Effect of acute administration of cocaine on pituitary gonadotrophin secretion in female rats

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The aim of this study was to characterize the acute effects of cocaine administration on pituitary gonadotrophin secretion in adult female rats. Ovariectomized, oestradiol-treated rats were infused i.v. with 0.1 ml normal saline or 2 mg cocaine hydrochloride kg⁻¹. Blood samples were collected immediately before cocaine infusion and at 3, 10, 30 and 60 min after cocaine infusion. Circulating LH concentrations were increased by 10 min after cocaine administration ($P < 0.05$ versus saline-treated controls) and decreased thereafter. Serum FSH concentrations were not significantly different from those of saline-infused controls at any time. In a second experiment, oestradiol-treated, ovariectomized rats were infused i.v. with: saline only, 2 mg cocaine hydrochloride kg⁻¹ in saline, 200 ng synthetic GnRH in 100 μ l PBS or 200 ng synthetic GnRH plus 2 mg cocaine hydrochloride kg⁻¹ in PBS. Blood samples were collected immediately before drug infusions

and 20 min later. Cocaine had no effect on either GnRH-stimulated LH or FSH secretion. In a third experiment, pituitary cells were obtained from oestradiol-treated, ovariectomized rats. The cell cultures were exposed to 25 ng cocaine hydrochloride ml⁻¹, 10^{-10} – 10^{-7} mol GnRH l⁻¹ with and without 25 ng cocaine hydrochloride ml⁻¹, or vehicle only. Medium was collected before and after exposure to GnRH to determine concentrations of secreted LH and FSH. Similar to the results of the second study, cocaine had no effect on GnRH-stimulated LH or FSH secretion from pituitary cells *in vitro*. On the basis of these results it is suggested that acutely administered cocaine stimulates release of hypothalamic GnRH, which, in turn, stimulates pituitary gonadotrophin secretion. Acute administration of cocaine does not appear to affect pituitary gonadotrophin secretion directly.

Introduction

Cocaine is the second most commonly used illicit drug (after marijuana) in the US and is the illicit drug mentioned most frequently by patients brought to emergency departments in most US cities (Drug Abuse Warning Network, 2000). In 1998, approximately 23 million Americans (11% of the country's total population) aged 12 years or older reported using cocaine in their lifetimes, 4 million (2%) reported cocaine use in the past year and 1.7 million (0.8%) reported current cocaine use (National Household Survey on Drug Abuse, 2000). Among non-pregnant women of childbearing age, >10% reported using illicit drugs including cocaine in the previous month. Clinical case studies indicate that cocaine alters normal reproductive function, including disruption of normal menstrual cyclicity and decreased libido in women who abuse this drug chronically (Siegel, 1982; Cocores *et al.*, 1986; Teoh *et al.*, 1994). Cocaine administration disrupts oestrous cyclicity and normal rates of ovulation in rats (King *et al.*, 1990, 1993) and menstrual cyclicity and ovulation rates in monkeys (Potter *et al.*, 1998, 1999). The effects of cocaine

on cyclic reproductive function, including circulating LH concentrations, oestrous cyclicity and rates of ovulation in rats are dose-dependent and, at higher doses, may be irreversible (King *et al.*, 1993). Despite the growing evidence that cocaine disrupts normal reproductive cyclicity, few studies have attempted to define the site of action of cocaine within the reproductive neuroendocrine axis.

Cocaine has no effect on GnRH-stimulated increases in serum LH and FSH concentrations in ovariectomized cynomolgus monkeys (Chen *et al.*, 1998) and cocaine inhibits both noradrenaline- and phorbol ester-stimulated GnRH release from slices of rat hypothalamus *in vitro* (King *et al.*, 1992, 1993). On the basis of the results of these studies, it was hypothesized that cocaine induces female reproductive dysfunction by disrupting release of GnRH from the hypothalamus, with resultant disturbances in cyclic reproductive function. In contrast, several studies have shown that serum gonadotrophin concentrations increase transiently within minutes of administration of cocaine to female rhesus monkeys (Mello *et al.*, 1990a,b) and women (Mendelson *et al.*, 2001). These investigators have suggested that acute exposure to cocaine stimulates a burst of hypothalamic GnRH release with a resultant

transient increase in circulating LH concentrations (Mello and Mendelson, 1997). Similar studies to examine the acute effects of cocaine on pituitary gonadotrophin secretion in a rodent model have not been reported. Characterization of the acute effects of cocaine on pituitary gonadotrophin secretion could provide valuable information concerning both the site and mechanism of action to explain the effects of this drug on cyclic reproductive function in females.

The aim of the present study was to determine whether acute administration of cocaine directly affects pituitary gonadotrophin secretion in female rats by characterizing the acute effects of cocaine on: (i) LH and FSH concentrations in ovariectomized, oestrogen-replaced rats; (ii) GnRH-stimulated pituitary LH and FSH secretion in ovariectomized, oestrogen-replaced rats; and (iii) GnRH-stimulated LH and FSH secretion from pituitary cells obtained from ovariectomized, oestrogen-treated rats.

Materials and Methods

Animals

All the rats used in these experiments were adult female Sprague–Dawley rats (125–135 g at delivery) (Charles River Laboratories Inc., Wilmington, MA). Rats were housed in groups of three per clear plastic cage and maintained in an automatically controlled cycle of 14 h light:10 h dark (lights on at 06:00 h daily). The rats were provided with food (Wayne Lab-Blox) and fresh tap water *ad libitum*. Oestrous cyclicity was monitored by daily (09:00–10:00 h) vaginal cytology through a minimum of two complete cycles for each rat. Rats with irregular oestrous cycles were excluded from the study. Each experimental protocol involving the use of animals was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), The University of Texas Health Science Center at San Antonio, San Antonio, Texas.

Animal surgery

Oestradiol benzoate-treated, ovariectomized rats were used to normalize ovarian steroid concentrations in these experiments. Rats in dioestrus were anaesthetized with Rompun cocktail (100 mg ketamine hydrochloride ml⁻¹: Ketaset, Aveco Company Inc., Fort Dodge, IA; 20 mg xylazine hydrochloride ml⁻¹: AnaSed, Lloyd Laboratories, Shenandoah, IA; 60:40, v/v) i.m. and ovariectomized bilaterally. On each of days 19–21 after ovariectomy, the rats were injected s.c. at 09:00 h with 50 µg oestradiol benzoate in sesame oil kg⁻¹ body weight (Sigma Chemical Co., St Louis, MO). On day 19 after ovariectomy, the rats were also anaesthetized i.m. with Rompun cocktail. The left jugular vein of each rat was cannulated with Silastic tubing that extended from the right atrium through the dorsum of the neck region. The rats were housed one per cage after this time. The tubing was later connected to additional tubing leading to the outside of the cage, allowing blood collection without the necessity of handling the rat. This

tubing was weighted lightly and attached to a swivel above the cage. As the rat moved around its cage, slack in the cannula was taken up by this system, thus preventing the rat from accessing and damaging the cannula. Patency of the cannulae was maintained by regular flushing with heparinized saline.

Gonadotrophin secretion in vivo

At 10:00 h on the day of the third oestradiol benzoate injection, a single 0.5 ml blood sample was collected over a period of 30–60 s from each rat via the indwelling venous cannula (time 0). The rats were divided into two experimental treatment groups: a group of eight rats infused via the indwelling jugular cannula with 0.2 ml saline vehicle and a group of eight rats infused via the indwelling cannula with 2 mg cocaine hydrochloride kg⁻¹ (Sigma Chemical Co.). This dosage of cocaine was chosen to provide serum concentrations of the drug comparable with those in humans who reported “feeling high” within minutes of self-administering the drug (Jatlow, 1987; Canez *et al.*, 1992). Blood samples (0.5 ml) were collected at 3, 10, 30 and 60 min after saline or cocaine infusion. Blood samples were centrifuged at 2300 *g* for 5 min as collected; the serum was removed and stored at –20°C until assayed for LH and FSH by radioimmunoassay.

In the second half of this experiment, oestradiol benzoate-treated, ovariectomized rats were infused at 10:00 h via the indwelling venous cannula with 100 µl PBS only (*n* = 9 rats) or 2 mg cocaine hydrochloride in PBS kg⁻¹ (*n* = 8 rats) (‘no-GnRH’ groups). After 20 min, a single 0.5 ml blood sample was collected within a 30–60 s time interval. Two additional groups of rats were infused via the indwelling cannula with 200 ng synthetic GnRH in 100 µl PBS vehicle (pH 7.0; ‘GnRH’ groups), followed immediately by 100 µl PBS or 2 mg cocaine hydrochloride kg⁻¹. An additional 0.5 ml blood sample was collected 20 min after GnRH infusion. The 20 min post-GnRH infusion interval was chosen to measure the maximum increase in GnRH-stimulated LH secretion and the stimulating effect of cocaine is still present (see Fig. 1) (Aiyer *et al.*, 1974; Libertun *et al.*, 1974). Blood samples were centrifuged at 2300 *g* for 5 min as collected; the serum was removed and stored at –20°C until assayed for LH and FSH by radioimmunoassay.

Gonadotrophin secretion in vitro

Oestradiol benzoate-treated, ovariectomized rats (*n* = 26) were killed by decapitation. The anterior pituitary glands were removed rapidly and pooled together in cold Hepes buffer solution (pH 7.2). The glands were minced finely with a sterile scalpel and scissors. The tissue was incubated with continuous stirring in a mixture of 0.1% (w/v) hyaluronidase (Gibco, Santa Clara, CA), 0.35% (w/v) collagenase (Gibco) and 3% (w/v) bovine serum albumin (fraction V; Sigma Chemical Co.) at 37°C for 45 min. After initial enzymatic cell dispersal, the mixture was centrifuged

at 500 *g* for 2 min. The resulting pellet was resuspended in HEPES buffer solution containing 0.25% (w/v) viocase (Sigma Chemical Co.) and stirred for 15–30 min at 37°C. The dispersed cells were counted using a haemocytometer and > 80% of the cells were diluted to a concentration of 2.5×10^5 viable cells ml^{-1} growth medium. Growth medium consisted of Dulbecco's modified Eagle's medium (DMEM) with the following substances: 10% horse serum, 2.5% fetal calf serum, 0.1 mmol glutamine l^{-1} , 1% (w/v) nonessential amino acids and penicillin–streptomycin (100 iu ml^{-1} and 100 $\mu\text{g ml}^{-1}$, respectively). The cells were added to Falcon multi-well dishes (Falcon Plastics, Los Angeles, CA) in a volume of 0.5 ml growth medium per well and incubated at 37°C in a water-saturated atmosphere of 5% CO_2 and 95% air. After 3 days to allow cell attachment, the original growth medium was discarded and the cells were washed three times with 500 μl DMEM.

DMEM (400 μl) containing final concentrations of 10^{-10} , 10^{-9} or 10^{-7} mol synthetic GnRH l^{-1} , or DMEM without GnRH, was added to the wells. This range of GnRH concentrations was chosen on the basis of published reports using synthetic GnRH to stimulate LH and FSH release from dispersed rat pituitary cells *in vitro* (Drouin *et al.*, 1976; Tang, 1980). DMEM (100 μl) containing cocaine hydrochloride at a final concentration of 25 ng ml^{-1} was added at this time to half of the wells. An equal volume of DMEM without cocaine hydrochloride was added to the other wells. After 30 min, the medium was removed from each well and stored at -20°C for subsequent measurements of LH and FSH by radioimmunoassay.

Gonadotrophin assays

Serum LH concentrations were measured in duplicate using a rabbit anti-ovine LH antibody (No. 15; G. Niswender, Colorado State University, Fort Collins, CO) at a final dilution of 1:120 000. Purified ovine LH was iodinated with ^{125}I to a specific activity of 304 $\mu\text{Ci mg}^{-1}$ according to the method of Hunter and Greenwood (1962). The standard was rat LH (NIDDK-LH-RP1) provided by A. Parlow (NIDDK, Bethesda, MD). Serum samples were diluted to a volume of 300 μl with 0.5% (w/v) BSA in 0.05 mol phosphate buffer l^{-1} . Primary antibody (100 μl) was added and the samples were incubated overnight at 4°C. Labelled LH (100 μl) was then added to each sample. A suspension (500 μl) of iron-containing polymer beads coated with donkey anti-rabbit gamma-globulin (1:3 dilution) was added to each sample and vortexed for 5 min. The incubation was continued for another hour at room temperature. The samples were placed on a magnetic base for 15 min to separate bound from free hormone (Amersham Magnetic Particle Separations System; Amersham Corporation, Arlington Heights, IL). Supernatants were discarded and the radioactivity in the magnetically retrieved pellet was measured in a gamma counter. Maximum binding and non-specific binding were 25 and 1.9%, respectively. Assay sensitivity was 0.88 ng per tube. Intra-

Fig. 1. Effects of acute administration of cocaine on pituitary (a) LH and (b) FSH secretion in ovariectomized, oestrogen-replaced rats. Rats were ovariectomized 3 weeks earlier and treated with oestrogen for the final 3 days of the experiment. Rats were divided into two treatment groups: i.v. saline administration (●; $n = 8$ rats) or i.v. administration of 2 mg cocaine hydrochloride kg^{-1} (○; $n = 8$ rats). Dashed line indicates time of administration. On the final day, an initial blood sample was collected via a jugular vein cannula. Serum LH concentrations increased in response to the acute administration of cocaine infusion in ovariectomized, oestrogen-replaced rats. Serum LH concentrations subsequently decreased over the next 60 min to pre-cocaine infusion concentrations. Acute exposure to cocaine had no significant effect on serum FSH concentrations in ovariectomized, oestrogen-replaced rats. Data are expressed as mean $\pm$ SEM. *Significantly different from saline-treated rats at this time ($P < 0.05$).

and interassay coefficients of variation were 5.5 and 6.7%, respectively.

Serum FSH concentrations were measured using a specific hFSH antiserum (A. Parlow, NIDDK) at a final dilution of 1:125 000. Purified rat FSH was iodinated with ^{125}I to a specific activity of 365 $\mu\text{Ci mg}^{-1}$, according to the method of Hunter and Greenwood (1962). The standard was rat FSH (NIDDK-FSH-RP1; A. Parlow, NIDDK). Serum samples with diluent added to a total volume of 300 μl were

Fig. 2. Effect of acute administration of cocaine on GnRH-stimulated (a) LH and (b) FSH secretion in ovariectomized, oestrogen-replaced rats. Rats were ovariectomized 3 weeks earlier and treated with oestrogen for the final 3 days of the study. On day 3, two sets of rats were infused at 10:00 h via an indwelling venous cannula with 100 μ l PBS only ($n = 9$ rats) or 2 mg cocaine hydrochloride in PBS kg^{-1} ($n = 8$ rats) ('no-GnRH' groups; $\square$). These rats were not infused with GnRH. After 20 min, a single 0.5 ml blood sample was collected within a 30–60 s time interval. Two additional groups of rats were infused via the indwelling cannula with 200 ng synthetic GnRH in 100 μ l PBS vehicle (pH 7.0; 'with GnRH'; $\boxtimes$), followed immediately by 100 μ l PBS ($n = 8$ rats) or 2 mg cocaine hydrochloride kg^{-1} ($n = 8$ rats). A 0.5 ml blood sample was collected 20 min after the infusion of GnRH for determination of serum LH and FSH concentrations. Serum LH (but not FSH) concentrations increased minimally in response to cocaine in rats that had not been infused with GnRH. GnRH infusion resulted in a significant increase in serum LH concentrations (* $P < 0.001$ versus the 'no GnRH' saline-infused group). However, no significant differences between saline-infused controls and cocaine-treated animals were observed for GnRH-stimulated LH or FSH release. Data are mean $\pm$ SEM.

preincubated for 48 h at 4°C. Iodinated FSH (100 μ l) was added and the incubation was continued for an additional 72 h at 4°C. At that time, 500 ml of a suspension of iron-containing polymer beads coated with donkey anti-rabbit gamma-globulin (1:2 dilution) was added to each sample. Bound and free hormone were separated (Amerlex-M Magnetic Separations System). After discarding the

supernatant, radioactivity was measured in a gamma counter. Maximum binding and non-specific binding were 30 and 1.5%, respectively. Assay sensitivity was 32 ng per tube. Intra- and interassay coefficients of variation were 5.2 and 5.9%, respectively.

Statistical analysis

Data are expressed as mean $\pm$ SEM. Differences within and among treatment groups were analysed initially by two-way ANOVA for repeated measures. Specific mean differences were evaluated further using a post-test for linear trends (Fig. 1; GraphPad: Prism, Version 2.0, San Diego, CA). Specific differences between controls and cocaine-treated groups were analysed further using the Bonferroni test (Figs 2 and 3; GraphPad: Prism, Version 2.0). The level of significance was $P \leq 0.05$.

Results

The acute effects of cocaine on serum concentrations of LH and FSH in ovariectomized, oestrogen-replaced rats are shown (Fig. 1). Mean serum LH and FSH concentrations were 20.5 ± 4.5 and 815 ± 82 ng ml^{-1} , respectively, in ovariectomized, oestrogen-treated rats. Within 10 min after i.v. administration of 2 mg cocaine hydrochloride kg^{-1} , circulating LH (Fig. 1a) and FSH (Fig. 1b) concentrations increased approximately 119% ($P < 0.05$) and 13% (not significant), respectively, versus saline-infused controls. Serum LH concentrations decreased during the next 60 min to concentrations similar to those observed before administration of cocaine.

The acute effects of cocaine on GnRH-stimulated increases in serum LH and FSH concentrations in ovariectomized, oestrogen-replaced rats are shown (Fig. 2). Basal concentrations of serum LH and FSH were 21.4 ± 2.3 and 780.2 ± 127.4 ng ml^{-1} , respectively, in ovariectomized, oestrogen-treated rats. Intravenous infusion of 100 ng synthetic GnRH ml^{-1} resulted in significant increases in both LH and FSH concentrations compared with basal concentrations. Administration of cocaine had no significant effect on GnRH-induced increases in either LH or FSH responses to GnRH infusion.

The acute effects of cocaine on GnRH-stimulated LH and FSH secretion from pituitary cells in culture are shown (Fig. 3). Basal concentrations of LH and FSH were 72 ± 12 and 89 ± 18 ng per 5×10^5 cells, respectively, in cultures of rat pituitary cells obtained from ovariectomized, oestrogen-treated rats. Addition of 25 ng cocaine hydrochloride ml^{-1} to the cell cultures had no effect on either LH or FSH release. With addition of synthetic GnRH at concentrations ranging from 10^{-10} to 10^{-7} mol l^{-1} , LH and FSH release increased in a dose-dependent fashion. Addition of 10^{-7} mol GnRH l^{-1} resulted in a 676–743% increase relative to basal LH release and 228–245% increase relative to basal FSH release. The addition of 25 ng cocaine hydrochloride ml^{-1} had no effect on the GnRH-stimulated, dose-

Fig. 3. Effect of cocaine on GnRH-stimulated LH and FSH secretion from cultured rat pituitary cells. Anterior pituitary glands were collected from oestradiol benzoate-treated, ovariectomized rats. Dispersed pituitary cells were maintained in culture at a concentration of 2.5×10^5 viable cells per ml growth medium. LH and FSH were measured in aliquots of medium collected 30 min after exposure of the cells to GnRH or cocaine or both. (a,b) Exposure of the cells to 2.5–100.0 ng cocaine hydrochloride (cocaine HCl) ml⁻¹ had no effect on LH or FSH release. (c,d) Exposure of the cells to 10^{-10} – 10^{-7} mol GnRH l⁻¹ stimulated LH and FSH release (□). Concurrent exposure of the cells to 25 ng cocaine hydrochloride ml⁻¹ had no effect on GnRH-stimulated LH or FSH release (■). Data are expressed as mean $\pm$ SEM. * $P < 0.01$ and ** $P < 0.001$.

dependent release of LH and FSH from cultured pituitary cells.

Discussion

The results of the present study demonstrate that acute administration of cocaine stimulates pituitary gonadotrophin secretion in ovariectomized rats transiently but has no effect on GnRH-stimulated pituitary gonadotrophin secretion in ovariectomized rats. Furthermore, cocaine has no apparent effect on GnRH-stimulated gonadotrophin secretion from pituitary cells in culture. On the basis of these results it is suggested that acutely administered cocaine stimulates the release of hypothalamic GnRH, which, in turn, stimulates pituitary gonadotrophin secretion. Acute administration of cocaine does not appear to affect pituitary gonadotrophin secretion directly.

Cocaine abuse is often associated with reproductive dysfunction in women (Siegel, 1982; Cocores *et al.*, 1986; Teoh *et al.*, 1994). Cocaine abuse has also been associated with altered pituitary volume (Teoh *et al.*, 1993) and secretion of pituitary hormones, including gonadotrophins, in humans (Mendelson *et al.*, 1989). However, potential sites of action and the mechanism of action for cocaine-induced reproductive dysfunction have not been defined precisely. Over the past two decades several research groups have attempted to define the effects of cocaine on pituitary function using animal models. Steger *et al.* (1981) reported that acute i.p. administration of 10 or 20 mg cocaine hydrochloride kg⁻¹ increased, whereas acute i.p. administration of 40 mg cocaine hydrochloride kg⁻¹ decreased, serum LH concentrations in ovariectomized rats. Dada and Horacek (1991) showed that i.v. injection of cocaine hydrochloride at concentrations of 1–3 mg kg⁻¹

decreased circulating LH concentrations but had no apparent effect on circulating FSH concentrations in orchidectomized rats. King *et al.* (1993) reported that daily s.c. administration of 10 mg cocaine hydrochloride kg^{-1} to rats undergoing regular oestrous cycles resulted in marked disruption of oestrous cyclicity and decreased ovulation rates. Likewise, Potter *et al.* (1999) demonstrated that daily i.v. administration of 1 or 2 mg cocaine hydrochloride kg^{-1} throughout the follicular phase (days 2–14 of the oestrous cycle) leads to disruption of menstrual cyclicity and ovulation rates in rhesus monkeys.

Mello *et al.* (1990a,b) demonstrated that acute i.v. administration of 0.4 and 0.8 mg cocaine hydrochloride kg^{-1} results in increased concentrations of circulating LH in female rhesus monkeys during the early follicular phase. In mid-luteal phase rhesus monkeys, administration of 0.4 mg cocaine kg^{-1} had no effect on serum LH or FSH concentrations, whereas administration of 0.8 mg cocaine kg^{-1} stimulated an increase in serum LH (but not FSH) concentrations (Mello *et al.*, 1993). Similarly, Mendelson *et al.* (2001) have shown that administration of 0.2 mg cocaine kg^{-1} to women did not affect pituitary LH secretion but that administration of 0.4 mg cocaine kg^{-1} stimulated significant increases in LH during both the follicular and luteal phases of their cycles. These studies indicate that the acute effects of cocaine on stimulation of pituitary gonadotrophin secretion are dependent on both dosage and cycle phase. Furthermore, Mello *et al.* (1990b, 1995) reported that i.v. administration of 0.4 mg cocaine kg^{-1} significantly enhanced GnRH stimulation of LH and FSH in rhesus monkeys during the early follicular phase, an effect that may have occurred either at the pituitary gonadotroph or the hypothalamic GnRH neurones. The apparent differences between our studies using rats and monkeys and those of Mello *et al.* (1990a,b, 1993) using monkeys may be a function of differences in drug concentrations or other experimental parameters. Apart from such methodological differences, cocaine clearly has significant effects on the hypothalamic GnRH–pituitary gonadotrophin axis and, thus, on cyclic reproductive function. What is not as obvious is whether cocaine exerts these effects on hypothalamic GnRH release or pituitary gonadotrophin secretion.

Mello *et al.* (1990b) have shown that cocaine enhances GnRH-stimulated LH secretion in female rhesus monkeys. Using deconvolutional pulse analysis, these workers noted that cocaine does not affect the elimination half-life of LH and suggested that cocaine may stimulate a burst of hypothalamic GnRH release with a resultant transient increase in pituitary LH release. The results of the present study using ovariectomized, oestrogen-replaced rats, in which cocaine treatment caused a transient increase in circulating LH (but not FSH) concentrations, support their conclusion. However, acute i.v. administration of 2 or 4 mg cocaine hydrochloride kg^{-1} decreased circulating concentrations of LH in ovariectomized rhesus monkeys but had no effect on GnRH-stimulated gonadotrophin secretion in

ovariectomized rhesus monkeys (Chen *et al.*, 1998). The results of Chen *et al.* (1998) compare favourably with the results of the present study in which cocaine had no effect on GnRH-stimulated LH or FSH release either *in vivo* or *in vitro*. Thus, the acute administration of cocaine does not affect pituitary gonadotrophs directly but instead stimulates a short-lived burst of hypothalamic GnRH release.

Cocaine could interfere with the normal secretion of hypothalamic GnRH via multiple mechanisms. Hypothalamic GnRH release is modulated by noradrenergic, dopaminergic and serotonergic systems (Kordon and Glowinski, 1972; Kalra and Kalra, 1983; Kalra, 1985). Changes in the patterns of aminergic activity regulating GnRH release could be expected to disrupt oestrous cyclicity in rats and menstrual cyclicity in primates. The effects of cocaine to alter aminergic neurotransmitter activity are well documented (for a review see Jatlow, 1987). Thus, it is not surprising that acute administration of cocaine, as shown in the present study, results in transient release of hypothalamic GnRH. In contrast, chronic cocaine administration could downregulate the aminergic regulation of hypothalamic GnRH neurones and, thus, disrupt normal pulsatile GnRH secretion. *In vitro*, noradrenaline-stimulated (King *et al.*, 1990) and phorbol ester-stimulated (King *et al.*, 1992) GnRH release is inhibited in slices of hypothalamic tissue obtained from rats injected s.c. with 10 mg cocaine kg^{-1} for 2 weeks. Thus, it is reasonable to postulate a hypothalamic site of action for both the acute and chronic effects of cocaine on the reproductive neuroendocrine axis.

In conclusion, serum LH concentrations increase within 10 min of i.v. administration of cocaine to ovariectomized, oestrogen-replaced rats, an effect that could be attributed to an effect of the drug on either hypothalamic GnRH neurones or pituitary gonadotrophs. However, acute administration of cocaine had no effect on GnRH-stimulated increases in either serum LH or FSH in ovariectomized, oestrogen-replaced rats. Furthermore, GnRH-stimulated LH and FSH secretion from pituitary cells in culture was unaffected by the addition of cocaine. These results indicate that acute exposure to cocaine does not have a direct effect on pituitary gonadotrophs in female rats. Without excluding possible direct effects of cocaine on ovarian function, it is suggested that an effect of this drug on hypothalamic GnRH neurones or the neurotransmitter systems regulating the hypothalamic GnRH neurones probably accounts for cocaine-induced cyclic reproductive dysfunction.

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