Endocrine, *vol. 19, no. 2, 169–176, November 2002* 0969–711X/02/19:169–176/$20.00

Estrogen-Induced Gonadotropin Surge in Rhesus Monkeys Is Not Inhibited by Cortisol Synthesis Inhibition or Hypoglycemia

Marla E. Lujan,[1] Peter J. MacTavish,[1] Alicja A. Krzemien,[2] Michael W. Bradstock,[1] and Dean A. Van Vugt[1,2]

Departments of [1]Physiology and [2]Obstetrics and Gynaecology, Queen's University, Kingston, Ontario, Canada

Acute administration of corticotrophin-releasing hormone (CRH) has been shown to inhibit gonadotropin secretion in several species including rodents, sheep, humans, and nonhuman primates. Similarly, a variety of acute stressors have been shown to inhibit tonic gonadotropin secretion and may do so through a CRH mechanism. Stress-induced inhibition of tonic gonadotropin secretion below levels required for follicular maturation would be expected to inhibit ovulation. An additional mechanism whereby acute stressors could interfere with ovulation is through inhibition of the preovulatory gonadotropin surge. In the present study, we determined the effect of acute activation of the hypothalamic-pituitary-adrenal (HPA) axis on phasic gonadotropin secretion in female rhesus monkeys. Activation of the HPA axis was achieved by either a hypoglycemic challenge or blockage of cortisol synthesis with metyrapone, 24 h after an estradiol benzoate challenge. Neither metyrapone nor insulin-induced hypoglycemia inhibited gonadotropin secretion. In fact, the initiation of the luteinizing hormone and follicle-stimulating hormone surge was advanced by 7.4 ± 0.4 h ($p < 0.001$) and 4.8 ± 1.4 h ($p = 0.04$) respectively, in metyrapone-treated monkeys compared with saline controls. By contrast, hypoglycemia did not affect the gonadotropin surge. The gonadotropin surge was preceded by increased progesterone secretion in metyrapone-treated but not insulin-treated monkeys. This difference in progesterone secretion likely explains the advancement of the gonadotropin surge in the metyrapone-treated animals.

Key Words: Corticotropin-releasing hormone; metyrapone; hypoglycemia; surge; rhesus monkeys.

Introduction

Acute activation of the hypothalamic-pituitary-adrenal (HPA) axis has been demonstrated to inhibit gonadotropin secretion in several species *(1)*. Acute stressors that inhibit gonadotropin secretion include restraint *(2–4)*, insulin-induced hypoglycemia (IIH) *(5–10)*, footshock *(11,12)*, and endotoxemia *(13–15)*. It is generally accepted that acute activation of the HPA axis inhibits gonadotropin secretion by inhibiting gonadotropin-releasing hormone (GnRH) secretion and that this effect is mediated, in part, by increased corticotropin-releasing hormone (CRH) secretion *(16–18)*. Exogenous CRH inhibits tonic luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion in several species, including nonhuman primates *(19)* and women *(20)*, and does so independent of increased adrenocorticotropic hormone (ACTH) or cortisol secretion *(21,22)*. CRH can inhibit GnRH, as demonstrated by both in vivo *(11)* and in vitro studies *(23)*. CRH may directly affect GnRH neurons since CRH-containing neurons have been reported to synapse on GnRH neurons *(24)*. In addition, mediation by endogenous opiates is likely since CRH-induced inhibition of LH secretion and hypothalamic multiunit activity associated with LH pulses are reversed by opioid antagonism *(22,25)*.

HPA axis activation could interfere with ovulation by inhibiting gonadotropins on a chronic basis, thereby preventing gonadotropin-dependent follicular maturation. In the absence of follicular maturation, estrogen levels would not reach the threshold required to elicit a gonadotropin surge. Alternatively, one could speculate that activation of the HPA axis of a more acute nature could inhibit ovulation by interrupting or blocking the preovulatory gonadotropin surge. Such an effect would suggest a mechanism independent of GnRH since elimination of GnRH input does not disrupt the LH surge in the nonhuman primate *(26,27)*.

The objective of the present study was to determine whether activation of the HPA axis inhibits phasic gonadotropin secretion in the nonhuman primate. Specifically, we determined whether either IIH or administration of metyrapone, two stimuli known to increase CRH secretion *(28–36)*, affected the timing or magnitude of the estrogen-induced gonadotropin surge in rhesus monkeys.

Received July 17, 2002; Revised September 9, 2002; Accepted September 16, 2002.

Author to whom all correspondence and reprint requests should be addressed: Dean A. Van Vugt, Ph.D. Department of Obstetrics and Gynaecology, 3022 Etherington Hall, Queen's University, Kingston, Ontario, Canada K7L 3N6. E-mail: vanvugtd@post.queensu.ca

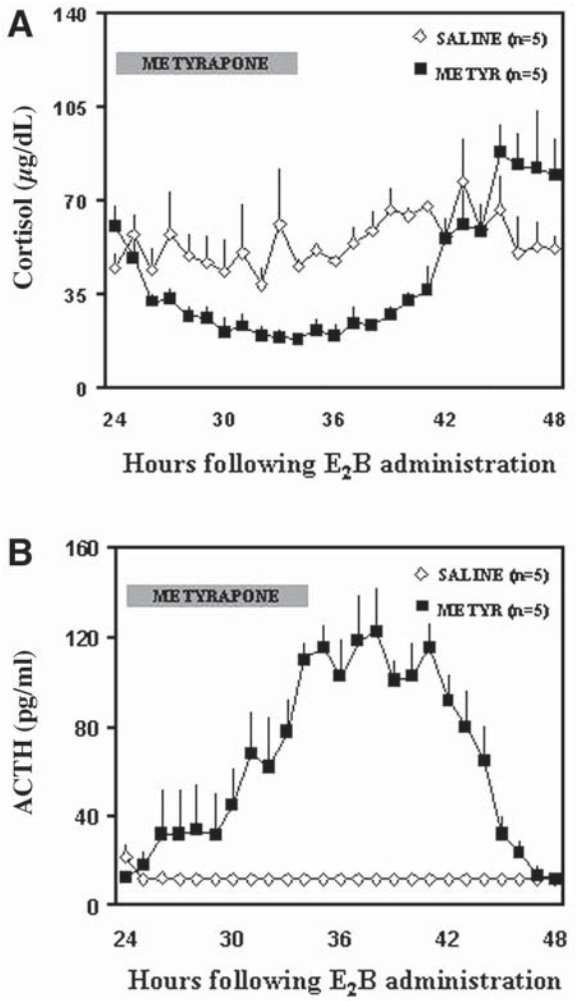

Fig. 1. Effect of administration of metyrapone (METYR) on circulating cortisol and ACTH concentrations. Mean responses (±SEM) in five OVX monkeys given a 10-h infusion of saline or metyrapone (5 mg/kg/h) starting at $t = 24$ h are shown. (**A**) Cortisol decreased following administration of metyrapone and remained below basal levels for 18 h. Total cortisol secretion from h 24 to 42 was significantly lower in metyrapone-treated monkeys compared with controls ($p < 0.001$). (**B**) Administration of metyrapone significantly increased ACTH ($p < 0.001$). Circulating ACTH increased beginning at h 30 (6 h following the onset of administration of metyrapone) and peaked at h 34. Peak concentrations were maintained for 8 h before declining precipitously from h 43 to 48. By contrast, ACTH levels in saline-treated animals were uniformly low, remaining near the detection limits of the assay for the duration of the experiment.

Results

Experiment 1: Effect of Metyrapone on HPA Axis and Estrogen-Induced Gonadotropin Surge

The effects of a 10-h infusion of metyrapone on the HPA axis of five ovariectomized (OVX) rhesus monkeys is depicted in Fig. 1. Cortisol levels declined from a baseline of 60.6 ± 7.9 to 18.4 ± 2.8 μg/dL during metyrapone infusion (Fig. 1A). Metyrapone maintained cortisol levels below baseline for 18 h while levels were unchanged in saline-treated animals. Total cortisol secretion over 18 h, expressed as area under the curve (AUC), was significantly reduced in metyrapone-treated animals (saline: 915.8 ± 71.4 μg·h/dL vs metyrapone: 460.3 ± 27.8 μg·hr/dL; $p < 0.001$). By contrast, administration of metyrapone significantly increased ACTH concentrations (Fig. 1B; analysis of variance [ANOVA]: $p < 0.001$). ACTH levels increased within 2 h of initiating metyrapone infusion and remained elevated for approx 16 h. ACTH levels in controls remained uniformly low and near the detection limits of the assay for the duration of the experiment. Approximately 8 h after stopping administration of metyrapone, ACTH levels declined precipitously while cortisol levels rapidly increased.

The effect of cortisol synthesis inhibition on mean LH and FSH responses to an estrogen challenge is presented in Fig. 2. The estradiol profile to an injection of estradiol benzoate (E_2B) (50 μg/kg) is included in Fig. 2A (shaded area). Serum estradiol concentrations increased from basal levels of 61 ± 17 pg/mL to a peak of 965 ± 371 pg/mL by h 30 before gradually declining to 222 ± 58 pg/mL at h 48. The estrogen challenge produced an LH surge in all five saline-treated and metyrapone-treated OVX monkeys (Fig. 2A). In the controls, the LH surge commenced 36.8 ± 1.4 h following administration of estrogen and reached a peak of 12.9 ± 2.7 ng/mL at h 46.2 ± 1.1. Total LH released over the sampling period (expressed as AUC) was 147.6 ± 32.5 ng·h/dL. The LH surge in the metyrapone group began at h 29.4 ± 1.1 (7.4 ± 0.4 h earlier than in controls; $p < 0.001$) and reached a peak of 13.2 ± 2.3 ng/mL at h 41.6 ± 2.6, which was 4.6 ± 2.2 h earlier compared with saline controls ($p = 0.12$). Total LH released following metyrapone treatment was increased but failed to reach statistical significance by a small margin (210.1 ± 42.2 ng·hr/mL; $p = 0.06$).

The mean FSH response is displayed in Fig. 2B. One animal was excluded because it did not exhibit an FSH surge under either experimental condition. The FSH surge commenced at h 37.5 ± 1.9 in controls. FSH levels reached a peak of 8.6 ± 1.0 ng/mL at h 46.0 ± 0.9. By contrast, the FSH surge began at h 32.8 ± 1.1 in the metyrapone group and levels peaked at 10.9 ± 1.6 ng/mL by h 39.5 ± 2.3. Therefore, metyrapone advanced the onset of the FSH surge by 4.8 ± 1.4 h ($p = 0.04$) and time to peak FSH concentrations by 6.5 ± 1.8 h ($p = 0.03$). As with LH, total FSH secretion was increased by metyrapone but this increase was not statistically significant (saline: 137.9 ± 5.1 ng·hr/mL vs metyrapone: 186.8 ± 21.8 ng·hr/mL; $p = 0.09$).

Experiment 2: Effect of Hypoglycemia on Estrogen-Induced Gonadotropin Surge

The glucose and LH responses in OVX monkeys challenged with estrogen under euglycemic and hypoglycemic conditions are shown in Fig. 3A. Blood glucose in insulin-treated animals declined from a resting value of 3.4 ± 0.4 mmol/L and was maintained below 1.4 ± 0.2 mmol/L for 10 h. Four of four euglycemic animals and three of four

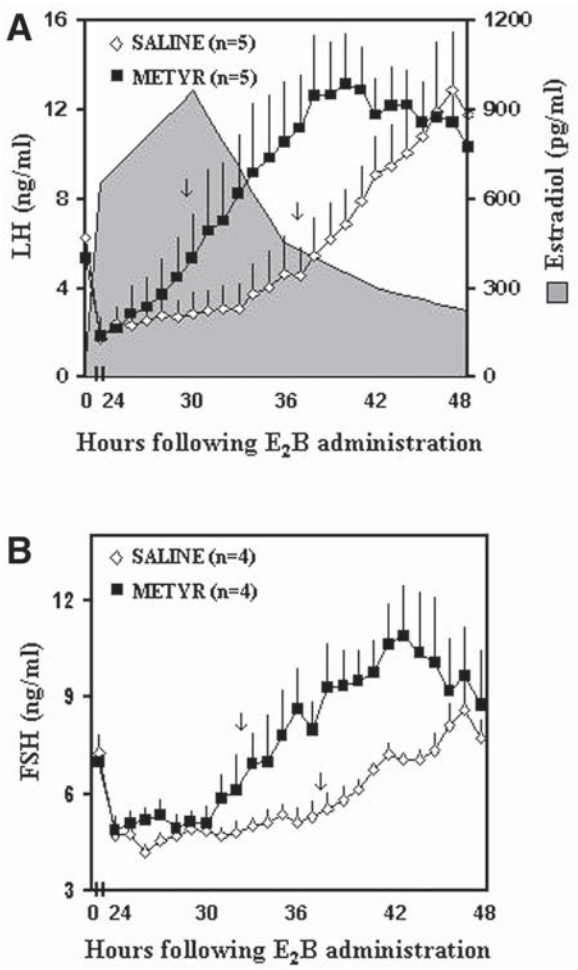

Fig. 2. Effect of metyrapone (METYR) treatment on the E_2B-induced LH and FSH surge. (**A**) The mean LH response (±SEM) in five OVX monkeys given an estrogen challenge at $t = 0$ h and infused with saline or metyrapone (5 mg/kg/h) from h 24 to 34 is shown. Also shown are the estradiol levels achieved in control animals following administration of estradiol benzoate (represented by shaded area). LH surges were detected in all monkeys under both experimental conditions. Metyrapone treatment advanced the LH surge by 7.4 ± 0.4 h ($p < 0.001$) and time to peak LH levels by 4.6 ± 2.2 h ($p = 0.12$). Total LH secretion was not significantly different between groups ($p = 0.06$). (**B**) Data from four monkeys demonstrating FSH surges under both experimental conditions are shown (one monkey did not exhibit an FSH surge). Administration of metyrapone advanced the FSH surge by 4.8 ± 1.4 h ($p = 0.04$) and time to peak FSH levels by 6.5 ± 1.8 h ($p = 0.03$). Total FSH release was not significantly different between groups ($p = 0.09$). The average time of surge onset is denoted by arrows.

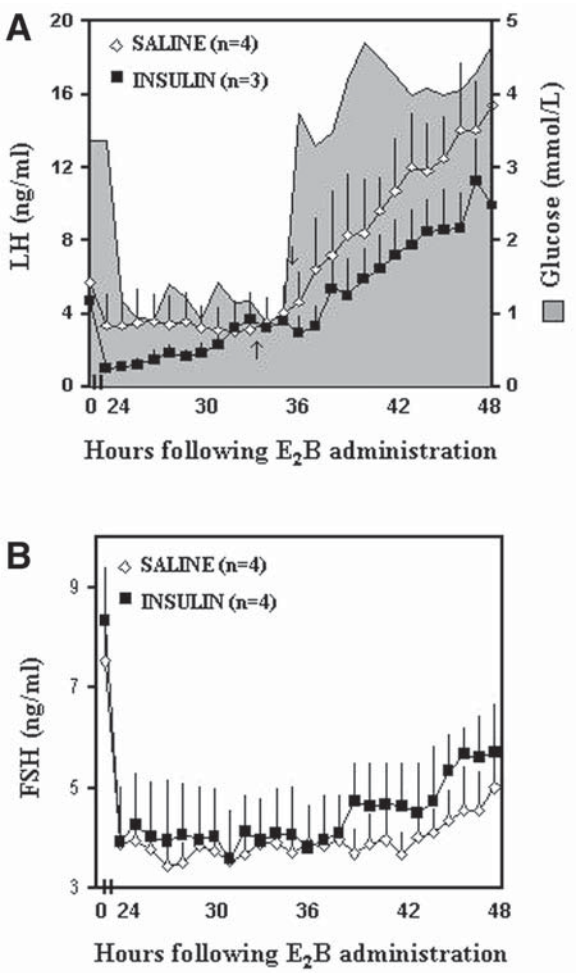

Fig. 3. Effect of hypoglycemia on E_2B-induced LH and FSH surge in OVX monkeys. (**A**) Mean LH responses (± SEM) in animals given an estrogen challenge at $t = 0$ h and either saline or insulin (0.7 U/kg) at h 24 are shown. Glucose levels (represented by shaded area) were maintained below 50% normal until h 34 in insulin-treated monkeys. All monkeys exhibited LH surges under euglycemic conditions and three of four under hypoglycemic conditions. Data from the four euglycemic and three hypoglycemic monkeys that demonstrated LH surges are shown. Onset of surge ($p = 0.45$), time to peak response ($p = 0.79$), and total LH released ($p = 0.40$) were not affected by hypoglycemia. The average time of surge onset is denoted by an arrow (saline: ↓; insulin: ↑). (**B**) Mean FSH responses (± SEM) in four monkeys given an estrogen challenge at $t = 0$ h and either saline or insulin (0.7 U/kg) from h 24 to 34 are shown. One of four euglycemic monkeys elicited an FSH surge. FSH surges were not detected in hypoglycemic monkeys. Hypoglycemia did not affect total FSH secretion ($p = 0.29$).

hypoglycemic animals exhibited an LH surge. In euglycemic animals, the LH surge began 35.5 ± 0.6 h postestrogen injection, and levels increased to 15.4 ± 4.9 ng/mL. Hypoglycemia did not affect the time to LH surge onset ($p = 0.45$), time to peak response ($p = 0.79$), or cumulative LH release ($p = 0.40$). The LH surge began at h 33.7 ± 1.9, and circulating LH increased to 11.3 ± 2.4 ng/mL in hypoglycemic animals. Peak levels occurred at approximately h 47 in both groups (saline: 47.3 ± 0.7 h vs insulin: 47.7 ± 0.3 h) and total LH released was 164.7 ± 49.7 and 111.1 ± 57.3 ng·hr/mL in euglycemic and hypoglycemic monkeys, respectively.

The FSH profile is shown in Fig. 3B. Only one euglycemic animal exhibited an FSH surge, while no FSH surges were detected in the hypoglycemic group. Data generated from all four animals are shown. While FSH values increased

in parallel with LH, at no time did mean values exceed baseline (t = 0 h) in either group. Hypoglycemia did not affect total FSH secretion (saline: 103.3 ± 7.7 ng·hr/mL vs insulin: 83.1 ± 16.8 ng·hr/mL; p = 0.29).

The effect of hypoglycemia on the gonadotropin response to E_2B in follicular-phase monkeys is shown in Fig. 4. Of the five animals in each group, four euglycemic and four hypoglycemic animals exhibited LH and FSH surges. Data from the monkeys that demonstrated LH surges are presented in Fig. 4A. In euglycemic animals, the LH surge was initiated 30.0 ± 2.1 h following administration of estrogen. A peak LH level of 8.3 ± 5.2 ng/mL occurred at h 39.3 ± 4.8, and total LH secretion over the sampling period was 154.8 ± 82.9 ng·hr/mL. In hypoglycemic monkeys, the LH surge began at h 28.8 ± 0.9 (p = 0.62), and peak LH levels (14.7 ± 9.0 ng/mL) occurred 43.8 ± 2.7 h following the estrogen challenge (p = 0.48). Mean cumulative LH release was 238.9 ± 102.7 ng·hr/mL (p = 0.55). No differences between groups were statistically significant. Glucose levels in insulin-treated animals were maintained below 50% of basal levels for 10 h (<1.4 ± 0.2 vs 2.8 ± 0.3 mmol/L). Figure 4B illustrates the FSH profiles for the four monkeys that surged. In euglycemic controls, the onset of the FSH surge occurred at h 31.7 ± 2.4. Peak FSH levels of 3.0 ± 0.3 ng/mL occurred at h 43.5 ± 1.4, and total FSH released was 46.3 ± 12.3 ng·hr/mL. Hypoglycemia did not affect any parameter of the FSH surge. The onset occurred at h 30.8 ± 0.9 (p = 0.53). Peak FSH levels of 3.6 ± 0.5 ng/mL occurred at h 43.3 ± 2.0 (p = 0.93), and cumulative FSH secretion was 76.7 ± 19.4 ng·hr/mL (p = 0.24).

Since progesterone has been shown to advance the estrogen-induced gonadotropin surge, progesterone concentrations following metyrapone or IIH were compared to saline controls (Fig. 5). Progesterone levels were significantly increased by metyrapone infusion in OVX monkeys (Fig. 5A; ANOVA: p = 0.008). Progesterone increased from basal levels of 2.7 ± 0.3 ng/mL at h 24 to 5.4 ± 1.1 ng/mL at h 34. By contrast, progesterone levels during administration of saline were unchanged (ANOVA: p = 0.389). Total progesterone released during the 10-h metyrapone infusion period was significantly increased compared to saline controls (saline: 28.5 ± 5.8 ng·hr/mL vs metyrapone: 47.8 ± 7.8 ng·hr/mL; p = 0.05). In experiment 2, progesterone levels significantly declined in both OVX and ovary-intact euglycemic controls (Fig. 5B; ANOVA: p < 0.001). Under hypoglycemic conditions, progesterone levels did not decline in either group. In fact, total progesterone released over 10 h was significantly greater in hypoglycemic follicular-phase monkeys compared with euglycemic controls (saline: 19.1 ± 4.0 ng·h/mL vs insulin: 35.3 ± 2.5 ng·h/mL; p = 0.01). Progesterone secretion was also increased in hypoglycemic OVX animals; however, this difference was not statistically significant (saline: 15.5 ± 1.5 ng·h/mL vs insulin: 25.1 ± 5.4 ng·h/mL; p = 0.23). Peak levels in hypoglycemic animals were 2.7 ± 0.8 ng/mL in OVX animals and 4.1 ± 1.0 ng/mL in follicular-phase animals. These peak values were lower than those seen in the metyrapone-treated group (5.4 ± 1.1 ng/mL).

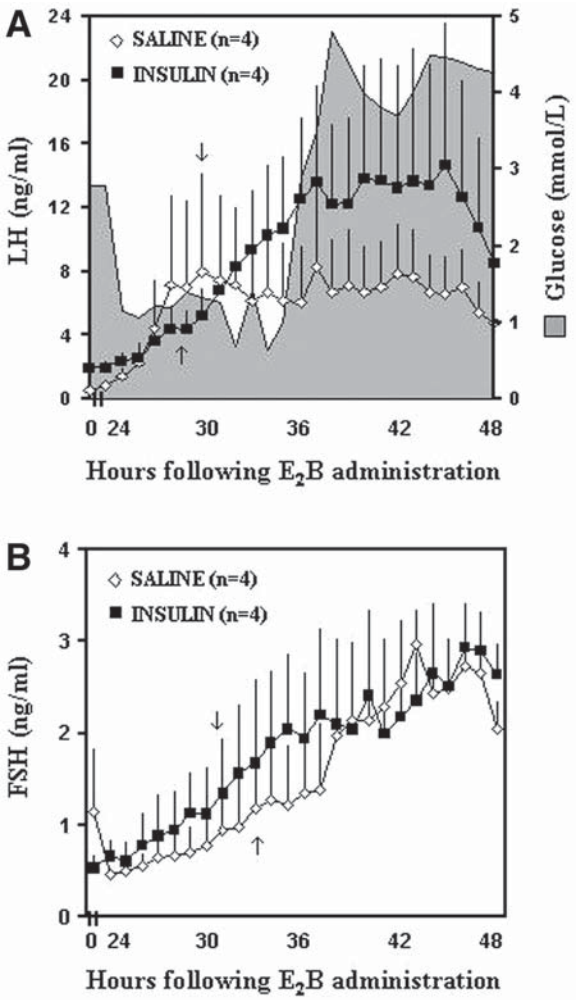

Fig. 4. Effect of hypoglycemia on E_2B-induced LH and FSH surge in ovary-intact monkeys. (**A**) Mean data (±SEM) from follicular-phase animals given an estrogen challenge at t = 0 h and either saline or insulin (0.7 U/kg) from h 24 to 34 are shown. Mean glucose levels (represented by shaded area) were maintained below 50% normal in insulin-treated monkeys for 10 h. Four of five saline-treated monkeys and four of five insulin-treated monkeys exhibited LH surges. Hypoglycemia did not alter the time of surge onset (p = 0.62), time to peak response (p = 0.48), or cumulative LH release (p = 0.55). The average time of surge onset is denoted by an arrow (saline: ↓; insulin: ↑). (**B**) Mean FSH responses (±SEM) in follicular-phase animals given an estrogen challenge at t = 0 h and either saline or insulin (0.7 U/kg) from h 24 to 34 are shown. Four saline-treated monkeys and four insulin-treated monkeys exhibited FSH surges. The FSH surge was not affected by hypoglycemia. No significant difference in time of surge onset (p = 0.53), time to peak response (p = 0.93), or cumulative FSH release (p = 0.24) was detected between groups. The average time of surge onset is denoted by an arrow (saline: ↑; insulin: ↓).

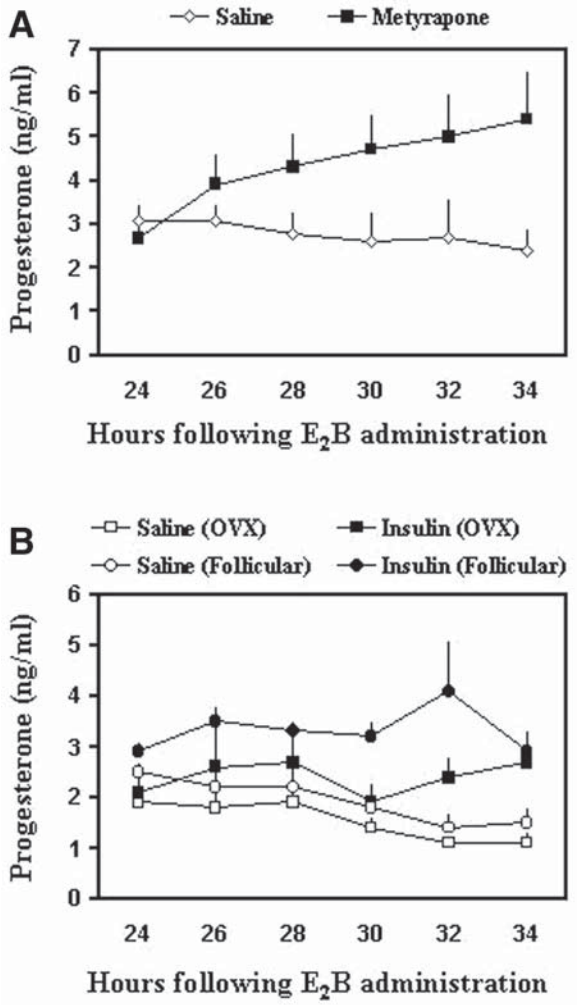

Fig. 5. Effect of cortisol synthesis inhibition and hypoglycemia on progesterone secretion. (**A**) Mean progesterone responses (± SEM) in five OVX monkeys given a 10-h infusion of saline or metyrapone (5 mg/kg/h) starting at $t = 24$ h are shown. Progesterone secretion significantly increased following metyrapone infusion ($p = 0.008$). Total progesterone secreted over a 10-h period was significantly greater compared to saline controls ($p = 0.05$). No significant alterations in progesterone concentrations were detected in saline-treated animals ($p = 0.389$). (B) Mean progesterone responses (± SEM) in four OVX monkeys and five follicular-phase animals given either saline or a 10-h hypoglycemic challenge (blood glucose maintained below 50% of baseline) starting at $t = 24$ h are shown. Progesterone levels moderately decreased with time in both OVX and follicular-phase controls ($p < 0.001$). Progesterone secretion over 10 h was significantly increased in hypoglycemic follicular phase animals compared with saline controls ($p = 0.01$). Although progesterone secretion was elevated in hypoglycemic OVX animals, this increase was not statistically significant ($p = 0.23$).

Discussion

Activation of the hypothalamic-pituitary component of the HPA axis by either metyrapone or IIH did not negatively impact the gonadotropin surge in the nonhuman primate. Quite unexpectedly, we observed an advancement of the LH and FSH surge in metyrapone-treated animals. Given that progesterone levels were significantly increased by metyrapone, and since progesterone is known to advance and augment the estrogen-induced gonadotropin surge *(37,38)*, increased circulating progesterone is the most likely explanation for our observation. In contrast to metyrapone, IIH did not advance the LH surge, nor did it increase progesterone concentrations to the same extent. This observation further supports our conclusion that advancement of the gonadotropin surge was owing to increased progesterone secretion following administration of metyrapone. We further conclude that the source of increased progesterone was the adrenal gland and not the ovary since this effect of metyrapone was produced in OVX animals. Metyrapone-induced progesterone secretion likely results from a combined effect of increased adrenal stimulation by ACTH and increased adrenal progesterone stores resulting from progesterone not being converted to cortisol *(39)*.

Both metyrapone and IIH have been shown to increase CRH in several animal models, including nonhuman primates *(29,36)*. Nevertheless, neither treatment interfered with the gonadotropin surge. Administration of CRH has been reported to inhibit the proestrous LH surge in sheep *(40)* and rats *(41)*. Hypoglycemia of a similar magnitude and duration to our study significantly delayed the estrogen-induced LH surge in OVX ewes *(42)*. In fact, induction of hypoglycemia at three different periods relative to an estrogen challenge delayed the LH surge in the ewe. While we tested the effect of IIH at only one time period relative to the estrogen challenge, it should be noted that the estrogen-induced LH surge began earlier in follicular-phase monkeys (30.0 ± 2.1 h vs 36.8 ± 1.4 and 35.5 ± 0.6 h in the OVX controls of experiments 1 and 2, respectively). Since the strength of the estrogen challenge is known to influence the onset of gonadotropin surge *(43)*, this earlier onset in follicular-phase monkeys may be owing to an additive effect of E_2B with endogenous estrogens. Therefore, the period of hypoglycemia in follicular-phase monkeys encompassed the period of surge initiation and elaboration, whereas the period of hypoglycemia in OVX monkeys encompassed a 10-h period immediately prior to surge initiation. Therefore, we are confident in the conclusion that unlike in the ewe, the estrogen-induced gonadotropin surge in the nonhuman primate is not blocked or delayed by IIH.

This difference between ewe and monkey is striking considering that tonic LH secretion in ewes and monkeys is similarly susceptible to hypoglycemia *(5,44)*, and similar GnRH surge dynamics associated with the LH surge have been demonstrated in both species *(45–47)*. Despite these similarities, the LH surge in primates appears to be more resistant to inhibition when compared with sheep and rodents. In distinction to rodents *(48,49)*, neither morphine *(50)* nor pentobarbital *(51)* blocked the estrogen-induced LH surge in monkeys. Although increased GnRH secretion precedes

the LH surge in monkeys *(45)*, gonadotropin surges can be generated in primates in the absence of acute GnRH stimulation *(26,27)*. Hence, a drug or stressor would interfere with the elaboration of the gonadotropin surge in the nonhuman primate *only* if its effects extended beyond inhibition of GnRH. Since neither IIH nor metyrapone inhibited the gonadotropin surge, this hypothesis is not supported by our findings.

The advancement of the gonadotropin surge by metyrapone treatment raised the concern that acute stressors could have detrimental effects on fertility. A premature surge could theoretically cause premature rupture of the follicle and release of an immature egg incapable of fertilization or, conversely, result in failed follicular rupture and luteinization because the follicular apparatus was incompletely developed. IIH did not advance the estrogen-induced gonadotropin surge in either OVX or follicular-phase monkeys. While hypoglycemia significantly increased progesterone concentrations in follicular-phase monkeys, progesterone concentrations were 25–35% lower compared with metyrapone-treated animals. This difference in progesterone may account for why the gonadotropin surge was not advanced. However, it is possible that hypoglycemia failed to advance the gonadotropin surge because the elapsed time between the first elevated progesterone value following IIH and the onset of the LH surge was only 4–6 h in follicular-phase monkeys. Injection of progesterone to estrogen-primed OVX monkeys consistently initiated an LH surge 6–9 h later *(38,52)*. Assuming that a similar latency period applies to our experimental conditions, it is unlikely that an effect of progesterone could be distinguished from an effect of E_2B in follicular-phase monkeys. However, the lack of an effect of IIH in OVX monkeys would argue against advancement of the gonadotropin surge following activation of the HPA axis by IIH.

The estrogen challenge in the OVX animals of experiment 2 elicited FSH surges in only one of four euglycemic and none of four hypoglycemic animals. By contrast, FSH surges were observed in four of five OVX animals in experiment 1, and five of five follicular-phase monkeys in experiment 2. The absence of FSH surges in OVX primates challenged with estrogen has been reported by other groups *(53,54)*. March et al. *(54)* documented FSH surges in oophorectomized women only after receiving both exogenous estrogen and progesterone. Estrogen alone failed to elicit FSH surges in these women. Comparison of progesterone levels among OVX controls in experiments 1 and 2 shows that progesterone levels were higher in experiment 1, in which most animals exhibited an FSH surge. Individual variations in progesterone secretion may account for why FSH surges were observed in some OVX monkeys.

In summary, activation of the HPA axis by either IIH or metyrapone did not inhibit the gonadotropin surge. This may reflect the autonomy of the surge mechanism rather than a failure of CRH or other peptidergic systems to inhibit GnRH secretion. The advancement of the gonadotropin surge by metyrapone is most likely owing to increased adrenal progesterone release. Hypoglycemia did not increase progesterone concentrations to the same extent as metyrapone. Hence, acute stressful events near the primate midcycle are unlikely to inhibit or advance the preovulatory gonadotropin surge to the detriment of oocyte maturation or ovulation.

Materials and Methods

Animal Husbandry

Studies were conducted in seven OVX and five ovary-intact rhesus monkeys (*Macaca mulatta*). Monkeys ranged in age from 5 to 18 yr and weighed between 5.2 and 10.2 kg. Animals were individually or group housed in a light- and temperature-controlled environment (lights on: 9:00 AM to 7:00 PM; temperature: 22ºC). Diets consisted of a twice-daily ration of Purina monkey chow (Ralston Purina, St. Louis, MO) supplemented with fruit and vegetables. Water was available ad libitum. Ovariectomy was performed at least 6 mo prior to experimentation. The follicular phase was determined from menses data and progesterone concentrations in blood samples collected three times a week. A progesterone value of <3.5 ng/mL was considered indicative of the follicular phase. This cutoff was based on progesterone measurements over six consecutive cycles in five rhesus monkeys with regular ovulatory menstrual cycles (mean follicular-phase progesterone levels = 1.8 ± 0.1 ng/mL and range = 1.0–3.7 ng/mL; mean luteal phase progesterone levels = 8.5 ± 0.4 ng/mL and range = 4.0–21.9 ng/mL). All animal husbandry practices and experimental procedures conformed to regulations set forth by the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

Experimental Protocol

Experiment 1

Experiment 1 compared the effects of metyrapone to saline administration on the estrogen-induced LH surge in five OVX monkeys. A baseline ($t = 0$ h) blood sample was taken by venipuncture at 10:00 AM followed by an im injection of E_2B (50 μg/kg) (Steraloids, Wilton, NH) dissolved in sunflower oil. Approximately 20 h later, monkeys were sedated with ketamine HCl (5–10 mg/kg) (Rogarsetic, Montreal, PQ, Canada) and transferred to primate chairs. Angiocatheters were inserted into a femoral vein for blood sampling and a saphenous vein for iv drug administration. Beginning 24 h after E_2B injection, monkeys received a 10-h infusion of metyrapone (5 mg/kg/h) (Sigma, St. Louis, MO); or an equivalent volume of saline. Hourly blood samples (3 mL) were taken from h 24 to h 48 for LH, FSH, ACTH, cortisol, estradiol, and progesterone measurements. Each animal in experiment 1 was randomly assigned to receive either metyrapone or saline. After a minimum of 4 wk, the opposite

treatment was given during a second estrogen challenge. The time of E_2B injection was constant for each animal.

Experiment 2

Experiment 2 compared the effects of hypoglycemia to euglycemia on the E_2B-induced gonadotropin surge in OVX ($n = 4$) and ovary-intact ($n = 5$) monkeys in the follicular phase. The experimental protocol was identical to that of experiment 1 except IIH was substituted for metyrapone infusion. Experiment 2 was conducted approx 1 yr following experiment 1. Two of the OVX animals in experiment 2 had been used in experiment 1. The three remaining animals used in experiment 1 had been euthanized in another study and, therefore, were not available for inclusion in experiment 2. Hypoglycemia was induced by a bolus of insulin (0.7 U/kg) (Eli Lilly Canada, Scarborough, Ontario, Canada) at h 24 relative to the E_2B challenge. Glucose levels were maintained between 25 and 50% of normal for 10 h with supplemental doses of insulin (0.05 U/kg) administered approximately every 2 h. Hourly blood samples (3 mL) were taken from h 24 to h 48.

Radioimmunoassays

From the 3 mL blood samples, a 1mL aliquot was placed in a chilled tube containing EDTA (1.4 µg) and centrifuged within 3 h of collection for ACTH measurements. The remaining blood (2 mL) was refrigerated overnight. Serum was isolated following centrifugation and stored at –20ºC until assayed. LH and FSH were assayed using reagents provided by the National Hormone and Pituitary Program. Unknowns were assayed in triplicate. Standard curves used LH and FSH reference preparations AFP 6936A and AFP 6940A, respectively. Serum samples were incubated with LH (AFP 6936A) or FSH (AFP 782594) antibodies followed by the addition of ^{125}I-radiolabeled LH (AFP 6936A) and FSH (AFP 6940A). A sheep anti–rabbit γ-globulin (Prince, Toronto, Ontario, Canada) was used to precipitate the antigen-antibody complex. Precipitation was facilitated by adding 12.5% Carbowax® (Sigma) prior to centrifugation. Assay sensitivity, defined as the amount of reference preparation required to reduce binding by 2 SDs below the zero standard divided by the sample volume, was 0.6 ng/mL for LH and 0.4 ng/mL for FSH. The interassay coefficient of variation (CV) determined from comparison of high- and low-serum pools was 11.7 and 11.5% for LH and FSH respectively, while the intraassay CV was 7.4 and 9.6%, respectively. ACTH, cortisol, estradiol, and progesterone were assayed using commercial radioimmunoassay kits (Diagnostic Products, Los Angeles, CA). Assay sensitivity was 8 pg/mL for ACTH, 22 pg/mL for estradiol, 0.3 µg/dL for cortisol, and 0.02 ng/mL for progesterone. Intraassay CV for ACTH, cortisol, estradiol, and progesterone assays were 3.2, 3.5, 4.3, and 3.6%, respectively, and interassay CVs were 6.4, 5.8, 6.8, and 3.9%, respectively. The progesterone assay was validated for use in the nonhuman primate as follows: the displacement curve produced by volumes of 5, 10, 25, 50, and 100 µL of a monkey serum pool was parallel to the progesterone standard curve regardless of whether or not the serum was extracted. Moreover, similar progesterone levels were achieved when an unextracted monkey serum pool was assayed by the commercial assay and when measured using Niswender 337 antiserum and tritiated progesterone following ether extraction.

Glucose levels were measured using a glucometer (Accu-Chek Advantage, Boehringer Mannheim, Laval, PQ, Canada) and expressed as millimoles per liter.

Data Analyses

Given that gonadotropin secretion is pulsatile during the surge, gonadotropin responses were smoothed by calculating a bin value for four consecutive samples (h 24–27, h 25–28, h 26–29, and so on). The onset of a surge was designated when a bin value exceeded the nadir by 3 SDs and subsequent bin values continued to increase to a peak. For the gonadotropin response to be considered a surge, peak levels had to exceed levels at h 0. The magnitude of the gonadotropin response was quantified by calculating the AUC for LH and FSH using the trapezoid rule. Differences in time of surge onset, time to peak response, and total gonadotropin release (AUC) between groups were analyzed by paired *t*-tests.

Changes in progesterone and ACTH levels subsequent to saline, metyrapone, or insulin treatment were analyzed using a repeated-measures ANOVA. In addition, AUC for cortisol and progesterone were calculated and paired *t*-tests were used to determine differences between treatment groups. Statistical significance was set at $p \leq 0.05$.

Acknowledgments

We are grateful to Dr. Parlow and the National Hormone and Pituitary Program for providing LH and FSH assay reagents. This work was supported by the Medical Research Council of Canada.

References

1. Chrousos, G. P., Torpy, D. J., and Gold, P. W. (1998). *Ann. Intern. Med.* **129,** 229–240.
2. Roozendaal, M. M., Swarts, J. J., van Maanen, J. C., Wiegant, V. M., and Mattheij, J. A. (1997). *Life Sci.* **60,** 735–742.
3. Norman, R. L., McGlone, J., and Smith, C. J. (1994). *Biol. Reprod.* **50,** 16–26.
4. Briski, K. P. and Sylvester, P. W. (1992). *Psychoneuroendocrinology* **17,** 125–133.
5. Clarke, I. J., Horton, R. J., and Doughton, B. W. (1990). *Endocrinology* **127,** 1470–1476.
6. Chen, M. D., Ordog, T., O'Byrne, K. T., Goldsmith, J. R., Connaughton, M. A., and Knobil, E. (1996). *Endocrinology* **137,** 2012–2021.
7. Chen, M. D., O'Byrne, K. T., Chiappini, S. E., Hotchkiss, J., and Knobil, E. (1992). *Neuroendocrinology* **56,** 666–673.
8. Goubillon, M. L. and Thalabard, J. C. (1996). *Neuroendocrinology* **64,** 49–56.

9. Heisler, L. E., Pallotta, C. M., Reid, R. L., and Van Vugt, D. A. (1993). *J. Clin. Endocrinol. Metab.* **76,** 1280–1285.
10. Heisler, L. E., Tumber, A. J., Reid, R. L., and Van Vugt, D. A. (1994). *Neuroendocrinology* **60,** 297–304.
11. Rivest, S. and Rivier, C. (1991). *Endocrinology* **129,** 2049–2057.
12. Petraglia, F., Vale, W., and Rivier, C. (1986). *Endocrinology* **119,** 2445–2450.
13. Williams, C. Y., Harris, T. G., Battaglia, D. F., Viguie, C., and Karsch, F. J. (2001). *Endocrinology* **142,** 1915–1922.
14. Harris, T. G., Battaglia, D. F., Brown, M. E., et al. (2000). *Endocrinology* **141,** 1050–1058.
15. Battaglia, D. F., Beaver, A. B., Harris, T. G., Tanhehco, E., Viguie, C., and Karsch, F. J. (1999). *Endocrinology* **140,** 2471–2479.
16. Rivier, C. and Rivest, S. (1991). *Biol. Reprod.* **45,** 523–532.
17. Chatterton, R. T. (1990). *Int. J. Fertil.* **35,** 8–13.
18. Ferin, M. (1993). *Ann. NY Acad. Sci.* **697,** 106–116.
19. Olster, D. and Ferin, M. (1987). *J. Clin. Endocrinol. Metab.* **65,** 262–267.
20. Barbarino, A., De Marinis, L., Tofani, A., et al. (1989). *J. Clin. Endocrinol. Metab.* **68,** 523–528.
21. Xiao, E. N. and Ferin, M. (1988). *Biol. Reprod.* **38,** 763–767.
22. Williams, C. L., Nishihara, M., Thalabard, J. C., Grosser, P. M., Hotchkiss, J., and Knobil, E. (1990). *Neuroendocrinology* **52,** 133–137.
23. Gambacciani, M., Yen, S., and Rasmussen, D. (1986). *Neuroendocrinology* **43,** 533–536.
24. MacLusky, N. J., Naftolin, F., and Leranth, C. (1988). *Brain Res.* **439,** 391–395.
25. Gindoff, P. and Ferin, M. (1987). *Endocrinology* **121,** 837–842.
26. Ferin, M., Rosenblatt, H., Carmel, P. W., Antunes, J. L., and Vande Wiele, R. L. (1979). *Endocrinology* **104,** 50–52.
27. Knobil, E., Plant, T. M., Wildt, L., Belchetz, P. E., and Marshall, G. (1980). *Science* **207,** 1371–1373.
28. Berkenbosch, F., De Goeij, D. C., and Tilders, F. J. (1989). *Endocrinology* **125,** 28–34.
29. Roy, B. N., Shridhar, S., and Van Vugt, D. A. (1999). In: Abstracts of the 81st Annual Meeting of the Endocrine Society, June 12–15, 1999, San Diego, California (Abstract P2-455).
30. Paulmyer-Lacroix, O., Anglade, G., and Grino, M. (1994). *J. Mol. Endocrinol.* **13,** 313–320.
31. Plotsky, P. M., Bruhn, T. O., and Vale, W. (1985). *Endocrinology* **117,** 323–329.
32. Robinson, B. G., Mealy, K., Wilmore, D. W., and Majzoub, J. A. (1992). *Endocrinology* **130,** 920–925.
33. Plotsky, P. M. and Sawchenko, P. E. (1987). *Endocrinology* **120,** 1361–1369.
34. Alexander, S. L., Irvine, C. H., Livesey, J. H., and Donald, R. A. (1993). *Endocrinology* **133,** 860–866.
35. Conte-Devolx, B., Guillaume, V., Boudouresque, F., et al. (1992). *Acta Endocrinol.* **127,** 435–440.
36. Van Vugt, D. A., Piercy, J., Farley, A. E., Reid, R. L., and Rivest, S. (1997). *Endocrinology* **138,** 2249–2258.
37. Lee, W. S., Smith, M. S., and Hoffman, G. E. (1990). *Endocrinology* **127,** 2604–2606.
38. Terasawa, E., Rodriguez-Sierra, J. F., Dierschke, D. J., Bridson, W. E., and Goy, R. W. (1980). *J. Clin. Endocrinol. Metab.* **51,** 1245–1250.
39. Grant, J. K. (1972). *Sci. Basis Med. Annu. Rev.* 103–117.
40. Polkowska, J. and Przekop, F. (1997). *Acta Neurobiol. Exp. (Warsz)* **57,** 91–99.
41. Rivier, C. and Vale, W. (1984). *Endocrinology* **114,** 914–921.
42. Medina, C. L., Nagatani, S., Darling, T. A., et al. (1998). *J. Neuroendocrinol.* **10,** 785–792.
43. Karsch, F. J., Weick, R. F., Butler, W. R., et al. (1973). *Endocrinology* **92,** 1740–1747.
44. Heisler, L. E., Pallotta, C. M., Reid, R. L., and Van Vugt, D. A. (1993). *J. Clin. Endocrinol. Metab.* **76,** 1280–1285.
45. Xia, L., Van Vugt, D., Alston, E. J., Luckhaus, J., and Ferin, M. (1992). *Endocrinology* **131,** 2812–2820.
46. Caraty, A. and Skinner, D. C. (1999). *Endocrinology* **140,** 165–170.
47. Moenter, S. M., Brand, R. C., and Karsch, F. J. (1992). *Endocrinology* **130,** 2978–2984.
48. Barraclough, C. A. and Sawyer, C. H. (1955). *Endocrinology* **57,** 329–337.
49. Butcher, R. L., Collins, W. E., and Fugo, N. W. (1975). *Endocrinology* **96,** 576–586.
50. Ferin, M., Wehrenberg, W. B., Lam, N. Y., Alston, E. J., and Vande Wiele, R. L. (1982). *Endocrinology* **111,** 1652–1656.
51. Knobil, E. (1974). *Recent Prog. Horm. Res.* **30,** 1–46.
52. Terasawa, E., Yeoman, R. R., and Schultz, N. J. (1984). *Biol. Reprod.* **31,** 732–741.
53. Clifton, D. K., Steiner, R. A., Resko, J. A., and Spies, H. G. (1975). *Biol. Reprod.* **13,** 190–194.
54. March, C. M., Goebelsmann, U., Nakamura, R. M., and Mishell, D. R. Jr. (1979). *J. Clin. Endocrinol. Metab.* **49,** 507–513.