary-adrenal hormones, is thought to be a critical determinant of relapse (Sinha, 2001; Sinha *et al*, 2003, 2006). Interestingly, progesterone did not significantly attenuate subjective responses to cocaine in men (Sofuoglu *et al*, 2004; Evans and Foltin, 2006; see for a review, Evans and Foltin, 2010). Progesterone also did not significantly reduce cocaine-positive urines over a 10-week placebo-controlled trial in methadone-stabilized, opioid-dependent men who abused cocaine (Sofuoglu *et al*, 2007). Sex differences in subjective responses to cocaine appear to be determined in part by the route of cocaine administration (see for a review, Mello and Mendelson, 2009; Evans and Foltin, 2010).

Findings from clinical laboratory studies of progesterone effects on cocaine self-administration have been less consistent than studies of subjective responses to cocaine. Two studies reported no effect of progesterone on the number of cocaine injections self-administered (Sofuoglu *et al*, 2004; Reed *et al*, 2011). In another study, progesterone decreased self-administration of smoked cocaine in women (Evans, 2007; Evans and Foltin, 2010). It is often the case that decreases in positive subjective effects may not predict decreases in drug self-administration (Sofuoglu *et al*, 2004; Haney and Spealman, 2008).

The effects of progesterone on cocaine self-administration by rhesus monkeys in this study are consistent with preclinical studies in rodents. Progesterone reduced acquisition of cocaine self-administration (Jackson *et al*, 2006; Yang *et al*, 2007) and escalation of cocaine self-administration during periods of extended access to cocaine in female rats (Larson *et al*, 2007). Similarly, in a cocaine-primed reinstatement paradigm, progesterone treatment attenuated responding during estrus, but not during proestrus or diestrus phases of the estrous cycle in gonadally intact female rats (Feltenstein *et al*, 2009). Progesterone treatment also reduced cocaine-primed reinstatement in ovariectomized rats after estradiol replacement (Anker *et al*, 2007).

Testosterone and cocaine interactions. In comparison to progesterone and estradiol, relatively little is known about the effects of testosterone on the abuse-related effects of cocaine. We were unable to locate any clinical studies of the interactions between cocaine and testosterone. As noted earlier, findings from preclinical studies of the effects of testosterone on cocaine-related increases in locomotor activity in rodents were inconsistent (Long *et al*, 1994; Martinez-Sanchis *et al*, 2002; Minerly *et al*, 2010). In this study, testosterone, like progesterone, produced a significant dose-dependent decrease in cocaine self-administration in female rhesus monkeys. Food-maintained responding was not altered by testosterone, indicating that these effects were selective for cocaine, and did not reflect a

disruption of operant responding. To the best of our knowledge, this is the first report that testosterone reduces cocaine self-administration in non-human primates. These findings are consistent with a report that testosterone delayed and reduced cocaine-related sensitization in rats (Chen *et al*, 2003). As discussed below, the mechanisms by which testosterone and progesterone altered cocaine self-administration are unclear.

Self-administration of neuroactive steroid hormones. The abuse-related effects of neuroactive steroids in non-human primates have received little scientific attention. It is unlikely that progesterone and testosterone reduced cocaine self-administration by substituting for cocaine's reinforcing effects. Progesterone did not maintain self-administration in rhesus monkeys, and this finding is consistent with its negative subjective effects in clinical studies (Soderpalm *et al*, 2004; Goletiani *et al*, 2007). However, pregnenolone, a precursor of progesterone and testosterone, had marginal reinforcing effects in male monkeys when substituted for the short-acting barbiturate, methohexital (Rowlett *et al*, 1999). Testosterone was not self-administered by non-human primates in this study, and clinical studies indicate that testosterone does not have acute intoxicating effects (Fingerhood *et al*, 1997; Kanayama *et al*, 2009). The clinical profile of testosterone is very different from that of progesterone. Testosterone is often self-administered by body builders (Kanayama *et al*, 2009; Pope and Brower, 2000) and is effective in reducing depression, and increasing general feelings of well-being and libido in oophorectomized women (Shifren *et al*, 2000) and elderly men (Pope *et al*, 2000; Kanayama *et al*, 2007).

In contrast to our findings in non-human primates, testosterone is a weak reinforcer in rats and hamsters (see for a review, Wood, 2004). Testosterone maintained modest levels of oral, i.v., and intracerebroventricular self-administration on an FR1 schedule in male rats and in male and female hamsters (Johnson and Wood, 2001a, b; Triemstra and Wood, 2004; Wood, 2004; Wood *et al*, 2004), and induced conditioned place preference in rodents (de Beun *et al*, 1992; Schroeder and Packard, 2000; Frye *et al*, 2002; Parrilla-Carrero *et al*, 2009). Testosterone injection into the nucleus accumbens also induced conditioned place preference, and this effect could be blocked by D1 and D2 antagonists (Packard *et al*, 1997; Schroeder and Packard, 2000). Moreover, this effect was produced only by stimulation of the shell, not the core, of the nucleus accumbens (Frye *et al*, 2002), but not under all conditions (Triemstra *et al*, 2008). These findings have been interpreted as evidence for non-genomic stimulation of dopaminergic activity by testosterone (Wood, 2004). The effects of progesterone and testosterone on dopamine synthesis and release in non-human primates are unknown.

Progesterone and testosterone effects on cocaine discrimination. Progesterone and testosterone did not alter the discriminative stimulus effects of cocaine at the same doses, and by the same route of administration that reduced cocaine self-administration in this study. Taken together, these data suggest that progesterone and testosterone did not interfere with the monkey's ability to recognize cocaine, but did decrease its reinforcing properties. However, progesterone and testosterone alone did not produce cocaine-like discriminative stimulus effects in female rhesus monkeys, and neither hormone maintained self-administration above saline levels. Progesterone often shares discriminative stimulus effects with drugs that act as general depressants. For example, progesterone produced pentobarbital-like discriminative stimulus effects in ovariectomized, but not in gonadally intact female or male rats (Heinsbroek *et al*, 1987). In monkeys, allopregnanolone, a major metabolite of progesterone, produced ethanol-like discriminative stimulus effects and the degree of substitution varied across menstrual cycle phase (Grant *et al*, 1997).

Strengths and limitations of this study. A strength of this study is that multiple doses of progesterone and testosterone were evaluated against cocaine self-administration dose–effect curves. Progesterone and testosterone both produced dose-dependent rightward and downward shifts in the cocaine self-administration dose–effect function. Controls for possible confounding effects of disruption of operant responding and sedation are consistent with the conclusion that the effects of progesterone and testosterone were selective for cocaine. For example, although progesterone often has sedative effects expressed as fatigue and drowsiness in clinical studies, it is unlikely that sedation accounted for progesterone's effects on cocaine self-administration in this study. Ratings on a sedation scale immediately following cocaine self-administration sessions indicated that monkeys were alert, responsive to the investigator, and would take food treats. Food-maintained responding also was not affected by progesterone and testosterone. We conclude that across the dose range studied, progesterone and testosterone did not produce significant sedative effects that compromised operant performance.

One limitation of this study is that only females were studied, and the extent to which these findings generalize to males is not known. As noted earlier, clinical evidence suggests that attenuation of the positive subjective effects and reinforcing effects of cocaine by progesterone is limited to females (Sofuoglu *et al*, 2002, 2007; Evans and Foltin, 2006, 2010; Evans, 2007). Interpretation of apparent sex differences in medication effects on cocaine is complicated by inconsistencies in sex differences in response to cocaine alone (Mello and Mendelson, 2002, 2009). The route of cocaine administration is one important determinant of sex differences in clinical studies (Collins *et al*, 2007; Evans and Foltin, 2010). How the effects of testosterone on the abuse-related effects of cocaine may differ as a function of sex remains to be determined.

Mechanisms of Neuroactive Steroid Actions and Implications for Cocaine Abuse Treatment

The mechanisms by which these neuroactive steroid hormones may decrease cocaine self-administration in rhesus females, cocaine seeking in rodents, and positive subjective reactions to cocaine in women are unclear. Some possibilities are considered below and are discussed in terms of progesterone's interactions with cocaine. Although the effects of progesterone and testosterone on the abuse-related effects of cocaine were very similar in this study, few

relevant studies of testosterone interactions with cocaine are available (see for a review, Wood, 2004).

Progesterone's effects on cocaine may reflect its actions as a $GABA_A$ modulator. Progesterone, and its metabolite allopregnanolone, are positive allosteric modulators of $GABA_A$ receptors (Reddy, 2003; Eser *et al*, 2006; Schumacher *et al*, 2007). $GABA_A$ receptor agonists and receptor modulators inhibit the activity of mesolimbic dopamine neurons that mediate the abuse-related effects of cocaine, and selectively reduced cocaine self-administration by rodents (Goeders *et al*, 1993; Roberts *et al*, 1996; Roberts and Andrews, 1997; Brebner *et al*, 2000). However, as in this study, reduction of cocaine self-administration did not predict effects on cocaine discrimination. For example, the high-efficacy $GABA_A$ modulator, triazolam, dose dependently reduced cocaine's discriminative stimulus effects, but the low-efficacy $GABA_A$ modulator, imidazenil, and the direct GABA agonist, muscimol, had no significant effect (Negus *et al*, 2000). Progesterone is a neuroactive neurosteroid and interacts with a broad range of receptors, including serotonin, NMDA, sigma, nicotine, and muscarinic receptors (Do Rego *et al*, 2009), so there is probably no simple explanation for its effects on the abuse-related effects of cocaine.

It is unlikely that estradiol significantly influenced the effects of progesterone or testosterone on cocaine self-administration, because estradiol levels did not change appreciably as progesterone and testosterone levels increased. Moreover, data from behavioral studies of estradiol–cocaine interactions have been inconsistent. Estradiol enhanced the abuse-related effects of low doses of cocaine in rodents under some conditions (see for a review, Lynch *et al*, 2002; Mello and Mendelson, 2002; Carroll *et al*, 2004). However, other studies have found no effect of estradiol on cocaine self-administration dose–effect curves in rats (Caine *et al*, 2004) and female rhesus monkeys (Mello *et al*, 2008). Although progesterone antagonizes the effects of estradiol under several conditions (Dierschke *et al*, 1973; Wildt *et al*, 1981; Van Vugt *et al*, 1992; Clark and Mani, 1994), this is unlikely to account for progesterone's effects on the abuse-related effects of cocaine.

In conclusion, the consistency of progesterone's effects on the abuse-related effects of cocaine in humans, non-human primates and rodents, and the parallel effects of testosterone in the present study, suggests that these neuroactive steroid hormones may offer a new approach to the pharmacological treatment of cocaine abuse. There has been considerable attention to the effects of cocaine and other drugs on reproductive function, but the impact of the neuroactive steroid hormones on drug abuse is a relatively new area of inquiry (see for a review, Mello and Mendelson, 2002, 2009). In addition to cocaine, progesterone also attenuates positive subjective reactions to smoked and i.v. nicotine, probably by its blockade of $\alpha4\beta2$ nicotinic receptors, and enhancement of GABAergic transmission (Sofuoglu *et al*, 2001, 2009; Lynch and Sofuoglu, 2010).

One exciting related discovery is that neuroactive steroids may have a role in the treatment of anxiety and depression (Rupprecht, 2003; Rupprecht *et al*, 2006; Eser *et al*, 2008). A novel approach to enhancing GABAergic neurotransmission is to administer a translocator protein ligand (18 kDa) to induce synthesis of endogenous neurosteroids (Rupprecht *et al*, 2009). One ligand (XBD173) dose-dependently reduced drug-induced panic reactions in rodents and humans, with minimal side effects, or withdrawal symptoms in comparison to a benzodiazepine, alprazolam (Rupprecht *et al*, 2009). If the anxiolytic and antidepressant effects of neuroactive steroids, like progesterone, prove to be superior to benzodiazepines in terms of minimal side effects or withdrawal effects, and more rapid onset of therapeutic effects (Rupprecht *et al*, 2009), these could be very useful for the clinical management of cocaine withdrawal symptoms and risk for relapse. Co-morbid anxiety and depression are often associated with cocaine abuse and dependence, and are prominent features of cessation of cocaine abuse (Gold, 1997; Mello and Mendelson, 2010), and luteal phase levels of endogenous progesterone are associated with lower levels of cue and stress-induced cocaine craving and anxiety (Sinha *et al*, 2007). Once cocaine withdrawal symptoms decrease, the risk of relapse remains high. A medication approach that attenuates cocaine's positive subjective effects as well as cocaine cue and stress-related craving and anxiety might be very effective for relapse prevention. The extent to which testosterone has similar effects on responses to cocaine remains to be determined in clinical studies. However, the possibility that neuroactive steroid hormones or their synthetic derivatives may be useful in the clinical treatment of cocaine abuse and dependence, and reduction of risk for relapse is an intriguing prospect that awaits clinical investigation.

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DISCLOSURE

The authors declare no conflict of interest.

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