Corticotropin-Releasing Hormone Effects on Luteinizing Hormone and Cortisol Secretion in Intact Female Rhesus Macaques

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ABSTRACT

It is generally accepted that corticotropin-releasing hormone (CRH) is the central mediator of stress-activated changes in the pituitary-adrenal axis because it results in the release of ACTH and ultimately increases the systemic levels of cortisol. And, because in some situations CRH also inhibits the hypothalamic release of GnRH, it has been presumed that it plays the central role in stress-related reduction in pituitary-gonadal function as well. We previously have shown that 6 h of restraint stress in intact female rhesus macaques suppresses plasma levels of LH in the follicular but not the luteal phase of the menstrual cycle and that this effect lasts beyond the period of restraint. Since CRH inhibits both the GnRH pulse generator and LH release in ovariectomized macaques and is generally thought to be the central mediator of stress-induced inhibition of gonadotropin release, we investigated the influence of CRH administration on LH in undisturbed intact female rhesus macaques. Blood samples were collected at 15-min intervals for 15 h from a remote site from female macaques in both the follicular and luteal phase. During this time, each animal received a 4-h infusion of CRH (100-µg bolus followed by 100 µg/h for 4 h) through an indwelling jugular catheter. Blood samples were collected for an additional 8 h after cessation of the CRH infusion. Cortisol levels were significantly elevated during and after the CRH infusion and were comparable to levels observed in animals that experienced restraint. However, CRH did not suppress LH levels in either the follicular or the luteal phase. These data suggest that CRH is not the only mediator of stress-induced inhibition of gonadotropin release in primates.

INTRODUCTION

We have recently shown that restraint stress inhibits LH release in the follicular phase of the menstrual cycle of female rhesus macaques [1]. Current opinion would favor corticotropin-releasing hormone (CRH) as the central mediator of this effect of stress since previous studies have demonstrated that CRH inhibits LH secretion in female rats by inhibiting the release of GnRH into the hypophysial-portal circulation [2] and can directly suppress GnRH release from hypothalamic tissue in vitro [3, 4]. Furthermore, LH release is diminished by i.v. administration of CRH in ovariectomized rhesus macaques [5, 6] and in intact women [7, 8], but not in agonadal women [9]. In addition, CRH administered i.v. to ovariectomized rhesus macaques decreases the frequency and duration of the intermittent volleys of electrical activity in the “GnRH pulse generator” located in the medial basal hypothalamus, and the frequency of the temporally associated LH pulses [10]. However, CRH given centrally to intact ewes causes a stimulation of LH release [11]. Whether this discrepancy between primate and ovine studies is due to the gonadal state of the animal or species difference is not known.

Although indirect evidence supports the hypothesis that CRH mediates the inhibitory effects of stress on gonadotropin secretion, direct evidence for the hypothesis is scant. The only study that directly implicates CRH demonstrated that suppression of pulsatile LH in male rats induced by footshock can be reversed by central administration of α-helical CRH, an antagonist to CRH, or by passive immunoneutralization with an antibody against CRH [12]. Evidence in primates that supports this hypotheses reveals that administration of ACTH to female rhesus macaques also disrupts the menstrual cycle [13, 14] and inhibits cyclic release of estrogens, LH, and progesterone. This effect in macaques can be duplicated by cortisol administration and appears to result from a direct effect of cortisol on the ovary since gonadotropin levels are not affected [14]. In addition, the cyclic fluctuations in both estrogen and progesterone observed in the menstrual cycle may also influence how the hypothalamic-pituitary-ovarian axis responds to stress since there appears to be a change in responsiveness of the hypothalamic-pituitary-adrenal axis that depends on levels of these ovarian hormones [15, 16].

To examine whether the stress-induced inhibition of the pituitary-ovarian axis could be partially due to the action of CRH, we determined how peripheral CRH administration affected the pituitary-adrenal axis and the pituitary-ovarian axis by measuring plasma levels of cortisol and LH in both the follicular and luteal phase of the menstrual cycle in rhesus macaques. These studies were conducted in the animal’s home cage and in the absence of any acute stressor.

MATERIALS AND METHODS

Subjects

Thirteen adult female rhesus macaques (Macaca mulatta), 5–8 kg in weight, were housed in the same room.

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TABLE 1. Individual animals included in each of the groups are indicated below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (100 µg)</th>
<th>CRH (200 µg)</th>
</tr>
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<tbody>
<tr>
<td>Follicular</td>
<td>8548 A159 T33</td>
<td>11892 L97 T39</td>
</tr>
<tr>
<td></td>
<td>9126 AG59 T33</td>
<td>12410 L125 AG59</td>
</tr>
<tr>
<td>Luteal</td>
<td>T17 T17</td>
<td>T27 A159 T33</td>
</tr>
<tr>
<td></td>
<td>T33 AG59 T39</td>
<td>T39 R83</td>
</tr>
</tbody>
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in individual cages under temperature (23 ± 2°C) and light-controlled conditions (lights-on 0645–1845 h). Animals were maintained on a diet of monkey chow and fresh fruit, with tap water available ad libitum. Routine daily animal care and maintenance consisted of feeding from 0700–0900 h, cage cleaning from 0900–1000 h, and an unrestricted afternoon feeding at 1600 h. Fresh fruit was provided three times a week. Entry into the primate room was restricted to the animal caretakers and personnel involved in the research project.

Experimental Procedures

Each subject was fitted with a cranial prosthesis surgically attached to the calvaria with dental acrylic [17]. After at least 2 wk of recovery from surgery, each animal was tethered with a stainless steel cable to a swivel device attached to the top of the cage. Most females adjusted quickly to the tether in that within a few days, they essentially ignored it and moved freely about the cage. Animals that did not adjust to the tether were not used. Tethering did not normally disturb the menstrual cycle, and animals were generally kept on the tether throughout the duration of the studies. Only animals that showed at least two consecutive normal menstrual cycles were used in these studies. When the animal had adapted to the tether, she was fitted with an indwelling cardiac catheter constructed from 18-gauge polyvinylchloride tubing. The catheter was introduced into the right atrium via the external or internal jugular vein. Once positioned in the heart, the free end of the catheter was tunneled s.c. to the cranial prosthesis and exteriorized via the tether unit. To allow remote collection of blood samples, an extension of the cardiac catheter attached to the swivel device at the top of the cage was passed through a small opening in the wall and connected to a three-way stopcock in the adjacent laboratory. Patency of the catheter was maintained by continuous infusion (0.6 ml/h) of heparinized physiological saline (10 U/ml).

On days when experiments were conducted, blood samples (1 ml) were collected through the indwelling catheter at 15-min intervals for 15 h beginning at 0700 h. The blood was immediately transferred from the plastic syringe into prechilled siliconized glass tubes containing 10 µl 14% EDTA. During the course of each experiment, blood samples were centrifuged at 5-h intervals, and the plasma was withdrawn and stored frozen at −20°C in polypropylene vials until assays were performed.

Human CRH (Bachem, Torrance, CA) was dissolved in sterile physiological saline (100 µg/5 ml) immediately before administration. After 3 h of control samples were taken, a 100-µg CRH bolus was administered to animals in the mid-follicular (Days 7–9; n = 4) or midluteal (Days 19–24; n = 4) phase of the menstrual cycle through an indwelling venous catheter at 1000 h followed by a constant infusion of 100 µg/h (13.3 to 14.4 µg/kg BW) for 4 h. The CRH was infused through the same catheter from which blood samples were collected. This resulted in an interruption of CRH administration for approximately 1–2 min each 15 min. The CRH and vehicle were withdrawn from the catheter, and approximately 0.5 ml of blood (in heparin) was taken before the sample for hormonal measurements was collected. The 0.5 ml of blood was replaced, and the catheter was cleared with heparinized saline before the CRH was rein-

![FIG. 1. LH and cortisol levels in blood samples collected at 15-min intervals for 15 h in representative animals in the follicular (8545) and luteal (T17) phase of the menstrual cycle.](image-url)
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Blood samples were collected for an additional 8 h after CRH treatment ceased. In addition, two animals in the mid-follicular phase of the cycle were given a 200-μg bolus followed by 200 μg/h for 4 h. Follicular (n = 5) and luteal phase (n = 4) control animals were given vehicle (sterile saline) instead of CRH. Animals included in each group are shown in Table 1. In animals that were used more than once, a period of at least 2 wk rest was allowed between trials. Throughout the experiment, normal human traffic related to animal husbandry was permitted. Otherwise, the animals were not disturbed throughout the experimental period.

FIG. 2. LH and cortisol levels in 4 animals given 100 μg CRH in both the follicular phase (left panel) and the luteal phase (right panel) of the menstrual cycle. CRH was administered i.v. for 4 h beginning at 1000 h as indicated by the box bracketed by the dashed lines.
FIG. 3. LH and cortisol levels in one of two animals given 200 μg CRH in the follicular phase of the menstrual cycle. CRH was administered i.v. for 4 h beginning at 1000 h as indicated by the box bracketed by the dashed lines.

**Assays**

Plasma concentrations of LH were measured in 5- or 10-μl plasma aliquots with a Leydig cell bioassay [18, 19]; results are expressed as ng NICHD rh LH RP-1(WDF-XV-20)/ml. Sensitivity of the assay was 0.1 ng with 10 μl, and only an occasional sample fell below this level; those points are plotted directly on the abscissa in the figures. Intra- and interassay coefficients of variation were 10% and 16%. Estradiol [20], progesterone [21], and cortisol [22] were measured in plasma by established RIAs after samples were extracted with ether. Recoveries after ether extraction averaged 85% for estradiol, 88% for progesterone, and 80% for cortisol; and the immunoassay data were corrected for recovery. Assay blanks were 3 pg for estradiol, 7 pg for progesterone, and 11 pg for cortisol. Because of the amount of serum required for the estradiol assay (200 μl), this hormone was measured only in the blood sample (2.0 ml) taken at the initiation of each trial. The intra- and interassay coefficients of variation were 6% and 15% for estradiol, 3% and 11% for progesterone, and 4% and 9% for cortisol.

**Data Analyses**

Group data for cortisol and LH, obtained by collapsing the plasma values for each animal across three intervals (0700–1000 h; 1000–1400 h; 1400–2200 h) based on the period of CRH administration (1000–1400 h), were submitted to split-plot ANOVA. The independent component of the design consisted of controls and CRH-treated groups, and the repeated measures component consisted of the three time intervals indicated above. Animals served as blocks in the main plots. Time intervals served as sub-plots. An F-protected, predicted-difference test was used to examine sub-plot-least squares means (means in the time-by-treatment interaction).

Because the time blocks were small (3–8 h) in the present study, we feel that changes in mean levels of the hormones are more indicative of relevant physiological changes than is pulse analysis. This is particularly true in the luteal phase when LH and progesterone pulses are less frequent than in the follicular phase.

FIG. 4. Mean ± SEM LH and cortisol levels in control (n = 5) and CRH-treated (n = 4) animals in the follicular phase of the menstrual cycle. Data were collapsed for three time periods before, during, and after CRH administration. Asterisks indicate a significant difference (p < 0.05) from the control level for that group.
RESULTS

Plasma levels of LH and cortisol are shown for representative control animals in both the follicular phase (8545) and luteal phase (T17) in Figure 1. Animals in the follicular phase had estradiol levels that averaged 100 ± 29 pg/ml and progesterone levels of < 0.2 ng/ml, whereas luteal phase animals had estradiol levels of 23 ± 6 pg/ml and progesterone levels of 1.4 ± 0.25 ng/ml. As shown in Figure 1, the LH pattern is typical of what we have previously reported for intact female rhesus macaques [1, 23]. The effects of 4 h of CRH (100-µg bolus followed by 100 µg/h) infusion on LH and cortisol levels are shown for both follicular and luteal phase animals in Figure 2. Although CRH caused a moderate increase in cortisol levels, no effect on LH secretion was observed. In both phases of the cycle, LH pulses continued throughout the period of CRH administration. The LH and cortisol response of one of the two animals that received 200 µg CRH is shown in Figure 3. Both of these animals responded essentially the same as the animals that received only 100 µg. The mean LH and cortisol levels for control and CRH-treated animals in the follicular phase are shown in Figure 4. Mean LH levels did not change significantly in follicular phase control animals across the three time periods analyzed. ANOVA revealed that there was no significant effect of treatment on mean LH levels ($F_{1,14} = 0.15; p = 0.71$) or of treatment by period ($F_{2,14} = 1.85; p = 0.19$). The mean LH level during CRH infusion was not different from that observed before CRH treatment.

Mean levels of progesterone, LH, and cortisol during the luteal phase are shown in Figure 5. ANOVA of the LH data in the luteal phase showed there was no effect of treatment ($F_{1,12} = 0.12; p = 0.74$) or of period by treatment ($F_{2,12} = 2.31; p = 0.14$). As was observed in the follicular phase, there was no significant difference in mean LH levels in luteal phase control animals across the three time periods analyzed (Fig. 4). Analysis of the progesterone data in the luteal phase showed no effect of treatment or of period by treatment. There was no change in mean progesterone levels in the control or treatment animals across the three time periods investigated.

There was a significant period effect on cortisol levels in animals in both the follicular ($F_{2,14} = 40.79; p = 0.0001$) and luteal ($F_{2,12} = 16.34; p = 0.0005$) phase and a significant treatment by period effect ($F_{2,12} = 8.61; p = 0.005$) in the luteal phase. Although cortisol showed a significant increase in untreated follicular phase controls (Fig. 4) during the same time period when CRH was given to the experimental group ($p = 0.005$), the increase was significantly

![Fig. 5. Mean ± SEM LH, progesterone, and cortisol levels in control (n = 4) and CRH-treated (n = 4) animals in the luteal phase of the menstrual cycle. Data were collapsed for three time periods before, during, and after CRH administration. Asterisks indicate a significant difference (p < 0.05) from the control level for that group.](image-url)
greater in the experimental group \( (p \leq 0.05) \). The expected afternoon decrease \( (p \leq 0.02) \) in cortisol was observed in the control group, but not in animals treated with CRH. The mean cortisol level in the CRH-treated group was still significantly elevated \( (p \leq 0.05) \) above controls for the 8-h period after CRH treatment. In the luteal phase, CRH stimulated a more dramatic rise in cortisol (Fig. 5).

**DISCUSSION**

Our observations that peripheral administration of CRH in either the follicular or the luteal phase of the menstrual cycle in rhesus macaques does not inhibit pulsatile LH release is surprising in the light of previous observations in agonadal macaques that a comparable regimen of CRH suppressed both LH and FSH secretion \([5, 6]\) and multiunit activity in what is presumed to be the GnRH pulse generator \([10]\). Because plasma levels of cortisol were elevated to nearly the same degree after CRH administration as we have previously observed in females that experienced restraint stress \([1]\), we conclude that CRH was effective in stimulating the pituitary-adrenal axis. Likewise, behavioral changes (increased vocalization, huddling, and environmental exploration) similar to those previously reported \([23]\) after i.v. administration of CRH to rhesus macaques were observed after CRH administration in the present study. Therefore, we presume that the peripherally administered CRH had access to at least all parts of the nervous system outside the blood-brain barrier.

There are two differences in the experimental design between the present study and previous studies in female macaques \([5, 6, 10]\) that could explain the disparate results. The first is that in previous studies, CRH was administered to animals adapted to primate chairs whereas we administered CRH through indwelling catheters in undisturbed animals in their home cages. Cortisol levels in the restrained ovariectomized macaques were elevated before CRH was administered \([5, 6, 10]\), were typical of what we have observed in restrained females \([1]\), and were 2–3-fold elevated over levels observed in unrestrained individuals \([24]\). Therefore, the pituitary-adrenal axis may have already been activated before the CRH was administered, which would presumably impart an additional inhibitory influence on the GnRH pulse generator.

The second difference between these previous studies and the present report is that intact animals were used in the present study whereas ovariectomized females were experimental subjects in the previous studies \([5, 6, 10]\). This difference could be important since estrogen and progesterone affect the morphology of the central nervous system. Raisman and Field \([25]\) demonstrated a hormone-dependent sex difference in the dendritic spine density in the preoptic area of the hypothalamus, and since that report other investigators have shown gonadal steroid-dependent changes in synaptic formation in hypophysiotropic areas of the hypothalamus in both rodents \([26–28]\) and primates \([29]\). Therefore, access of CRH in the peripheral circulation to areas of the hypothalamus regulating GnRH release might be different between intact and agonadal individuals.

Another explanation for the lack of effect of CRH on LH release in this study and in male macaques \([30]\) is that CRH is not the sole mediator of the effects of stress on gonadotropin release, and that the inhibitory effect of this hypophysiotropic hormone is more pronounced in castrated animals. The participation of factors other that CRH in the suppression of gonadotropin secretion in response to a psychological or psychosocial stress is supported by the observation that ACTH release in subordinate rats living in a hierarchically structured colony is controlled primarily by vasopressin (arginine vasopressin) rather than CRH \([31]\). In addition, vasopressin has been implicated as a mediator of the interleukin-1α inhibition of gonadotropin secretion in female macaques \([32]\). Therefore, the release of other central mediators of the physiological response to stress in addition to CRH could explain the difference between the LH response in restrained animals \([1]\) and unstressed animals given CRH in the present study.

In similar studies in women, peripheral CRH lowered gonadotropin secretion in intact individuals in the follicular and luteal phases of the menstrual cycle \([7, 8]\), but had no effect on gonadotropin secretion in agonadal women \([9]\). In the latter study, the results might be explained by the fact that CRH was administered in a pulsatile fashion and at a much lower dose (2 μg/kg/90 min) than in the previous and present studies (10–20 μg/kg/h) in macaques. However, there is no apparent reason why a similar low dose of CRH (~2 μg/kg/h) infused over 3 h in intact women inhibited LH release \([7, 8]\), when a much higher dose (13–20 μg/kg/h) in the present study had no effect in intact female macaques. During CRH administration in both women \([7, 8]\) and female macaques (Figs. 4 and 5), cortisol levels were elevated approximately twofold over values obtained before CRH administration, suggesting a similar stimulation of the pituitary-adrenal axis.

In summary, the present data are consistent with the hypothesis that CRH is not the sole mediator of stress-induced inhibition of gonadotropin release. The present study is in agreement with similar results in intact male macaques where restraint inhibits LH and testosterone \([33]\) release but i.v. CRH administration in otherwise undisturbed animals does not inhibit LH release \([30]\).

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