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The Inhibitory Action of Corticotropin-Releasing Hormone on Gonadotropin Secretion in the Ovariectomized Rhesus Monkey Is Not Mediated by Adrenocorticotropic Hormone[1]

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ABSTRACT

Earlier observations in our laboratory indicated that i.v. infusion of human/rat corticotropin-releasing hormone (hCRH) suppresses pulsatile luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release in ovariectomized rhesus monkeys. Since cortisol secretion increased significantly as well, it was not possible to exclude the possibility that this inhibitory effect of hCRH on gonadotropins was related to the activation of the pituitary/adrenal axis. The purpose of the present study was to determine the role of pituitary/adrenal activation in the effect of hCRH on LH and FSH secretion. We compared the effects of 5-h i.v. infusions of hCRH (100 μg/h, n = 7) and of human adrenocorticotropic hormone (ACTH) (1–24) (5 μg/h, n = 3; 10 μg/h, n = 3; 20 μg/h, n = 3) to ovariectomized monkeys on LH, FSH, and cortisol secretion. As expected, during the 5-h ACTH infusions, cortisol levels increased by 176–215% of baseline control, an increase similar to that observed after CRH infusion (184%). However, in contrast to the inhibitory effect observed during the CRH infusion, LH and FSH continued to be released in a pulsatile fashion during the ACTH infusions, and no decreases in gonadotropin secretion were observed.

The results indicated that increases in ACTH and cortisol did not affect LH and FSH secretion and allowed us to conclude that the rapid inhibitory effect of CRH on LH and FSH pulsatile release was not mediated by activation of the pituitary/adrenal axis.

INTRODUCTION

It has long been known that stress, which activates the pituitary/adrenal axis, is disruptive to normal reproductive function (Ruisseau et al., 1978; Bullen et al., 1985; Rivier et al., 1986). Excess administration of adrenocorticotropic hormone (ACTH) or of glucocorticoids may interrupt normal gonadotropin secretion and interfere with menstrual cyclicity (Kim et al., 1974; Cunningham et al., 1975; Sakakura et al., 1975; Baldwin, 1979; Mann et al., 1982; Li and Wagner, 1983; Ringstrom and Schwartz, 1985; Matteri et al., 1986). In the male monkey, prolonged cortisol administration has been reported to inhibit pulsatile luteinizing hormone (LH) secretion (Dubey and Plant, 1985).

We have previously reported that peripheral human/rat corticotropin-releasing hormone (hCRH) administration rapidly inhibits pulsatile LH and follicle-stimulating hormone (FSH) secretion in the ovariectomized rhesus monkey (Olster and Ferin, 1987). Predictably, this decrease in gonadotropins parallels an increase in serum cortisol concentrations. Thus, whether the inhibitory action of hCRH is a direct one or is the result of activation of the adrenal axis remains to be determined. In the present study, we have compared the effects of human $ACTH^{1-24}$ and hCRH i.v. infusions on gonadotropins and cortisol levels in ovariectomized rhesus monkeys to determine whether activation of the pituitary/adrenal axis can account for the observed rapid effects of CRH on LH and FSH release.

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MATERIALS AND METHODS

Animals

Seven adult female rhesus monkeys *(Macaca mulatta)* were ovariectomized at least 6 wk before the experiments began. Animals were housed individually in light- and temperature-controlled rooms (12L:12D). Monkey chow was supplemented with fruit and vegetables. Water was available ad libitum.

Experimental Protocol

The study compared the effects of 5-h i.v. infusions of hCRH and of ACTH on LH, FSH, and cortisol secretion in 7 animals. (The effects of hCRH in 5 of these monkeys have been reported in a previous paper [Gindoff and Ferin, 1987].) Each animal received an infusion of CRH and one or more infusions of ACTH. Each of these infusions was administered on a separate day, with at least a 2-wk interval between two experimental protocols. On the evening before the experiment, monkeys were briefly sedated with ketamine-HCl (Warner Lambert Co., Morris Plains, NJ) (30–40 mg) while 2 catheters (20–22 g angiocath, one for blood collection, one for infusion) were inserted in the femoral and the saphenous veins, respectively. The animals were then placed in a primate chair, and the experiment was started the following morning. Peripheral blood samples were drawn at 15-min intervals throughout the experimental period (10h). After a 3-h control period, a 5-h infusion of hCRH (provided by Dr. N. Ling, Salk Institute San Diego, batch #145-268-13G-32-37) (100 μg/h; n = 7) was started. The infusion was followed by a 2-h observation period. Three doses of ACTH (human $ACTH^{1-24}$, Bachem Inc, Torrance, CA, Lot #7649) were used (5 μg/h, n = 3; 10 μg/h, n = 3; 20 μg/h, n = 3) following the above experimental protocol. Both hCRH and ACTH were dissolved in heparinized saline. A bolus of the same hourly dose was given prior to the infusion to achieve, without delay, a reasonable concentration of CRH and ACTH.

Hormone Assays

Sera were kept at −20°C until assayed for LH, FSH, and cortisol. LH and FSH were measured in duplicate in all samples by double-antibody radioimmunoassay (RIA), using reagents provided by the National Hormone and Pituitary Program (Olster and Ferin, 1987). In the LH assay, cercopithecus monkey pituitary LH as the ^{125}I-labeled antigen, partially purified rhesus monkey pituitary LH (NICHD rhLH) as the standard, and antiserum raised to human chorionic gonadotropin (hCG) were used. Intra- and interassay coefficients of variation (CV) were 6% and 9%, respectively. In the FSH assay, hFSH (NIH FSH-3) as the ^{125}I-labeled antigen, cynomolgus monkey FSH (NICHD-RPI), and antiserum raised to hFSH were used. Intra- and interassay CVs were 5% and 9%, respectively. Levels of LH and FSH are expressed in ng/ml. Cortisol concentrations were measured by a competitive protein-binding assay (Beardwell et al., 1968). Intra- and interassay CVs were 6% and 10.3%, respectively.

Data Analysis

The mean of all LH and FSH concentrations during the preinfusion period was calculated to yield baseline levels. LH and FSH secretion was also evaluated by a computer-assisted calculation of area under the curve. This was done by multiple-surface area calculations of trapezoids generated under the curve by the data points. Hourly changes in area under the curve during the infusion and postinfusion periods were then calculated as percentages of the preinfusion baseline. Significance between the different infusion groups was calculated by analysis of variance and Newman-Keul's test.

RESULTS

During the 3-h preinfusion control period, LH was released in the pulsatile pattern typical of ovariectomized monkeys (Dierschke et al., 1970). Mean baseline LH levels in all animals were 157.1 ± 16.1 ng/ml (± SEM). During the 5-h CRH infusion, LH levels declined progressively in all 7 monkeys, as reported previously by our laboratory (Gindoff and Ferin, 1987). By Hour 5 of infusion, LH secretion, as evaluated by area under the LH curve, was 44.7 ± 8.2% of the preinfusion value. In contrast, during the infusion of ACTH at doses of 5, 10, or 20 μg/h, LH levels did not significantly decrease, and LH secretion did not differ from that following a 5-h infusion of physiological saline (Gindoff and Ferin, 1987). By Hour 5, LH secretion in all animals receiving ACTH was significantly different from that in the animals infused with CRH (Table 1A).

During the 3-h preinfusion control period, mean baseline FSH levels were 69.6 ± 10 ng/ml. By Hour 5

TABLE 1. Effects of 5-h infusions of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion in ovariectomized monkeys.

Infusion	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7
A. LH							
CRH 100 μg/h	90.8 ± 4.7	69.0 ± 5.1	60.6 ± 7.9	47.2 ± 4.8	44.7 ± 8.2	45.0 ± 10.5	60.3 ± 10.7
ACTH 5 μg/h	98.8 ± 4.9	98.9 ± 5.8*	90.0 ± 10.1	99.6 ± 2.7**	93.8 ± 9.1**	90.9 ± 4.1*	93.2 ± 4.1
ACTH 10 μg/h	99.8 ± 1.6	93.7 ± 3.5*	89.9 ± 7.0*	92.8 ± 8.6**	89.4 ± 4.0**	86.0 ± 3.3*	88.1 ± 2.2
ACTH 20 μg/h	110.2 ± 8.0	91.9 ± 6.6*	103.7 ± 4.0*	100.2 ± 3.8**	95.6 ± 4.7**	94.3 ± 10.9*	97.6 ± 13.0
B. FSH							
CRH 100 μg/h	95.8 ± 1.8	88.1 ± 4.0	82.8 ± 4.8	81.2 ± 4.4	74.0 ± 3.7	72.0 ± 5.4	72.0 ± 6.4
ACTH 5 μg/h	106.9 ± 7.9	97.9 ± 4.7	92.5 ± 2.2	102.1 ± 7.6*	103.8 ± 6.4**	95.6 ± 6.0	99.5 ± 7.5
ACTH 10 μg/h	103.4 ± 3.5	92.5 ± 2.5	100.3 ± 4.7	104.1 ± 1.1*	94.6 ± 0.6*	92.6 ± 3.0	93.6 ± 2.2*
ACTH 20 μg/h	88.3 ± 6.4	87.1 ± 3.7	97.4 ± 0.8	97.0 ± 5.2	99.3 ± 1.7**	88.8 ± 5.6	98.1 ± 4.6*

[a]Mean (+SEM) hourly changes under the curve during the infusion (Hours 1–5) and postinfusion (Hours 6–7) periods are presented as a percentage of the preinfusion baseline area.

*$p<0.05$ vs. CRH.

**$p<0.01$ vs. CRH.

of the CRH infusion, FSH secretion was 74.0 ± 3.7% of the preinfusion value. No effects of ACTH infusions (at the 3 doses used) were observed. By Hour 5, FSH secretion in animals receiving ACTH was significantly different from that in the animals receiving CRH (Table 1B).

Mean baseline cortisol levels in our experimental setting (07.30–10.30) are shown on Table 2. Cortisol increased in all animals receiving either hCRH or ACTH. By Hour 5, cortisol levels were 183.9% (± 5.3%), 214.7% (± 23.8%), 176.0% (± 24.2%), and 171.8% (± 2.9%) of baseline control for the CRH, ACTH (5 μg/h), ACTH (10 μg/h), and ACTH (20 μg/h), respectively.

Figure 1 illustrates comparisons in 3 individual monkeys of the effects of CRH and of each of the three ACTH infusion doses.

DISCUSSION

In this study, we report that 5-h i.v. infusions of ACTH, at doses that comparatively elicit a maximal adrenal cortisol response in the human (Landon et al., 1964), do not interfere with normal pulsatile gonadotropin release in the ovariectomized monkey. Since these infusions also result in large increases in cortisol, one can conclude that overall stimulation of the pituitary/adrenal axis in itself does not acutely interfere with pituitary/gonadal function. Although several reports describe inhibitory effects of ACTH or glucocorticoids on gonadotropin secretion, these observations usually have been made after chronic administration of the compounds. For instance, in the orchidectomized rhesus monkey treated with hydrocortisone acetate, circulating LH and FSH began to decline only 35 days after initiation of the treatment (Dubey and Plant, 1985).

The absence of acute effects of ACTH on gonadotropin secretion in the monkey contrasts markedly with those observed following CRH infusion. The present result adds to our previous observations of an

TABLE 2. Effects of 5-h infusions of corticotropin-releasing hormone (CRH) and adrenocorticotropin hormone (ACTH) (Hours 1–5) on cortisol secretion in ovariectomized rhesus monkeys.*

Infusion	Baseline	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6	Hour 7
CRH 100 μg/h	27.5 ± 2.5	32.9 ± 3.4	42.1 ± 4.3	45.9 ± 5.1	50.0 ± 3.5	52.4 ± 6.1	51.9 ± 3.8	45.7 ± 6.2
ACTH 5 μg/h	31.0 ± 3.4	39.2 ± 3.6	44.8 ± 5.6	51.0 ± 7.1	55.8 ± 7.2	68.4 ± 15.8	58.0 ± 8.8	46.7 ± 10.2
ACTH 10 μg/h	34.1 ± 6.0	41.5 ± 4.4	49.8 ± 6.1	52.6 ± 5.4	56.8 ± 3.8	56.6 ± 3.8	59.7 ± 6.2	43.3 ± 6.6
ACTH 20 μg/h	35.3 ± 1.2	49.6 ± 4.6	67.4 ± 5.9	66.8 ± 5.2	66.0 ± 6.7	60.4 ± 2.9	52.5 ± 7.6	47.8 ± 6.9

*Cortisol levels are expressed as mean/h + SEM (μg/dl).

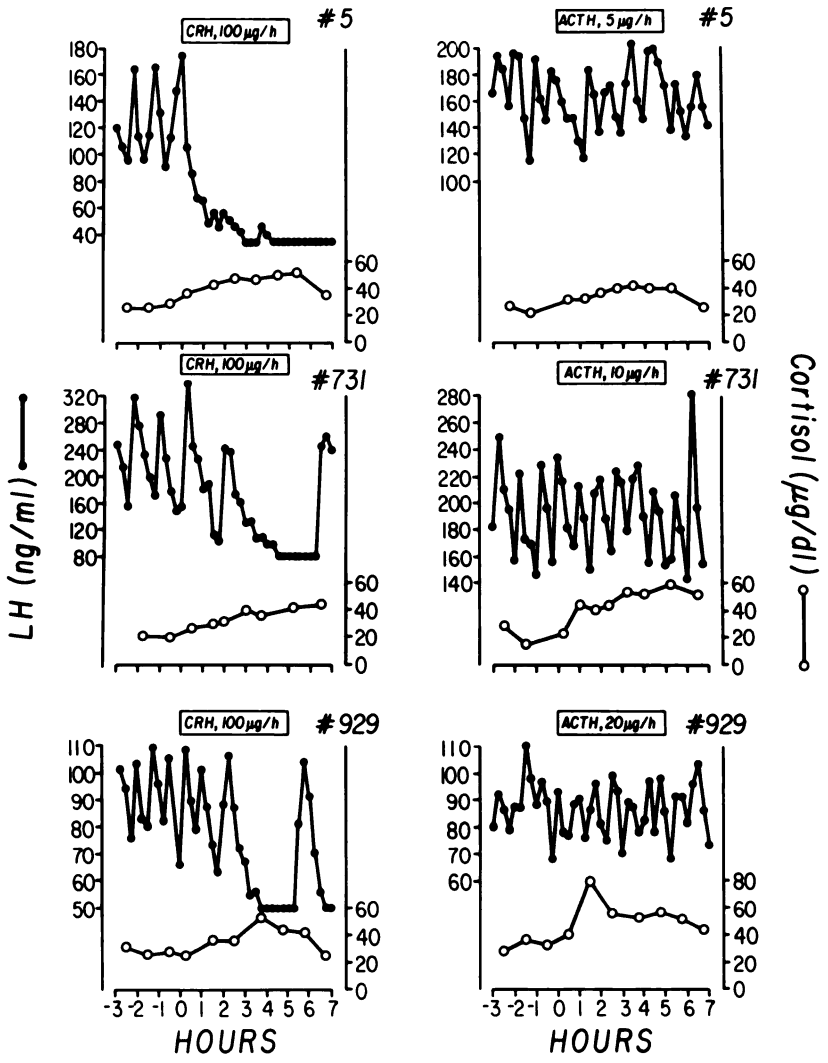

FIG. 1. Luteinizing hormone and cortisol responses to a 5-h i.v. infusion of human corticotropin-releasing hormone (*bCRH*: 100 μg/h) *(left)* or of adrenocorticotropin hormone (*ACTH*: 5 μg/h, 10 μg/h or 20 μg/h) *(right)* in 3 individual ovariectomized rhesus monkeys.

acute inhibitory effect of i.v. CRH administration on LH and FSH secretion in the rhesus monkey (Gindoff and Ferin, 1987; Olster and Ferin, 1987). Similar inhibitory effects were observed after intraventricular injections of CRH in the rodent (Ono et al., 1984; Rivier and Vale, 1984), and monkey (unpublished observation). The main purpose of the present experiment was to determine whether the pituitary/adrenal axis plays a role in this phenomenon in the primate. Our results indicate that this is unlikely, since the CRH effect cannot be mimicked by ACTH infusion under identical experimental conditions. Although we have used high doses of ACTH that stimulate cortisol secretion maximally, it is always possible that insufficiently high concentrations of ACTH have been reached locally within the pituitary gland or median eminence to simulate those obtained following CRH stimulation. Barring this possibility, which cannot be excluded at present, we conclude that, as also suggested in the rodent (Rivier and Vale, 1984; Petraglia et al., 1987), the rapid inhibition of gonadotropin secretion induced by CRH is mediated centrally within the hypothalamo/pituitary axis and not through activation of the adrenal axis. That this is so is further emphasized by previous observations in the primate (Gindoff and Ferin, 1987) and rodent (Petraglia et al., 1986), which demonstrate that the effect of CRH on LH secretion is modulated by

endogenous opioid peptides, since opioid antagonists or antisera can prevent the decrease in gonadotropins induced by CRH.

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REFERENCES

Baldwin DM, 1979. The effect of glucocorticoids on estrogen-dependent luteinizing hormone in the ovariectomized rat and on gonadotropin secretion in the intact female rat. Endocrinology 105:120–28

Beardwell CG, Burke CW, Cope CL, 1968. Urinary free cortisol measured by competitive protein binding. J Endocrinol 42:79–89

Blake CA, 1975. Effects of "stress" on pulsatile luteinizing hormone release in ovariectomized rats. Proc Soc Exp Biol Med 148: 813–15

Bullen BA, Skyinar GS, Beitins IZ, von Mering G, Turnbull BA, McArthur JW, 1985. Induction of menstrual disorders by strenuous exercise in untrained women. N Engl J Med 312:1349–53

Cunningham GR, Caperton EM, Goldzieher JW, 1975. Antiovulatory activity of synthetic corticoids. J Clin Endocrinol Metab 40: 265–67

Dierschke JD, Bhattacharya AN, Atkinson LE, Knobil E, 1970. Circhoral oscillations of plasma LH levels in the ovariectomized rhesus monkey. Endocrinology 87:850–53

Dubey AK, Plant TM, 1985. A suppression of gonadotropin secretion by cortisol in castrated male rhesus monkey (Macaca mulatta) mediated by the interruption of hypothalamic gonadotropin-releasing hormone release. Biol Reprod 33:423–31

Gindoff PR, Ferin M, 1987. Endogenous opioid peptides modulate the effect of corticotropin-releasing factors on gonadotropin release in the primate. Endocrinology 121:837–42

Kim MH, Hosseinian AH, Dupon C, 1974. Plasma levels of estrogen, androgens and progesterone during normal and dexamethasone-treated cycles. J Clin Endocrinol Metab 39:706–12

Landon J, James VHT, Cryer RJ, Wynn V, Frankland AW, 1964. Adrenocorticotropic effects of a synthetic polypeptide beta 1-24 corticotropin in man. J Clin Endocrinol Metab 24:1206–13

Li PS, Wagner WC, 1983. In vivo and in vitro studies on the effect of adrenocorticotropin hormone or cortisol on the pituitary response to gonadotropin releasing hormone. Biol Reprod 29:25–37

Mann DR, Jackson GG, Blank MS, 1982. Influence of adrenocorticotropin and adrenalectomy on gonadotropin secretion in immature rats. Neuroendocrinology 34:20–26

Matteri PL, Moberg GD, Watson JG, 1986. Adrenocorticotropin-induced changes in ovine pituitary gonadotropin secretion in vitro. Endocrinology 118:2091–96

Olster D, Ferin M, 1987. Corticotropin-releasing hormone (CRH) inhibits gonadotropin secretion in the ovariectomized rhesus monkey. J Clin Endocrinol Metab 65:262–67

Ono N, Lumpkin MD, Samson WK, McDonald JK, McCann SM, 1984. Intrahypothalamic action of corticotropin-releasing factor (CRF) to inhibit growth hormone and LH release in the rat. Life Sci 35:1117–23

Petraglia F, Sulton S, Vale W, Plotsky P, 1987. Corticotropin-releasing factor decreases plasma luteinizing hormone levels in female rats by inhibiting gonadotropin-releasing hormone release into hypophyseal-portal circulation. Endocrinology 120:1083–88

Petraglia F, Vale W, Rivier C, 1986. Opioid acts centrally to modulate stress-induced decrease in luteinizing hormone in the rat. Endocrinology 119:2245–50

Ringstrom SJ, Schwartz NB, 1985. Cortisol suppresses the LH but not the FSH response to gonadotropin-releasing hormone after orchidectomy. Endocrinology 116:472–74

Rivier C, Rivier J, Vale W, 1986. Stress-induced inhibition of reproductive functions: role of endogenous corticotropin-releasing factor. Science 231:607–09

Rivier C, Vale W, 1984. Influence of corticotropin-releasing factor on reproduction in the rat. Endocrinology 114: 914–21

Ruisseau PD, Tache Y, Brazeau P, Collu R, 1978. Pattern of adenohypophyseal hormone changes induced by various stressors in female and male rats. Neuroendocrinology 27:257–71

Sakakura M, Takebe K, Nakagawa S, 1975. Inhibition of luteinizing hormone secretion induced by synthetic LRH by long-term treatment with glucocorticoids in human subjects. J Clin Endocrinol Metab 40:774–79