Stimulatory Effects of Stress on Gonadotropin Secretion in Estrogen-Treated Women*

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ABSTRACT

Although stress is known to inhibit the hypothalamic-pituitary-gonadal axis, recent studies in the monkey show that, under certain conditions, in the presence of estrogen, stress may actually stimulate LH release. We investigated the effects of a mild inflammatory stress (2.0–3.0 ng/kg endotoxin) on LH release in five postmenopausal women with and without transdermal estradiol (E2, 0.1 mg) replacement. In another five E2-treated women, LH release was studied when the adrenal was stimulated directly by a 3-h ACTH infusion (Cortrosyn, 50 µg/h). Mean E2 levels were less than 12 pg/mL in the unreplaced subjects and were 86 ± 10 pg/mL and 102 ± 18 pg/mL in the two groups of E2-replaced subjects. Blood was sampled every 15–20 min for 2 h before and for 7 h after endotoxin or ACTH injection. Mean cortisol and progesterone levels increased in all three groups over time ($P < 0.001$). In the women without E2 replacement, basal LH was 26.8 ± 5.3 mIU/mL and did not change significantly, over

time, after endotoxin ($P = 0.58$). In the same women on E2, however, a significant increase in LH occurred after endotoxin ($P = 0.02$), from a mean hourly baseline of 15.3 ± 5.4 mIU/mL to a peak of 50.0 ± 25.2 mIU/mL. During the ACTH infusion, there was a significant stimulation of LH release in the E2-replaced subjects ($P < 0.001$), from a mean hourly baseline of 13.3 ± 3.0 mIU/mL to a peak of 44.1 ± 11.7 mIU/mL. In both groups, this increase occurred 2–4 h after the initial rise in progesterone and persisted to the end. We conclude that, in the presence of sufficient estrogen, activation of the hypothalamic-pituitary-adrenal axis leads to a stimulation of LH release. This is likely related to a rise in adrenal progesterone and its known stimulatory effect on LH release in the presence of E2. These studies provide a potential mechanism in the human by which an acute stress during the follicular phase of the menstrual cycle might lead to a premature LH surge and thereby interfere with follicular maturation and ovulation. (*J Clin Endocrinol Metab* 85: 2184–2188, 2000)

STRESS IS KNOWN to interfere with the menstrual cycle and may lead to chronic anovulation and amenorrhea (1, 2). This is generally thought to be caused by a decrease in the activity of the hypothalamic GnRH pulse generator with subsequent inhibition of the pituitary-gonadal axis (3–5). There is considerable evidence that stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis plays an important role in suppressing the hypothalamic-pituitary-gonadal (HPG) axis (6). In animals, CRH has been shown to suppress the GnRH pulse generator, resulting in a decrease in pulsatile LH release (7, 8). CRH antagonism has also been shown to prevent the inhibitory effect of stress on the HPG axis in the rodent and in the monkey (3, 9). Women with hypothalamic amenorrhea have higher basal cortisol levels and a blunted cortisol response to exogenous administration of CRH, suggesting that the increase in cortisol secretion may reflect increased endogenous CRH activity (10). These women with functional hypothalamic amenorrhea also show significant slowing of their LH pulse frequency (11).

Recent data in the monkey, however, demonstrate that the effect of stress on LH release depends on the stage of the

menstrual cycle and the level of circulating estradiol (E2). In ovariectomized monkeys without E2 replacement, inflammatory stress, induced by intracerebroventricular (ICV) injection of the cytokine interleukin-1 α (IL-1 α), suppressed LH by a CRH-dependent mechanism (9). However, in estrogen-replaced ovariectomized monkeys and in intact monkeys during the midfollicular phase of the menstrual cycle, administration of IL-1 α actually stimulated LH secretion (12, 13). The IL-1 α -induced stimulation of adrenal cortisol release was accompanied by a parallel increase in progesterone levels. It was postulated that the rise in plasma progesterone, of adrenal origin, which occurs in response to HPA activation, synergizes with circulating E2 to enhance LH secretion (5). This was further supported by the finding that the progesterone antagonist RU486 prevented the IL-1 α -induced increase in LH in the monkey (12).

It is not known whether estrogen can similarly affect the LH response to stress in humans. We therefore studied the effects of a mild inflammatory stress, in the form of endotoxin, on LH release in postmenopausal women with and without estrogen replacement. We also investigated the LH response in estrogen-treated postmenopausal women when the adrenal was activated directly by an ACTH infusion.

Subjects and Methods

Experimental protocol

Endotoxin study. Five healthy female subjects, 51–68 yr old, were studied. Three subjects were at least 1 yr after natural menopause, and two had undergone a bilateral oophorectomy 3–4 yr before. Subjects received low-dose endotoxin on

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two occasions separated by 1–2 months. Subjects were studied in random order, either without estrogen replacement or with estrogen, which was administered for 1 month before the study, via a transdermal E2 patch (0.1 mg). Mean ($\pm$ SEM) E2 levels were 6.4 ± 0.9 pg/mL before the study in the unreplaced subjects and 86 ± 10 pg/mL after E2. Unreplaced subjects were documented to have low E2 levels for at least 2 months before receiving endotoxin.

An indwelling iv catheter was inserted at 0800 h on the day of the study. Purified endotoxin was given iv at 1000 h, and blood was sampled every 20 min for 2 h before and for 7 h after endotoxin injection. All women experienced mild myalgias, occasional nausea, chills, and an increase in mean peak temperature, to 38.2°C vs. 38.3°C , with and without E2, respectively. Two endotoxin preparations were used. The first four subjects received endotoxin (purified lipopolysaccharide prepared from *Escherichia coli*, US Pharmacopeia Endotoxin Reference Standard, EC-5), obtained from the US Pharmacopoeia (Bethesda, MD). The first woman received 3 ng/kg, whereas the subsequent three subjects received 2.5 ng/kg. Because the EC-5 preparation became unavailable, the fifth patient received a more recently purified endotoxin preparation (US Standard Reference Endotoxin, PDS no. 67801), obtained from the Pharmaceutical Development Section, NIH (Bethesda, MD). This patient received 2 ng/kg, because the latter endotoxin preparation was more potent. Each person received the same dose and preparation of endotoxin in both parts of the study.

ACTH study. Five healthy postmenopausal women, 36–59 yr old, were studied. None of these women had previously participated in the first study. Four subjects were at least 1 yr after natural menopause, and one had undergone a bilateral oophorectomy 1 yr before. Each subject was treated with a transdermal E2 patch (0.1 mg) for 1 month. E2 levels were measured 4 days before the study and, if less than 50 pg/mL, an additional E2 patch was added. On the day of the study, mean E2 levels were 102 ± 18 pg/mL. Cortrosyn (ACTH 1–24, manufactured by Organon Inc., West Orange, NJ) was administered as a 50- μg iv bolus at 1000 h, followed by a 3-h infusion of 50 $\mu\text{g}/\text{h}$. Blood was collected at 15-min intervals for 2 h before and 7 h after the initial bolus. In both studies, blood samples were centrifuged within 1 h, and serum was stored at -20°C until assay.

Informed consent was obtained from all subjects, and both studies were approved by the Columbia-Presbyterian Medical Center Institutional Review Board.

Assays

Serum cortisol was assayed in unextracted plasma by solid-phase RIA (Diagnostic Products Corp., Los Angeles, CA). Progesterone and LH were measured by a commercial solid-phase, chemiluminescent immunoassay (Immulite; Diagnostic Products Corp.). The polyclonal antibodies used are highly specific for each hormone, with an assay sensitivity of 0.2 ng/mL for progesterone and 0.7 mIU/mL for LH, respectively. Two assays were used for E2 measurements: All samples were measured by a commercial solid-phase, chemiluminescent immunoassay (Immulite; Diagnostic Products Corp.) with an assay sensitivity of 20 pg/mL. In samples with E2 levels of less than 20 pg/mL, the measurement was repeated using a sensitive double-antibody RIA for E2 (Diagnostic Products Corp.). Sensitivity of this assay was 5 pg/mL.

E2 levels were measured twice at the start of each study, before

endotoxin or ACTH injection, whereas cortisol and progesterone were measured at hourly intervals, and LH levels were measured on all samples obtained at 15- to 20-min intervals.

Data analysis

Mean LH levels per hour were calculated for each individual. For each subject, hourly changes were calculated as a percentage of the 2-h baseline. Mean ($\pm$ SEM) hourly LH, progesterone, and cortisol concentrations were calculated for each group. The area under the LH concentration curve was calculated by trapezoid analysis. Changes in hormone concentrations over time were analyzed by ANOVA with repeated measures. Statistical significance was set at $P < 0.05$.

Results

Endotoxin study

Mean ($\pm$ SEM) basal cortisol and progesterone concentrations were 8.1 ± 0.7 $\mu\text{g}/\text{dL}$ and 0.3 ± 0.03 ng/mL without E2 treatment and 9.0 ± 0.9 $\mu\text{g}/\text{dL}$ and 0.3 ± 0.1 ng/mL with E2, respectively. Mean cortisol and progesterone levels rose significantly over time, from their 2 h baseline ($P < 0.001$ in both groups), and peaked 4–5 h after endotoxin injection in both the unreplaced group (31.7 ± 3.6 $\mu\text{g}/\text{dL}$ and 1.7 ± 0.3 ng/mL) and in the group on E2 (30.5 ± 3.3 $\mu\text{g}/\text{dL}$ and 1.5 ± 0.4 ng/mL) (Figs. 1B and 2B). In the women without E2 replacement, basal LH was 26.8 ± 5.3 mIU/mL and did not change significantly over time ($P = 0.58$) (Fig. 1A). In the E2-replaced group, however, there was a significant stimulation of LH release ($P = 0.02$), which occurred 3–4 h after the initial rise in progesterone. Mean hourly LH increased from a baseline of 15.3 ± 5.4 mIU/mL and peaked at 50.0 ± 25.2 mIU/mL (Fig. 2A). In one individual, LH increased markedly, to a maximum of 185 mIU/mL. The peak LH increase in the E2-treated women ranged from 162–738% of their baseline values.

The progesterone and LH responses to endotoxin in an individual subject, a 68-yr-old woman who had undergone bilateral oophorectomy, are shown in Fig. 3A. She received the highest dose of endotoxin (3 ng/kg) and was the only subject noted to have a suppression of pulsatile LH release in the absence of E2. On E2 replacement, her LH increased from a baseline of 2.38 mIU/mL to a peak of 20.6 mIU/mL, or a mean hourly rise of 738% at hour 7, compared with her baseline (Fig. 3B).

ACTH study

After the Cortrosyn infusion, mean cortisol and progesterone concentrations increased significantly over time, in all subjects ($P < 0.001$). Cortisol and progesterone increased from a 2-h baseline of 9.3 ± 1.7 $\mu\text{g}/\text{dL}$ and 0.6 ± 0.2 ng/mL to a peak of 39.4 ± 2.3 $\mu\text{g}/\text{dL}$ and 1.9 ± 0.2 ng/mL, respectively, 3–4 h after the Cortrosyn injection (Fig. 4B). This rise in progesterone and cortisol was accompanied by a significant stimulation of LH release ($P < 0.001$), which was noted 2–3 h after the initial rise in progesterone. Mean hourly LH rose from a baseline of 13.3 ± 3.0 mIU/mL to a peak of 44.1 ± 11.7 mIU/mL (Fig. 4A). The peak mean hourly LH increase over baseline ranged from 158–770%.

Discussion

Our data show, for the first time in humans, how a reproducible specific stressor has a markedly different effect on

FIG. 1. Mean ($\pm$ SEM) LH (A), cortisol, and progesterone (B) responses to an endotoxin injection in five postmenopausal women without E2 replacement (mean E2 = 6.4 ± 0.9 pg/mL). Cortisol and progesterone levels rose significantly over time ($P < 0.01$), whereas LH plasma concentrations did not change significantly over time ($P = 0.58$).

the HPG axis in the presence or absence of estrogen. Endotoxin was chosen as the inflammatory stressor because of its stimulatory effects on the release of endogenous cytokines (IL-1, IL-6, tumor necrosis factor), which in turn activate the HPA axis and lead to the suppression of the HPG axis (6, 14–19).

Whereas, in the first part of our study, both cortisol and progesterone rose significantly after endotoxin injection in women with and without estrogen replacement, LH did not change significantly over time, in the unreplaced group as a whole. In contrast, most previous animal studies report a decrease in LH in response to endotoxin or IL-1 α . Ovariectomized rhesus monkeys show a suppression in basal pulsatile LH secretion after endotoxin infusion, given in much higher amounts than have been administered to humans (20). A similar effect was seen in the monkey after IL-1 infusion (9) and in the male (14) and female (15, 17) rat. Our failure to see a decrease in LH after endotoxin in the estrogen-deficient subjects is most likely related to the relatively low dose of endotoxin used in our subjects. In support of this is the observation that in the subject who received the highest dose of endotoxin, LH did indeed decrease.

The suppression of LH secretion by IL-1 α in the monkey

FIG. 2. Mean ($\pm$ SEM) LH (A), cortisol, and progesterone (B) responses to an endotoxin injection in five postmenopausal women with E2 replacement (mean E2 = 86 ± 10 pg/mL). Cortisol and progesterone levels rose significantly over time ($P < 0.001$). This was accompanied by a significant stimulation of LH release ($P = 0.02$).

can be blocked by a CRH antagonist, supporting a role for CRH in this process (9). Other forms of stress have also been shown to decrease LH in animals by a CRH mediated-mechanism (3). Although there are no direct data in humans, hypothalamic CRH levels are thought to be elevated in women with hypothalamic amenorrhea and in highly trained runners, because both groups have elevated basal cortisol levels and blunted cortisol responses to CRH (8, 21). Elevated CRH levels have been reported in the CSF of amenorrheic women with anorexia nervosa (22).

In contrast to the low estrogen state, endotoxin administration did lead to a significant stimulation of plasma LH levels in the same postmenopausal women after 1 month of E2 replacement. In animals, there is evidence that a critical E2 concentration may be necessary for stress-induced stimulation of the HPG axis to occur. In both E2-replaced ovariectomized monkeys (with plasma E2 concentrations characteristic of the late follicular phase of the menstrual cycle) and in intact monkeys during the midfollicular phase, ICV IL-1 α administration stimulated LH release (12, 13). However, no significant change in LH levels was observed when IL-1 α was administered to intact monkeys during the early follic-

FIG. 3. Illustration of the effects of endotoxin on progesterone concentration and pulsatile LH release in an ovariectomized woman without (A, E2 < 5 pg/mL) and with (B, E2 = 112 pg/mL) E2 replacement.

ular phase (13). In addition, ICV injection of CRH into gonadectomized ewes, in the presence of exogenous E2, resulted in a significant increase in both LH pulse frequency and mean LH concentrations (23).

To determine whether the endotoxin-induced stimulation of LH release in the E2-treated subjects was primarily caused by adrenal stimulation, we performed a second study, in which we investigated the effects of direct adrenal stimulation by a Cortrosyn infusion in E2-replaced postmenopausal women. As in the first study, the rise in cortisol and progesterone in response to Cortrosyn infusion was accompanied by a significant LH increase. In both studies, the rise in progesterone preceded the LH rise by several hours, similar to what has been reported previously in animal studies. These findings are consistent with a synergistic role of progesterone in stimulating LH secretion. In monkeys, infusion of a CRH antagonist prevented both the increase in progesterone and the increase in LH seen after IL-1 α treatment. In addition, an increase in LH did not occur when the E2-replaced animals were pretreated with RU486, a progesterone antagonist, although the increase in progesterone itself was not prevented (12). It is thus postulated that progester-

FIG. 4. Mean ($\pm$ SEM) LH (A), cortisol, and progesterone (B) responses to an ACTH infusion in five postmenopausal women with E2 replacement (mean E2 = 102 $\pm$ 18 pg/mL). Cortisol and progesterone levels rose significantly over time (P < 0.001). This was accompanied by a significant stimulation of LH release (P < 0.001).

one facilitates estrogen's positive feedback effects on LH secretion. There is evidence that progesterone can stimulate LH secretion by acting directly on the pituitary (24). Interestingly, in all of the above studies, the progesterone is most likely of adrenal origin, because the increase was observed in ovariectomized monkeys, as well as in several women in our study who were also ovariectomized. In the monkey and in our human studies, the plasma progesterone level did not exceed 2 ng/mL. In contrast to primate studies, IL-1 administration to ovariectomized, E2-replaced rodents did not increase LH. This phenomenon has been attributed to the very high (>50 ng/mL) progesterone levels found in the rat after an inflammatory stimulus (15, 16). At high concentrations, progesterone is known to inhibit LH release.

There are a few clinical studies in humans that support our observation of a rise in LH after stress. When untrained eumenorrhoeic women were stressed by exercise during the midfollicular phase of the cycle, a small increase in maximal peak amplitude of plasma LH was observed (25). In another study, eumenorrhoeic athletes were compared with sedentary women with normal menstrual cycles. The athletes had higher mean serum LH levels, resulting primarily from an increase in LH pulse amplitude, although there was a de-

crease in LH pulse frequency (26). When eumenorrheic runners were studied in the follicular phase of the cycle, an increase of LH was seen immediately after a race, but this effect was not seen when the same women were studied 12–24 h after a run (27).

The physiologic significance of an acute LH rise in the follicular phase of the cycle is not known. There are no data specifically related to the effects of a stress-induced premature increase of LH in the mid- to late follicular phase. Several reports, however, indicate that elevated LH concentrations at that stage of the menstrual cycle may have adverse effects on the maturing follicle and on the developing oocyte and may result in early pregnancy loss (28) or in reduction in the fertilization rate of mature oocytes in women undergoing *in vitro* fertilization (29). One of the major causes of decreased fertility of unstimulated cycles is the occurrence of premature LH surges (30, 31). A potential negative effect of poorly timed increase in LH may be premature luteinization. It could thus be speculated that stress in the follicular phase may cause subtle interference with the menstrual cycle and fertility without interrupting the cycle completely.

Even though we only studied the immediate effects of acute stress, data in the nonhuman primate suggest that the effects of a more prolonged exposure to stress might have similar effects and that these effects may last beyond one menstrual cycle. Monkeys who received 5 days of endotoxin in the follicular phase subsequently had prematurely elevated levels of LH and FSH and experienced a number of menstrual abnormalities. These abnormalities included a prolonged follicular phase and instances of decreased follicular function during the treatment cycle itself and signs of luteal deficiency in the form of decreased progesterone secretion in the menstrual cycles thereafter (32).

In summary, our data provide evidence that, in the presence of adequate estrogen levels, stress-induced activation of the HPA axis is associated with the stimulation of LH release. The increase in LH is most probably related to the increase in adrenal progesterone that accompanies the release of cortisol in response to stress. Our results provide a mechanism in the human by which an acute stress in the mid- to late follicular phase of the menstrual cycle might provoke a premature LH surge and thereby interfere with proper follicular maturation and ovulation.

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