The Effects of Chronic Cocaine Self-Administration on the Menstrual Cycle in Rhesus Monkeys

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ABSTRACT

Clinical studies suggest that cocaine disrupts reproductive function, but because cocaine abusers often abuse opiates and alcohol, it has been difficult to determine the contribution of cocaine alone. The effects of chronic cocaine self-administration on menstrual cycle duration and basal levels of progesterone were examined in eight female rhesus monkeys and compared with the effects of occasional administration of single cocaine doses (0.4 or 0.8 mg/kg) in six otherwise drug-free controls. All monkeys had normal ovulatory menstrual cycles before cocaine exposure. Monkeys self-administered cocaine (0.10 mg/kg/injection) and food (1 gm banana pellets) in 4 daily sessions on a second-order schedule (fixed ratio 2 [variable ratio 16:S]). Cocaine intake was limited to 8 mg/kg/day. During the first cocaine exposure (256–776 days), monkeys self-administered 3.51 (± 0.77) to 7.41 (± 0.27) mg/kg/day. During the second cocaine exposure (103–623 days), monkeys self-administered 6.18 (± 0.77) to 7.41 (± 0.27) mg/kg/day. In these prospective longitudinal studies, 48% of the menstrual cycles were of abnormal duration in the cocaine self-administration group, whereas only 6% of the menstrual cycles were abnormal in the control group. There were 19 episodes of amenorrhea (61–190 days of no menses). During cocaine self-administration, approximately one-third of the menstrual cycles were anovulatory with low mid-luteal progesterone levels of 2.04 (± 0.6) to 4.13 (± 0.5) ng/ml. Over 25% of menstrual cycles were anovulatory during cocaine withdrawal with mid-luteal progesterone levels below 5 ng/ml. These data indicate that chronic cocaine exposure can disrupt the menstrual cycle in rhesus monkeys and that menstrual cycle abnormalities often persist during cocaine withdrawal. These data are consistent with clinical studies and reports of cocaine-induced disruption of the estrous cycle in rodents.

There is accumulating evidence that cocaine abuse is associated with disruptions of menstrual cycle regularity as well as abnormalities of prolactin regulation (Cocores et al., 1986; Dackis and Gold, 1985; Gawin and Ellinwood, 1988; Mello, 1997; Mello and Mendelson, 1997; Mendelson, 1988, 1989; Teoh et al., 1994). Clinical studies indicate that women who abuse cocaine may have a variety of menstrual cycle disorders, including amenorrhea and luteal phase dysfunction, which compromise fertility (Mello, in press; Mendelson and Mello, in press; Smith and Smith, 1990; Smith et al., 1984; Teoh et al., 1994). However, it is difficult to attribute these disorders to cocaine abuse alone, because many cocaine abusers also abuse alcohol, opiates and marijuana, and each of these drugs has been shown to disrupt reproductive function (Braude and Ludford, 1984; Cicero, 1980; Mello and Mendelson, 1997; Mello et al., 1992b; Mendelson and Mello, in press; Smith and Smith, 1990; Teoh et al., 1992; 1994).

One advantage of animal models is that the effects of chronic cocaine exposure on the menstrual cycle can be studied under controlled conditions, without the confounding influence of polydrug abuse or related medical disorders. In rodent models, it has been consistently observed that chronic cocaine exposure disrupts the estrous cycle (King et al., 1990; King et al., 1993; Roberts et al., 1989). In a study designed to evaluate the effects of estrous cycle phase on cocaine self-administration, female rats developed irregular estrous cycles after 18 days of cocaine exposure (Roberts et al., 1989). Administration of cocaine (10 mg/kg/day s.c.) for 3 to 6 weeks also resulted in irregular estrous cycles characterized by repetitive days of estrus, absence of proestrus and prolonged periods of diestrus (King et al., 1990). Subsequently, this pattern of estrous cycle disruption was found to be cocaine dose-dependent (King et al., 1993). In a carefully designed study, the effects of 4 weeks of no treatment or saline control treatment were compared with those of four doses of cocaine. A group that had restricted access to food (matched with the high-dose cocaine group) was also included to control for cocaine’s anorectic effects. Estrous cyclicity was significantly disrupted after 10 and 20 mg/kg/day of cocaine, but not after 1 or 5 mg/kg/day of cocaine, saline control treatment or food restriction (King et al., 1993). Ovulation was also signifi-
cantly reduced in the high-dose cocaine groups, as inferred from oocyte retrieval after sacrifice (King et al., 1993). Over half the rats given 10 mg/kg/day of cocaine resumed normal estrous cycles once cocaine treatment was discontinued, but few rats that received 20 mg/kg/day of cocaine returned to normal estrous cycles over 5 to 6 weeks of observation (King et al., 1993).

Although the estrous cycle in rodents is a valuable model of reproductive function, in part because a complete estrous cycle occurs every 4 days (Freeman, 1988), there appear to be many differences between the neuroendocrine regulation of the estrous cycle in rodents and that of the menstrual cycle in women and higher primates (Knobil, 1974, 1980; Knobil and Hotchkiss, 1988; Yen, 1991). The rhesus monkey (Macaca mulatta) has long been a model of choice in reproductive biology because neuroendocrine control of the menstrual cycle is similar to that in women (Ferin et al., 1984; Goodman and Hodgen, 1983; Knobil, 1974, 1980). Moreover, the rhesus monkey model permits long-term evaluation of the effects of chronic cocaine exposure on menstrual cycle duration and hormonal indices of menstrual cycle adequacy. The reproductive lifespan of female rhesus monkeys lasts about 15 years, and 2 to 3 years of cocaine self-administration represents about 20 percent of the reproductive life and corresponds roughly to a period of 5 to 7 1/2 years in the human reproductive cycle.

In addition, rhesus monkeys can be trained to self-administer cocaine using operant behavioral procedures (for review see Mello and Negus, 1996). Cocaine self-administration procedures can be used to simulate naturalistic patterns of cocaine abuse reported by humans. Under these conditions, monkeys self-regulate the daily dose of cocaine and maintain stable cocaine self-administration patterns for months or years. Furthermore, there is now compelling evidence that drug self-administration procedures, in which animals control the frequency and amount of cocaine injected, have an important advantage over investigator-determined drug administration procedures (Dworkin et al., 1995). Response-independent cocaine administration resulted in higher rates of lethality in rats than self-administration of the same doses of cocaine (Dworkin et al., 1995). In previous studies, we used operant drug self-administration procedures to evaluate the effects of chronic alcohol exposure on the menstrual cycle in rhesus monkeys (Mello et al., 1983). We found that during chronic alcohol self-administration, rhesus females developed amenorrhea, anovulation and luteal phase dysfunction, conditions comparable to the menstrual cycle disorders reported in alcoholic women (Mello et al., 1983, 1989b, 1992b).

To the best of our knowledge, there have not been any comparable studies of the effects of chronic cocaine exposure on the menstrual cycle in rhesus monkeys (see Mello, 1997; Mello and Mendelson, 1997 for review).

In the present study, we examined the effects of chronic cocaine self-administration on menstrual cycle duration and basal levels of gonadotropins and ovarian steroid hormones in formerly drug-naive rhesus monkeys. The effects of chronic cocaine exposure were compared with the effects of occasional cocaine exposure in an otherwise drug-free control group. We now report the effects of chronic cocaine self-administration on menstrual cycle duration and ovulation in eight female rhesus monkeys. In these prospective longitudinal studies, 48% of the menstrual cycles were of abnormal duration in the cocaine self-administration group, whereas only 6% of the menstrual cycles were abnormal in the control group. We observed recurrent episodes of amenorrhea, anovulation and luteal phase defects during cocaine self-administration and during cocaine withdrawal. These data indicate that chronic cocaine exposure can disrupt the menstrual cycle in rhesus monkeys and that menstrual cycle abnormalities often persist during cocaine withdrawal.

Materials and Methods

Subjects

Fourteen adult female rhesus monkeys (Macaca mulatta) (5.0–9.0 kg) lived in individual cages and were maintained at ad libitum weight. Eight females were subjects in studies of the effects of chronic cocaine self-administration on the menstrual cycle (Group 1). Six control females were drug-free except for exposure to single acute doses of cocaine (0.4 or 0.8 mg/kg i.v.) at intervals of 2 or more months (Group 2). The control monkeys were subjects in studies to evaluate the acute effects of cocaine on anterior pituitary hormones (Mello et al., 1990a and b, 1993a). Monkeys in Groups 1 and 2 lived in adjacent rooms and were maintained under identical conditions. A 12-hr light-dark cycle (lights on 7 A.M. to 7 P.M.) was in effect, and constant temperature and humidity levels were maintained. Monkeys were given multiple vitamins, fresh fruit and vegetables and Lab Diet Jumbo monkey biscuits (PMI Foods, Inc., St. Louis, MO.) to supplement a nutritionally fortified banana pellet diet. Water was continuously available.

Monkeys were obtained from a commercial supplier, and after quarantine, all monkeys were adapted to the laboratory for at least 6 months before these studies began. The onset and duration of menstrual bleeding was monitored daily with vaginal swabs. Monkeys were adapted to venipuncture procedures, and blood samples were collected for analysis of anterior pituitary and gonadal hormones on alternate days, starting at day 8 of the menstrual cycle and continuing until the onset of the next menstruation. Blood samples were collected at the same time each day between 1 and 2 P.M. Monkeys were periodically evaluated with laboratory tests to monitor the status of liver function, lipid and carbohydrate metabolism, electrolyte homeostasis and hematologic function.

Animal maintenance and research were conducted in accordance with the guidelines provided by the NIH Committee on Laboratory Animal Resources. The facility is licensed by the U.S. Department of Agriculture, and protocols were approved by the Institutional Animal Care and Use Committee. The health of the monkeys was monitored periodically by consultant veterinarians expert in primatology. Monkeys had visual, auditory and olfactory contact with other monkeys throughout the study. Environmental enrichment was provided by exposure to music, television and toys for at least 2 hr/day. Operant food and drug acquisition procedures described below provided an additional opportunity for environmental stimulation and enrichment (Line, 1987; Line et al., 1989).

Sequence of Conditions

Each monkey in Group 1 was studied as her own control before, during and after a period of chronic cocaine self-administration. During the drug-free base-line period, monkeys were trained to work at a simple operant task for food as described below, and the capacity for normal ovulatory menstrual cycles was evaluated. The basis for inferring normal ovulatory function was a mid-cycle LH surge and an elevation in progesterone levels during the mid-luteal phase. Subsequently, monkeys were surgically implanted with an i.v. catheter to permit cocaine self-administration. Details of the training and surgical procedures are described in the following sections.

Six of the 8 monkeys in Group 1 were exposed to two successive periods of cocaine self-administration and cocaine withdrawal. Dur-
ing the first period of cocaine availability, each monkey continued cocaine self-administration for as long as the i.v. catheter remained patent. Then the effects of cocaine abstinence on menstrual cycle duration were observed for an average of six menstrual cycles (range 2–10) or until menstrual cycle duration returned to normal length. Subsequently, another i.v. catheter was implanted, and monkeys were again given access to i.v. cocaine until the catheter was no longer patent. Then monkeys were observed during a second period of cocaine abstinence.

Rationale for Cocaine Self-Administration Procedures

Monkeys were allowed to control the frequency of cocaine injections and, consequently, the total dose of cocaine self-administered each day, within certain limits described below. This procedure was used instead of an investigator-determined cocaine administration regimen to simulate cocaine use patterns reported clinically and to minimize the possibility of toxic effects. Cocaine’s potentially adverse effects on cardiovascular as well as cerebrovascular function in humans are well known (Cregler and Mark, 1986; Holman et al., 1991; Jacobs et al., 1989). The relative safety of drug self-administration procedures compared with non-response contingent methods of drug self-administration has recently been demonstrated (Dworkin et al., 1995). Cocaine was significantly more lethal in a rodent model when drug administration was response-independent than when it was controlled by the animal (Dworkin et al., 1995). This difference in lethality occurred despite the fact that all rats received identical amounts of cocaine at identical frequencies, but one group self-administered cocaine and a yoked control group received response-independent cocaine injections at the same time (Dworkin et al., 1995). Similarly, in rhesus monkeys, response-independent phencyclidine administration resulted in lethality, whereas self-administration of higher doses of phencyclidine did not (Johanson and Schuster, 1981). We have used the same cocaine and food self-administration procedures described here in a number of behavioral studies and have found that monkeys remained healthy and well nourished under these subject-controlled drug access conditions (Mello et al., 1989a, 1990c, 1992a, 1993b).

Operant Behavioral Procedures and Apparatus

Monkeys lived in a well-ventilated stainless steel chamber equipped with an operant panel, a banana pellet feeder and a water dispenser. Drug injections were delivered by a syringe pump in a single pulse that dispensed 0.1 ml of fluid over 0.9 sec. The operation of the syringe pump (Model 981210, Harvard Apparatus, Inc., South Natick, MA) was audible to the monkey. Schedules of reinforcement were programmed by custom-designed software and run on Apple II GS computers.

Monkeys worked at an operant task for food and for i.v. cocaine injections on a second-order schedule of reinforcement (FR2 [VR: 16S]) that required an average of 32 responses for each food pellet or cocaine injection. Food availability and cocaine availability conditions were associated with different colored stimulus lights (S+) projected on a translucent Plexiglas response key (2-in. diameter) in the center of the operant panel. The key was dark during time-out periods when responses had no programmed consequences. When a food pellet or drug injection was delivered, the appropriate colored stimulus light (S+) (red or green) was illuminated for 1 sec on one of the three circles (3/4-in. diameter) located in a vertical column below the response key. Flashes of the 1-sec colored stimulus lights (S+) also signaled the completion of each successive VR component of a second-order schedule response requirement. When cocaine was not available because of catheter loss, the response key was dark except during food sessions.

Each experimental day consisted of four food availability and four drug availability sessions. Food sessions began at 11 A.M., 3 P.M., 7 P.M. and 7 A.M. each day, and drug sessions began 1 hr later at 12 noon, 4 P.M., 8 P.M. and 8 A.M. Consecutive food and drug sessions were separated by time-out periods 2 hr (1–3 P.M., 5–7 P.M. and 9–11 A.M.) or 10 hr in duration (9 P.M.–7 A.M.). The response key was dark during time-out periods, and responses had no programmed consequences. Each food or drug session lasted 1 hr or until 100 banana pellets (1 gm) or 20 cocaine injections (0.10 mg/kg/injection) were delivered. Cocaine injections were limited to 80 per day (8 mg/kg/day) to minimize the possibility of adverse drug effects.

Surgical Procedures

After operant performance for food was stable on the final schedule of reinforcement and there was evidence of normal ovulatory base-line menstrual cycles, each monkey was surgically implanted with an i.v. double lumen silicon catheter (I.D. 0.028 in., O.D. 0.080 in.) under aseptic conditions. Monkeys were sedated with ketamine (5 mg/kg s.c.), and anesthesia was induced with sodium thiopental (10 mg/kg i.v.). Atropine (0.05 mg/kg) was given to reduce salivation. After insertion of an intratracheal tube, a surgical level of anesthesia was maintained with halothane (1–1.5% in oxygen). Catheters were implanted in the jugular or femoral vein and exit in the mid-scapular region. After surgery, monkeys were given 200,000 units of Combiotic Dihydrostreptomycinv and Penicillin G i.m. on alternate days for a total of 5 injections. The i.v. catheter was protected by a tether system consisting of a custom-fitted nylon vest connected to a flexible stainless steel cable and fluid swivel (Spaulding Medical Products, Birmingham, AL), which permitted monkeys to move freely. Catheter patency was maintained by i.v. cocaine administration and a saline flush. Fluid swivel and catheter patency were checked manually each day. A short-acting barbiturate, methohexital sodium (3 mg/kg i.v.), was used to evaluate catheter patency, if necessary. The catheter was considered to be patent if i.v. administration of methohexital produced a loss of righting within 10 sec of its administration.

Preparation of Drug Solution

Cocaine hydrochloride was obtained in crystalline form from the National Institute on Drug Abuse (NIDA). The purity was certified by Research Triangle to be greater than 98%. Cocaine was dissolved in Sterile Saline U.S.P. for injection, to make a stock solution at a concentration of 50 mg/ml. The solution was then filter-sterilized using a 0.22-micron Millipore filter and stored in sterile, pyrogen-free vials. Doses for cocaine self-administration were calculated on the basis of each monkey’s weight so that a final dilution of the stock solution (with Sterile Saline for U.S.P. injection) resulted in a unit dose of 0.10 mg/kg/injection in a volume of 0.1 ml/injection.

Radioimmunoassay Procedures

LH. Plasma LH concentrations were determined in duplicate by a double-antibody radioimmunoassay procedure similar to that described by Midgley (1966), using materials prepared by Dr. W. Peckham and following his suggestions. Purified ceropithecus pituitary LH for radioiodination (WP-XV-117-3239), rabbit antiserum (WP-R13, pool D) to human choriongonadotropin and rhesus pituitary LH reference preparation (NICH-D-RH, also known as WP-XV-20) were provided by the National Hormone and Pituitary Program, supported by the National Institute of Child Health and Human Development and the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases. Radioiodination was performed using the chloramine-T (Greenwood et al., 1963) with sodium iodide-125 purchased from DuPont New England Nuclear Products (Billerica, MA). Goat antirabbit gammaglobulin was obtained from Behring Diagnostics (San Diego, CA). Results are expressed in ng/ml per milliliter in terms of the reference preparation. The assay sensitivity was 5.6 ng/ml. Intra- and interassay CVs were 4.6% and 13.9%, respectively.

Progestrone. Plasma progesterone concentrations were measured in duplicate by a direct double-antibody radioimmunoassay method using a kit purchased from ICN Biomedicals, Inc. (Costa
Changes in average cocaine dose during the first and second cocaine exposure and the effect of first and second cocaine exposure on changes in menstrual cycle length from the base-line average were also compared with ANOVA for repeated measures. Probability levels of $P < .05$ were considered statistically significant, and Huynh-Feldt Epsilon factors were used for correction of degrees of freedom for repeated measures.

**Endocrine characteristics of the menstrual cycle.** The endocrine characteristics of the menstrual cycle were evaluated by measuring changes in progesterone through time. The following criteria were used to classify each menstrual cycle as ovulatory, anovulatory and/or with luteal phase dysfunction. Ovulation can occur in menstrual cycles of any duration, including near the end of an amenorrheic cycle.

1) **Normal ovulatory menstrual cycles:** Cycles of normal duration relative to the precocaine base-line cycles in which there was evidence of a mid-cycle periovulatory surge in LH followed by an elevation in progesterone to levels of 8.5 ng/ml or higher (Filicori et al., 1984).

2) **Anovulation:** Failure to ovulate was inferred from low levels of progesterone during the mid-luteal phase ($< 5$ ng/ml) and absence of a mid-cycle LH surge.

3) **Luteal phase defects:** Two types of luteal phase defects have been described in the clinical and primate literature (diZerega and Hodgson, 1981; diZerega and Wilks, 1984; Goodman and Hodgson, 1983; Stouffer, 1990). An *inadequate luteal phase* was defined by abnormally low progesterone levels during the mid-luteal phase, but a mid-cycle LH surge was detected and the menstrual cycle was of normal length. A *short luteal phase* was inferred from an abnormally short cycle accompanied by low progesterone levels.

**Results**

Eight rhesus females self-administered cocaine for an average of 27 ± 3.6 menstrual cycles, and the experimental history of each monkey is summarized in table 1. The first exposure to cocaine lasted for 256 to 776 days, and monkeys self-administered an average of 3.51 to 6.89 mg/kg/day of cocaine. The average dose of cocaine self-administered by these rhesus monkeys was equivalent to or greater than cocaine use reported by human cocaine abusers. In clinical studies, cocaine abusers reported using about 2 g/week of i.v. cocaine, which is equal to 4.08 mg/kg/day in a 70-kg man or 5.71 mg/kg/day in a 50-kg woman (Mendelson et al., 1988, 1989). The second period of cocaine exposure lasted for 103 to 623 days, and monkeys self-administered significantly higher average doses of cocaine ($P < .02$) than during the first exposure (table 1). Body weight during base-line menstrual cycles did not change in average cocaine dose during the first and second cocaine exposure and the effect of first and second cocaine exposure on changes in menstrual cycle length from the base-line average were also compared with ANOVA for repeated measures. Probability levels of $P < .05$ were considered statistically significant, and Huynh-Feldt Epsilon factors were used for correction of degrees of freedom for repeated measures.

**TABLE 1**

**Experimental history**

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Duration of Cocaine Exposure (days)</th>
<th>Cocaine Dose (mg/kg/day) (mean ± S.E.)</th>
<th>Food Pellets per Day (mean ± S.E.)</th>
<th>Body Weight (kg) (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First Exposure</td>
<td>Second Exposure</td>
<td>First Exposure</td>
<td>Second Exposure</td>
</tr>
<tr>
<td>13312</td>
<td>334</td>
<td>389</td>
<td>6.89 ± 0.26</td>
<td>7.38 ± 0.22</td>
</tr>
<tr>
<td>9965</td>
<td>366</td>
<td>623</td>
<td>4.88 ± 0.64</td>
<td>7.41 ± 0.27</td>
</tr>
<tr>
<td>CH553</td>
<td>431</td>
<td>442</td>
<td>5.83 ± 0.31</td>
<td>6.87 ± 0.25</td>
</tr>
<tr>
<td>CH577</td>
<td>467</td>
<td>103</td>
<td>5.67 ± 0.32</td>
<td>6.18 ± 0.77</td>
</tr>
<tr>
<td>12740</td>
<td>535</td>
<td>474</td>
<td>4.94 ± 0.27</td>
<td>6.47 ± 0.27</td>
</tr>
<tr>
<td>CH 548*</td>
<td>654</td>
<td>—</td>
<td>6.01 ± 0.25</td>
<td>—</td>
</tr>
<tr>
<td>CH712</td>
<td>776</td>
<td>509</td>
<td>6.62 ± 0.37</td>
<td>7.21 ± 1.60</td>
</tr>
<tr>
<td>CH698*</td>
<td>256</td>
<td>—</td>
<td>3.51 ± 0.77</td>
<td>—</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>477 ± 60.9</td>
<td>423 ± 71.63</td>
<td>5.54 ± 0.38</td>
<td>6.92 ± 0.21</td>
</tr>
</tbody>
</table>

* These monkeys completed only one episode of cocaine exposure because of poor venous access.
differ significantly from body weight during the first or second exposure to cocaine (P = .1146). Although the average number of banana pellets self-administered varied between subjects, all monkeys consistently ate the supplemental fruit, vegetables and chow provided each day.

**Menstrual cycle duration during the precocaine base-line.** All females had two or more normal ovulatory menstrual cycles during the drug-free base-line conditions before chronic cocaine exposure (Group 1) or initiation of acute endocrine studies (Group 2). Base-line menstrual cycle duration did not differ significantly between the two groups. In the cocaine self-administration group, base-line menstrual cycles averaged 27.4 ± 0.80 days (range 23.5 ± 0.5 to 35.6 ± 1.2 days). In the control group, base-line menstrual cycle duration averaged 26.7 ± 0.57 (range 23.3 ± 0.32 to 30.3 ± 0.8 days).

**Menstrual cycle duration during chronic cocaine self-administration.** Figure 1 compares the distribution of menstrual cycles of different duration between the control group and the cocaine self-administration group during chronic cocaine exposure and cocaine withdrawal. The control group had significantly more menstrual cycles of normal duration than the cocaine self-administration group (P < .001). In the control group, 94% of the 155 cycles were of normal duration, and the number of cycles that were one standard deviation longer or shorter than the base-line cycles accounted for 5% and 1%, respectively. Moreover, the control group did not have any amenorrheic cycles (defined as 60 days or more without menses). In contrast, during cocaine self-administration, menstrual cycle duration was quite variable, and 48% of the 217 cycles were abnormally short, abnormally long or amenorrheic (fig. 1).

The distribution of normal, short, long and amenorrheic cycles during the first and second cocaine exposure was compared in 6 of the 8 monkeys (data not shown). There was a higher incidence of cycles of abnormal duration during the second cocaine exposure (53%) than during the first cocaine exposure (40%), but this difference was not statistically significant. There were more long cycles (24%) during the second cocaine exposure than during the first cocaine exposure (16%), but the numbers of short cycles were equivalent (18% vs. 20%). The incidence of amenorrheic cycles also was slightly higher during the second cocaine exposure (9%) than during the first cocaine exposure (5%). These data suggest that biologically significant tolerance to cocaine’s effects on menstrual cycle duration did not occur as a function of chronic cocaine exposure.

**Menstrual cycle duration after abrupt withdrawal from cocaine.** Cocaine self-administration continued until the i.v. catheter became occluded, requiring implantation of a new catheter. The distribution of abnormal menstrual cycle durations during cocaine withdrawal was similar to that during cocaine self-administration (fig. 1) and also differed significantly from the distribution of menstrual cycle durations in normal controls (P < .001). During cocaine withdrawal, 44% of the 82 cycles were of abnormal duration, compared with the precocaine base-line. Amenorrheic cycles ranging from 61 to 190 days in duration accounted for 6% of the menstrual cycles during cocaine self-administration, whereas during cocaine withdrawal, 7% of the cycles were amenorrheic. The transition cycles, when catheter occlusion occurred part way through a menstrual cycle, often were associated with amenorrhea.

**Menstrual cycle duration in individual monkeys.** The effects of chronic cocaine self-administration and cocaine withdrawal on the duration of consecutive menstrual cycles in six individual monkeys are summarized in figures 2 to 4. The pattern of menstrual cycle disruptions varied within individuals across time, and there was no consistent tendency for cycle length to normalize during an episode of chronic cocaine exposure. Figure 2 shows changes in menstrual cycle duration and average cocaine self-administration (mg/kg/day) during consecutive menstrual cycles for two monkeys that had several abnormally short, as well as long, menstrual cycles during cocaine exposure. The top panel shows menstrual cycle duration and cocaine dose over 17 menstrual cycles (431 days) for monkey CH553. During the first menstrual cycle when cocaine was available, this monkey self-administered an average of 3.81 (± 0.09) mg/kg/day of cocaine, and her menstrual cycle was only 17 days long. Cocaine self-administration increased to 4.29 (± 0.43) and 6.14 (± 0.24) mg/kg/day during the second and third menstrual cycles and then decreased to 3.3 (± 0.52) mg/kg/day during menstrual cycle 4, which lasted only 19 days. Her seventh and eighth menstrual cycles were 16 and 18 days long, and she self-administered an average of 7.45 (± 0.23) and 5.93 (± 0.45) mg/kg/day of cocaine, respectively. In the ensuing months, her menstrual cycles varied from 21 to 39 days in length, and cocaine self-administration averaged between 4.66 (± 0.38) and 7.20 (± 0.23) mg/kg/day.

During cocaine withdrawal, the second menstrual cycle lasted 57 days, but with the exception of one 14-day cycle, the remainder of the menstrual cycles observed during 210 days of cocaine abstinence were of normal length. The second exposure to cocaine lasted for 442 days (13 menstrual cycles), and this monkey self-administered an average of 6.87 (±
0.25) mg/kg/day of cocaine. Two abnormally short cycles (16 and 12 days in length) alternated with an abnormally long cycle (47 days) and an amenorrheic cycle (84 days). This monkey continued to self-administer cocaine for an additional 4 months, but these data were not included in the analysis because the monkey became sick during cocaine cycle 16, and exploratory surgery revealed a severe impaction of the colon that required euthanasia.

The lower panel of figure 2 shows data for another monkey over 23 consecutive menstrual cycles (654 days) of cocaine self-administration. This monkey increased cocaine intake from 3.22 (± 0.18) to 5.89 (± 0.37) mg/kg/day during the second menstrual cycle, and this menstrual cycle was abnormally long (37 days). After seven consecutive menstrual cycles (222 days of cocaine exposure), this monkey had an abnormally short cycle (17 days) during which she self-administered 6.34 (± 0.16) mg/kg/day of cocaine. Menstrual cycle 10 was only 15 days long, and the monkey self-administered an average of 7.87 (± 0.64) mg/kg/day of cocaine.

Throughout the remaining 13 cycles of cocaine exposure and seven cycles of cocaine withdrawal, most menstrual cycles were longer than base-line, but none met the criteria for amenorrhea (fig. 2, lower panel).

Figure 3 shows menstrual cycle durations and average cocaine self-administration for two monkeys that developed amenorrheic cycles during cocaine self-administration and withdrawal. Monkey 12740 self-administered cocaine for 11 menstrual cycles over 535 days (fig. 3, top). Cocaine self-administration averaged 4.94 (± 0.27) mg/kg/day (range 3.04 ± 0.19 to 6.05 ± 0.17 mg/kg/day), and 6 of the 11 menstrual cycles were abnormal in length. This monkey had two very short cycles and four long or amenorrheic cycles of 50, 140, 61 and 190 days in length. The i.v. catheter became occluded on day 58 of cocaine cycle 11, and no menses were detected for 190 days. During cocaine withdrawal, the 190-day amenorrheic cycle was followed by a second amenorrheic cycle 96 days in duration and by several short cycles. During the second cocaine exposure, this monkey self-administered
an average of 6.47 (± 0.27) mg/kg/day of cocaine for nine menstrual cycles (474 days). A 170-day amenorrheic cycle occurred during the second cycle of cocaine self-administration, and another amenorrheic cycle (71 days) occurred during the fifth cocaine cycle, when this monkey self-administered an average of 7.37 (± 0.07) mg/kg of cocaine per day. During the subsequent cocaine withdrawal period of 223 days, the second menstrual cycle was amenorrheic and lasted for 63 days.

A similar pattern of menstrual cycle disruption was evident in monkey CH712 (fig. 3, lower panel). During the first two cycles of cocaine exposure, she took less than 2 mg/kg/day of cocaine, and then she doubled and tripled her cocaine intake during cycles 3, 4, and 5. Increased cocaine self-administration was associated with three consecutive abnormally short menstrual cycles of 22, 18 and 14 days, respectively. During cycle 6, this monkey had a 117-day amenorrheic cycle. Subsequently, her cocaine intake increased to average over 7 mg/kg/day, and several cycles of normal length were followed by a series of abnormally long cycles that lasted for 44, 59 and 50 days, respectively (cycles 14, 16 and 17). The next three menstrual cycles were relatively short compared with this monkey’s base-line; they averaged 23, 22 and 23 days, respectively. The first menstrual cycle during cocaine withdrawal was a 95-day amenorrheic cycle. Subsequently, menstrual cycles returned to base-line length (24–26 days). When cocaine self-administration resumed, this monkey self-administered relatively high doses of cocaine averaging 7.21 (± 1.60) mg/kg/day. The third menstrual cycle was amenorrheic (77 days) and was followed by a series of menstrual cycles of normal length.

Two other monkeys also had abnormal menstrual cycles during cocaine self-administration and withdrawal (data not shown). During the first exposure to cocaine (15 cycles over 366 days), monkey 996B began to self-administer high doses of cocaine (6.49 ± 0.36 mg/kg/day) during cocaine cycle 5, and the next four cycles were shorter than the base-line cycle average. The first abnormally long cycle (42 days) occurred during cycle 10, and this monkey self-administered high doses of cocaine averaging more than 7 mg/kg/day. During
cocaine cycle 14, the first amenorrheic menstrual cycle of 63 days occurred. The second exposure to cocaine (20 menstrual cycles over 623 days) also was associated with abnormally long cycles, including two amenorrheic cycles of 61 and 69 days, respectively. An amenorrheic cycle of 92 days occurred during the first cocaine withdrawal cycle after the catheter became occluded on day 8 of the menstrual cycle.

Monkey CH696 administered relatively low levels of cocaine (3.51 ± 0.77 mg/kg/day) for seven menstrual cycles (256 days). During the second period of cocaine access, cocaine intake increased from an average of 3.23 (± 0.26) mg/kg/day to 7.30 (± 0.39) mg/kg/day, and the menstrual cycle was abnormally short (21 days). This monkey subsequently decreased cocaine self-administration to an average of 4.9 (± 0.61) mg/kg/day during cycle 3 and had a 98-day amenorrheic cycle during cycle 4, when cocaine self-administration averaged 3.29 mg/kg/day.

Not all monkeys had abnormal menstrual cycles during chronic cocaine self-administration. Figure 4 summarizes menstrual cycle durations and average cocaine self-administration (mg/kg/day) during successive menstrual cycles for two monkeys (13312 and CH577) that were relatively unaffected by cocaine initially. During the first exposure to cocaine (13 cycles over 334 days), monkey 13312 self-administered an average of 6.89 (± 0.26) mg/kg/day of cocaine (fig. 4, top). Despite consistent high levels of cocaine intake, her average menstrual cycle duration remained about the same as during the precocaine base-line. During six cycles of cocaine withdrawal, menstrual cycles continued to average 25.5 (± 1.28) days, equivalent to the precocaine base-line (fig. 4, top). When cocaine self-administration resumed, this monkey again maintained consistent high levels of cocaine self-administration (7.38 ± 0.22 mg/kg/day). Except for one abnormally long cycle of 55 days (cocaine cycle 4), her menstrual cycles ranged between 25 and 30 days in duration throughout 389 days of cocaine exposure.

Menstrual cycles of normal length were also observed in monkey CH577 over a comparable time period (fig. 4, lower panel). This monkey took relatively low doses of cocaine during the first 3 cycles of exposure (2.84–3.8 mg/kg/day) and
then abruptly increased cocaine self-administration to 6 (± 0.39) mg/kg/day during the fourth cycle. However, during the first 431 days of cocaine self-administration (5.67 ± 0.32 mg/kg/day), her 18 menstrual cycles averaged 26.7 ± 1.6 days, equivalent to her precocaine baseline. Menstrual cycle 18, during the first cocaine exposure, lasted for 52 days, and the catheter became partially occluded on day 30, so this long cycle may have occurred as a consequence of cocaine withdrawal. During the subsequent 10 cycles of cocaine abstinence (293 days), menstrual cycles averaged 26.8 (± 0.65) days. When cocaine self-administration resumed, the i.v. catheter remained patent for only 103 days, and the monkey took an average of 6.18 (± 0.77) mg/kg/day of cocaine. One abnormally short menstrual cycle (19 days) and one long cycle (37 days) occurred during this period. During the next five withdrawal cycles (181 days), an amenorrheic cycle of 63 days was followed by a long cycle of 37 days, and then cycles returned to normal length.

Endocrine Characteristics of the Menstrual Cycle

Ovulation and luteal phase adequacy during cocaine self-administration and withdrawal. Ovulation was inferred from a mid-luteal phase increase in progesterone to 8.5 ng/ml or above (Filicori et al., 1984). After ovulation, a corpus luteum forms at the site of the ruptured oocyte and secretes increasing levels of progesterone during the early luteal phase. The mid-luteal phase elevation of progesterone levels usually lasts for more than 6 days (Knobil, 1980; Knobil and Hotchkiss, 1988), and this is a reliable indicator of the occurrence of ovulation. The periovulatory LH surge usually lasts for only 24 hr, and in the present study, collection of blood samples on alternate days did not permit consistent detection of peak LH levels at mid-cycle. In the control group, peak progesterone levels averaged 15.36 ± 0.95 ng/ml during menstrual cycles classified as ovulatory.

Table 2 summarizes average peak progesterone levels during cocaine self-administration and withdrawal. In the cocaine-exposed monkeys, peak progesterone levels during menstrual cycles classified as ovulatory ranged from 11.6 to 16 ng/ml. Peak progesterone levels during ovulatory cycles were similar during cocaine self-administration and withdrawal. During cycles classified as anovulatory, peak progesterone levels ranged from the lower limit of assay sensitivity to 4.5 ng/ml. Progesterone levels during anovulatory cycles were similar during normal and long menstrual cycles but tended to be lower during short menstrual cycles (table 2).

The distribution of menstrual cycles classified as ovulatory or anovulatory as a function of cycle length is shown in figure 5. Sixty-eight percent of the 217 cocaine self-administration cycles and 66% of the 82 cocaine withdrawal cycles could be classified as ovulatory or anovulatory with certainty on the basis of mid-luteal phase elevations in progesterone. Amenorrheic cycles were usually anovulatory and are described separately below.

During cocaine self-administration, the frequency of ovulation was significantly higher in menstrual cycles of normal length than in short or long cycles (P < .05) (fig. 5, top panel). In menstrual cycles of normal duration, 78% had progesterone elevations consistent with ovulation. In contrast, only 45% of the short cycles and 66% of the long cycles were ovulatory. The frequency of anovulatory cycles was similar during first and second cocaine exposure (26% and 32%) (data not shown). During cocaine withdrawal, the frequency

<table>
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<td>Peak progesterone levels (ng/ml) as a function of menstrual cycle length during cocaine self-administration and withdrawal</td>
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<th>Normal Menstrual Cycles</th>
<th>Short Menstrual Cycles</th>
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<tr>
<td>Ovulatory</td>
<td>Anovulatory</td>
<td>Ovulatory</td>
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<tr>
<td>Cocaine self-administration</td>
<td>13.54 ± 0.5</td>
<td>4.13 ± 0.5</td>
</tr>
<tr>
<td>Cocaine withdrawal</td>
<td>15.02 ± 1.0</td>
<td>4.59 ± 0.8</td>
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of ovulation did not differ significantly in normal, short and long cycles (fig. 5, lower panel). Eighty-seven percent of the menstrual cycles of normal duration were ovulatory and 64% and 78% of the short and long menstrual cycles, respectively, were ovulatory.

Cycle length and ovulation as a function of cocaine dose. The average dose of cocaine self-administered during the menstrual cycle did not reliably predict menstrual cycle length or the presence or absence of ovulation (fig. 6, top panel). Monkeys self-administered an average of 6.36 (± 0.19) mg/kg/day of cocaine during ovulatory cycles and an average of 6.35 (± 0.25) mg/kg of cocaine during anovulatory cycles (fig. 6, top, column 1). The amount of cocaine self-administered during the menstrual cycle that immediately preceded an anovulatory cycle also did not predict ovulation or anovulation (fig. 6, lower panel). Monkeys self-administered an average of 5.75 (± 0.27) mg/kg/day of cocaine during the menstrual cycles that preceded ovulatory cycles and 5.78 (± 0.42) mg/kg/day during the menstrual cycles that preceded anovulatory cycles (fig. 6, lower panel, column 1). Cocaine dose during the same cycle and during the immediately preceding cycle was also analyzed as a function of menstrual cycle duration (fig. 6, columns 2, 3 and 4). There were no statistically significant differences between the average cocaine dose self-administered during ovulatory and anovulatory menstrual cycles regardless of cycle duration (fig. 6).

Amenorrhea during cocaine self-administration and withdrawal. Amenorrheic cycles occurred with approximately equal frequency during cocaine self-administration and withdrawal (see fig. 1). Sixteen of the 19 amenorrheic cycles appeared to be anovulatory, and peak progesterone values averaged 2.31 (± 0.37) ng/ml (range 0.69–4.24 ng/ml). Only three amenorrheic cycles appeared to be ovulatory, and each of the ovulatory cycles occurred during cocaine self-administration. Ovulation was inferred from peak progesterone levels of 14, 14.7 and 15.8 ng/ml, which were measured 5 to 8 days before the end of the amenorrheic cycle. Figure 7 shows elevations in progesterone during the three ovulatory amenorrheic cycles that lasted for 63 to 117 days. The antecedent LH surge was not detected, perhaps because of the relative infrequency of blood sample collection. Monkey CH712 self-administered 6.7 (± 0.18) and 7.9 (± 0.19) mg/kg/day of cocaine during these amenorrheic cycles, and monkey 996B self-administered 5.58 (± 0.19) mg/kg/day of cocaine. At the beginning of these amenorrheic cycles, monkey CH712 had self-administered cocaine for a total of 138 and 830 days, respectively, and monkey 996B had self-administered cocaine for a total of 291 days.

We also examined the distribution of amenorrheic cycles across months of the year, because in the Northern hemisphere, the fall breeding period is sometimes preceded by anovulatory/amenorrheic cycles during the summer. Figure 8 shows the month of onset and the duration of each of the 19 amenorrheic cycles observed during cocaine self-administration and withdrawal. It is apparent that the initiation of amenorrheic cycles was distributed across the year. The three ovulatory amenorrheic cycles shown in figure 8 began in March, April and October. Thus the onset and duration of amenorrheic cycles did not appear to be related to any particular season.

**Discussion**

Chronic cocaine self-administration and cocaine withdrawal were both associated with severe disruptions of menstrual cycle regularity compared with control conditions (fig. 1), and there was considerable variability in the pattern of menstrual cycle abnormalities observed within and between individuals (figs. 2–4). Ovulatory menstrual cycles of normal length were interspersed with anovulatory menstrual cycles and cycles of abnormal duration (figs. 2 and 3). Abnormally short cycles with low progesterone levels suggestive of luteal phase dysfunction were frequently observed (table 2; figs. 5 and 6). However, 2 of 8 monkeys were relatively resistant to the disruptive effects of cocaine (fig. 4). Chronic cocaine exposure, as well as the abrupt discontinuation of cocaine availability, was sometimes associated with amenorrhea 61 to 190 days in duration (figs. 7 and 8). The persistent menstrual cycle abnormalities observed during cocaine withdrawal suggest that cocaine’s disruptive effects on the menstrual cycle do not reflect acute intoxication alone. Rather, daily cocaine
exposure may induce long-lasting disruptions of the neuroendocrine regulation of the menstrual cycle. Persistent effects of cocaine after chronic exposure have been observed in several models (Mello and Mendelson, 1997). In human cocaine abusers, hyperprolactinemia may occur during cocaine abstinence as well as during occasional cocaine use (Cocores et al., 1986; Mendelson et al., 1988). In rats, estrous cycles did not return to normal after high doses of cocaine over 5 to 6 weeks of observation (King et al., 1993).

Daily cocaine exposure had variable effects on the menstrual cycle in these rhesus monkeys, and similar variations are reported in humans. Not all cocaine-dependent women report irregular menstrual cycles, and many cocaine abusers become pregnant, as evidenced by recent concerns over the teratogenic effects of cocaine abuse (Hutchings, 1989; Mayes et al., 1992). However, because many human cocaine abusers are also polydrug abusers, it is impossible to attribute any menstrual cycle disorders or impairment of fertility to cocaine alone (Mello and Mendelson, 1997; Smith and Smith, 1990; Teoh et al., 1994). Cocaine-induced menstrual cycle disruptions in rhesus monkeys are consistent with reports that chronic exposure to high doses of cocaine (10–20 mg/kg/day) for up to 6 weeks disrupted estrous cyclicity in rats and decreased rates of ovulation (King et al., 1990, 1993).

Factors Contributing to Menstrual Cycle Abnormalities

The effect of cocaine dose and duration of exposure on the menstrual cycle. The cocaine dose level was not well correlated with the extent or type of menstrual cycle disruptions observed in individual monkeys. Some monkeys self-administered high daily doses of cocaine with minimal toxic effects (fig. 4), whereas in other monkeys, menstrual cycles were disrupted by both low and high levels of cocaine self-administration (figs. 2 and 3). These findings suggest that there may be a threshold for dose and duration of cocaine exposure that is sufficient to disrupt the menstrual cycle but that this threshold varies across individual monkeys. Analysis of group data also indicated that the average dose of cocaine self-administered did not reliably predict either menstrual cycle duration or anovulation (fig. 6). Because events during one menstrual cycle may influence the subsequent menstrual cycle, we also examined the average dose of cocaine self-administered during the cycles immediately before ovulatory and anovulatory cycles of different lengths. However, the dose of cocaine self-administered did not differ significantly between menstrual cycles that preceded ovulatory and anovulatory menstrual cycles (fig. 6).

The duration of cocaine exposure did not significantly alter the characteristics of the menstrual cycle. When monkeys were given access to cocaine on two occasions, the extent and type of menstrual cycle disruptions observed during the second exposure did not differ significantly from disruptions observed during the first exposure (figs. 2 and 3). Menstrual cycle function was resilient in some monkeys (fig. 4) despite months of high-dose cocaine self-administration. Because
normal ovulatory cycles alternated unpredictably with abnormal cycles and there was no progressive normalization of cycles through time (figs. 2 and 3), it is unlikely that tolerance developed to cocaine’s toxic effects on reproductive function.

**Effects of other factors on menstrual cycle abnormalities.** Because a number of factors other than chronic drug exposure can influence menstrual cycle duration and ovulation, it is important to consider the possibility that the abnormalities observed in the present study might not be attributable to cocaine alone. For example, malnutrition, concurrent medical conditions, excessive exercise, and stress may result in amenorrhea in women (Bullen et al., 1985; Frisch, 1982; McArthur et al., 1980; Sherman, 1984; Warren, 1992). In rhesus monkeys, seasonal factors may also contribute to anovulatory menstrual cycles under some conditions (Hartman, 1932; Walker et al., 1983). However, on the basis of the following considerations, we conclude that chronic cocaine self-administration, and not other, uncontrolled factors, was primarily responsible for the menstrual cycle disruptions observed.

1) These monkeys were not malnourished or food-deprived. Although cocaine may induce transient anorectic effects, tolerance to its initial effects developed very rapidly, and food intake remained stable across the period of observation. Food self-administration and body weight during cocaine self-administration and withdrawal did not differ significantly from precocaine baseline levels (table 1). All monkeys continued to eat supplemental daily feedings of chow, fruit and vegetables throughout the study. Both fasting and anorexia are associated with amenorrhea in women (Bullen et al., 1985; Frisch, 1982; Sherman, 1984; Warren, 1992), so it is important to rule out malnutrition as a factor in menstrual cycle disruptions. However, because a wide spectrum of menstrual cycle abnormalities (anovulation, amenorrhea and abnormally short cycles), as well as normal ovulatory cycles, often occurred in the same monkey during stable food intake, it is unlikely that nutritional factors could account for these variations.

2) Seasonal factors probably did not contribute to the menstrual cycle abnormalities observed. Monkeys entered the laboratory, began cocaine self-administration and developed menstrual cycle abnormalities at different times of the year. Monkeys were maintained under constant temperature and humidity conditions and a constant 12-hr light-dark cycle, and these environmental conditions remained constant across the period of observation. These conditions were designed to minimize the influence of seasonal variations in light-dark cycles that influence breeding cycles in monkeys housed in outdoor colonies (Barsotti et al., 1980; Wehrenberg and Dyrenforth, 1983; Wilks et al., 1977). Previous studies have shown that animals acquired from outdoor compounds rapidly adjust to climate-controlled conditions and rarely have anovulation or amenorrhea (Barsotti et al., 1980). Examination of the onset of amenorrheic cycles in relation to the normal breeding period for rhesus macaques in the Northern hemisphere indicated that there were no consistent seasonal variations (fig. 8).

3) It is also unlikely that these monkeys had antecedent menstrual cycle abnormalities that were not related to cocaine exposure. All monkeys were adapted to laboratory conditions for at least 6 months after quarantine, and all had normal ovulatory menstrual cycles before initiation of these studies. In addition, more than 90% of the menstrual cycles observed in the six control monkeys were of normal length (fig. 1). Thus occasional exposure to single doses of cocaine approximately once every 2 months was not associated with menstrual cycle disruptions in the control monkeys. The significant difference between these two groups in the extent of menstrual cycle disruptions indicates that chronic daily cocaine exposure was more toxic than occasional single doses of cocaine (fig. 1).

4) The general health status of the monkeys was monitored by consultant veterinarians. According to these evaluations, all monkeys were in good health throughout the study, except the animal that developed an impacted colon (fig. 2).

**Possible mechanisms underlying cocaine’s effects on the menstrual cycle.** The ways in which cocaine disrupts neuroendocrine regulation of the menstrual cycle are poorly understood (Mello, in press; Mello and Mendelson, 1997; Teoh et al., 1994). Moreover, analysis of these disorders is complicated by the fact that each clinically defined syndrome may result from hormonal disruptions that occurred earlier in the same menstrual cycle or in the previous menstrual cycle. Acute administration of cocaine changes basal levels of anterior pituitary hormones that are important for menstrual cycle normalcy. For example, acute administration of cocaine stimulates the release of LH, FSH and ACTH and suppresses prolactin in rhesus monkeys (Mello et al., 1990a and b; Sarnyai et al., 1996) as well as humans (Heesch et al., 1996; Mendelson et al., 1989; Teoh et al., 1994). Frequent repetition of these acute hormonal effects of cocaine might contribute to the menstrual cycle abnormalities observed during chronic cocaine self-administration. Data relating elevations in gonadotropins and ACTH and changes in prolactin levels to regulation of the menstrual cycle are summarized below.

**Gonadotropin and Ovarian Steroid Hormones.** Abnormally high levels of LH and/or estradiol during the follicular phase may suppress FSH and delay follicle maturation and subsequent ovulation (Dierschke et al., 1985, 1987; Zeleznik, 1981). Acute cocaine administration increases LH (Mello et al., 1990a and b, 1993a), so it is possible that recurrent LH stimulation during chronic cocaine exposure disrupts follicle development. Although FSH is only one determinant of normal folliculogenesis, adequate FSH levels are necessary for follicle development and maturation of the preovulatory follicle (Goodman and Hodgson, 1983). Suppression of FSH during the follicular phase also may result in luteal phase dysfunction after timely ovulation. High estrogen levels during the early luteal phase may lead to premature regression of the corpus luteum, resulting in a short menstrual cycle (Hutchison et al., 1987). Cocaine’s interactions with progesterone remain to be determined, but suppression of progesterone could contribute to the luteal phase defects observed. In clinical endocrinology, criteria for the differential diagnosis and pathogenesis of luteal phase dysfunction remain controversial (McNeely and Soules, 1988; Stouffer, 1990).

**ACTH.** It is also possible that the acute stimulatory effects of cocaine on ACTH and, by inference, on CRH contribute to the menstrual cycle disorders observed during chronic cocaine self-administration. It is well established that CRF administration to rhesus monkeys suppresses the release of LH and FSH, which are essential for normal ovulation (Oster and Ferin, 1987; Xiao and Ferin, 1988). Thus repeated stimulation of ACTH during chronic cocaine self-administration may contribute to anovulation as well as amenorrhea.
The effects of CRF on the hypothalamic-pituitary-adrenal axis in rodent models have recently been reviewed (Rivest and Rivier, 1995).

**Prolactin.** Abnormally low or high prolactin levels may also be associated with luteal phase dysfunction (McNeely and Soules, 1988). Hyperprolactinemia is sometimes associated with chronic cocaine abuse as well as with cocaine abstinence (Cocores et al., 1986; Dackis and Gold, 1985; Mello et al., 1994; Mendelson et al., 1989). Hyperprolactinemia also may be associated with amenorrhea, but both conditions can occur independently (Buchanan and Tredway, 1979; Mello et al., 1988; Tolis, 1980). Cocaine-related hyperprolactinemia would seem to be inconsistent with cocaine's acute effects on prolactin. Single doses of cocaine decrease prolactin levels in rhesus males and females (Mello et al., 1990a; 1993a) and cocaine-naive men (Heesch et al., 1996), presumably as a result of increasing dopaminergic control (Ben-Jonathan, 1985; Neill et al., 1981; Yen 1979, 1991), and cocaine acts as an indirect dopamine agonist by binding to the dopamine transporter and blocking the reuptake of dopamine (Kuhar et al., 1988; Ritz et al., 1987). It has been suggested that chronic cocaine exposure may lead to a “down-regulation” of dopamine receptors that impairs the sensitive regulatory feedback relationship between hypothalamic dopamine and prolactin to result in hyperprolactinemia (Dackis and Gold, 1985; Wyatt et al., 1988).

In conclusion, regulation of the reproductive system is very complex and depends on co-modulatory interactions among the hypothalamus, the anterior pituitary, the ovary and the adrenal (see Knobil, 1980; Knobil and Hotchkiss, 1988; Mello and Mendelson, 1997; Yen, 1991). It seems unlikely that cocaine acts primarily at a single target site. However, cocaine-induced changes in basal levels of several hormones may affect the functional integration and regulation of the neuroendocrine system. By analogy, recent studies suggest that a progesterone antagonist (RU 486) may inhibit ovulation by interfering with estradiol stimulation of the hypothalamic release of gonadotropin-releasing hormone (GnRH) rather than by altering gonadotropin release at the level of the anterior pituitary (Heikinheimo et al., 1995). It is likely that any drug-induced imbalance in the interactions among anterior pituitary, gonadal and adrenal hormones would lead to disruption of the normal menstrual cycle.

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