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Estradiol and progesterone-induced slowing of gonadotropin-releasing hormone pulse frequency is not reversed by subsequent administration of mifepristone

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Abstract

Following suppression of LH (GnRH) pulse frequency by progesterone (P) and estradiol (E₂), LH pulse frequency remains slow for 7 days after P withdrawal if mid-luteal E₂ concentrations are maintained. This may reflect an ability of E₂ to potentiate the suppressive effects of low P levels. We explored this notion in a similar experimental paradigm by administering a P-receptor antagonist (mifepristone) after P withdrawal while continuing E₂. Studies were performed in seven ovulatory, non-obese women. Transdermal E₂ (0.2 mg/day) and oral micronized P (100 mg every 8 hours) were started within 24 hours of the LH surge and continued for 10 days. Subjects then underwent a 13-hour blood sampling protocol for determination of LH pulse characteristics and various hormone concentrations. Oral P was then discontinued, and oral mifepristone (50, 100, or 200 mg daily) and transdermal E₂ (0.2 mg/day) were administered for 7 days, after which the above sampling protocol was repeated. Results with all mifepristone doses were similar and therefore pooled. Mean LH, LH amplitude, and mean FSH markedly decreased after 7 days of mifepristone, but LH pulse frequency did not change (3.3 ± 1.5 vs. 2.4 ± 1.5 pulses/13 hours). Prolactin and androstenedione increased between the first and second admissions, with no changes in E₂, cortisol, testosterone, or DHEAS. In conclusion, blockade of P action by mifepristone does not reverse a suppressed LH pulse frequency within 7 days when E₂ concentrations are maintained, suggesting that P withdrawal alone may not explain the luteal-follicular increase of GnRH pulse frequency.

Index entries/key words

gonadotropin-releasing hormone; luteinizing hormone; progesterone; mifepristone; estradiol; luteal-follicular transition

INTRODUCTION

Pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates pituitary synthesis and pulsatile secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Differential secretion of LH and FSH throughout ovulatory cycles is in part related to variations of GnRH pulse frequency, which can differentially regulate LH β and FSH β transcription and mRNA expression [1–5]. Thus, the regulation of GnRH pulse frequency is of critical importance. Progesterone (P) appears to be the principal mediator of

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DISCLOSURES

None of the authors has any potential conflicts of interest to declare.

GnRH pulse frequency slowing in women, as LH (and by inference GnRH) pulse frequency slows concurrent with luteal increases of P [6,7], and P administration to women during the follicular phase slows GnRH pulse frequency [8]. This effect of P requires estradiol (E₂) as a permissive factor [9–11], likely related to E₂-induction of hypothalamic P receptors [12–15].

The role of estradiol (E₂) alone in regulating GnRH pulse frequency remains unclear. In postmenopausal women, E₂ administration has been reported to decrease LH pulse frequency [16,17], although one study found no change in α -subunit (and by inference GnRH) pulse frequency with E₂ administration [18]. Estradiol also reduces both hypothalamic electrical activity and LH pulsatility in ovariectomized monkeys [19]. However, during the late follicular phase in women—when E₂ concentrations are quite elevated—LH pulse frequency is maximal at approximately one pulse per hour [6,20]. Moreover, E₂ alone does not decrease LH pulse frequency in sheep [10], but instead is required for maximal LH pulse frequency during the follicular phase [21].

While most agree that the luteal-follicular increase in GnRH pulse frequency reflects decreasing P and E₂ levels, others have considered the subsequent follicular increase in frequency [6,7] to represent positive feedback actions of rising E₂ concentrations, similar to what occurs in sheep [21]. However, it is also possible that the follicular increase in GnRH pulse frequency represents a gradual loss of suppression by low concentrations of P. To investigate the relative roles of P and E₂ in slowing luteal GnRH pulse frequency, Nippoldt and colleagues performed a study in which exogenous E₂ alone, P alone, or E₂ plus P was started during the midluteal phase and continued for an average of 8.7 days [11]. Administration of E₂ alone, but not of P alone, maintained a slow LH pulse frequency, suggesting that E₂ may directly inhibit GnRH pulse frequency. Alternatively, these results may reflect continuing sensitivity of the GnRH pulse generator to low P levels related to E₂-induced increases in hypothalamic P receptors. To assess this further, our group performed a study in which exogenous P and E₂ was started immediately following the LH surge [22]. After 10 days of P and E₂, P was then discontinued, but E₂ continued for an additional 3 weeks. LH pulse frequency remained low 7 days after P discontinuation, when P had fallen to < 0.5 ng/ml. However, in the continued presence of E₂, LH pulse frequency thereafter increased. These results were consistent with an effect of E₂ to temporarily augment the efficacy of low levels of P on the GnRH pulse generator, with a waning effect of P over time.

We investigated this notion further using the P-receptor antagonist mifepristone as a physiological probe. Specifically, we employed a similar paradigm of E₂ and P administration for 10 days following the LH surge, after which LH pulse frequency was assessed. Progesterone was exchanged for mifepristone while E₂ was continued, with determination of LH pulse frequency 7 days thereafter. We hypothesized that mifepristone would block any continuing action of low plasma P at the hypothalamus (more completely than P withdrawal alone), resulting in a rapid increase in GnRH pulse frequency despite maintenance of E₂ concentrations.

RESULTS

Testing prior to first GCRC admission

For each participant, ovulation was confirmed prior to menses during the control cycle (mean P concentration 9.0 ± 2.8 ng/ml, range 4.8–12.5 ng/ml). On the initial day of urinary LH test positivity (study cycle), LH was 38.7 ± 25.9 IU/liter, FSH 8.4 ± 4.0 IU/liter, E₂ 206 ± 106 pg/ml, and P 1.1 ± 0.6 ng/ml. Five days after initiation of exogenous E₂ and P, serum concentrations of E₂ and P were 315 ± 79 pg/ml and 24.0 ± 17.5 ng/ml, respectively.

GCRC admissions

A representative subject's LH concentration-time series during GCRC admissions one and two are shown in Figure 1. Composite LH pulse characteristics over 13-hours are shown in Table 1. Whether assessed by Cluster 7 or deconvolution, the mean number of LH pulses did not significantly change from admission one to admission two, although there was a trend toward slower frequency by deconvolution analysis ($P = 0.0938$). Mean LH pulse amplitude (Cluster 7) decreased by 88% from admission one to admission two ($P = 0.0313$), and LH burst mass (deconvolution) decreased by 87% ($P = 0.0156$). Mean LH and mean FSH both decreased by approximately 80% ($P = 0.0156$ for both).

Mean 13-hour P decreased from 14.9 ± 7.2 ng/ml during admission one to 0.6 ± 0.3 ng/ml during admission two ($P = 0.0156$). Prolactin and androstenedione increased 1.7- and 1.9-fold, respectively ($P = 0.0156$ for both), but there were no significant changes of E_2 , T, cortisol, or DHEAS between admissions one and two (Table 1).

Safety tests and adverse events

One woman complained of feeling dizzy and lethargic while taking P and E_2 , but these symptoms resolved promptly after reducing the P dose to 60 mg three times daily. Adverse events potentially related to mifepristone were mild. One participant taking 50 mg mifepristone daily described mild headache and fatigue, and one participant taking 100 mg mifepristone daily experienced mild, transient lightheadedness early in the mifepristone course. All of these symptoms resolved quickly despite continued mifepristone and E_2 administration. All women had normal basic metabolic and liver panels after one week of mifepristone. Each participant experienced menstrual bleeding within a few days of mifepristone initiation: some reported that bleeding was lighter than usual, but none reported abnormalities otherwise.

DISCUSSION

The synthetic steroid mifepristone is a potent P-receptor antagonist, with a binding affinity for the P receptor that is approximately 5-fold higher than P [23]. We hypothesized that, in normal women given exogenous P and E_2 to achieve midluteal concentrations for 10 days beginning within 24 hours of the LH surge, subsequent withdrawal of P plus administration of mifepristone would result in a rapid (within 1 week) increase in GnRH pulse frequency to late follicular phase values despite maintenance of elevated E_2 concentrations. This hypothesis was predicated on the notion that P is the chief mediator of GnRH pulse frequency suppression throughout the menstrual cycle in women, in addition to the concept that the increase of LH frequency across the follicular phase [6,7] is related to a gradual loss of suppression by low P concentrations of P. However, the current results suggest that mifepristone does not rapidly reverse a suppressed (luteal) LH pulse frequency when E_2 concentrations remain elevated.

There are few data regarding the effects of P-receptor antagonism on follicular phase LH secretion. One study revealed no changes of LH pulse frequency or amplitude with 3 days of mifepristone administration (3 mg/kg/day) starting in the early follicular phase (cycle day 1–2) [24]. However, other studies of mifepristone (25–50 mg daily) beginning in the early follicular phase revealed that LH values increased approximately 2-fold within 5–7 days, remained 2- to 2.5-fold elevated for 2–4 weeks, and then returned to baseline [25–27]. These latter studies did not include assessment of LH pulsatility (e.g., LH frequency) when LH was elevated.

To our knowledge, the current study is the first to evaluate LH pulsatility a week after starting mifepristone. Our results suggest that mifepristone does not reverse a slow luteal LH pulse frequency within one week, at least when E_2 concentrations remain elevated. In our study,

mifepristone was started immediately after P administration, but P levels were concurrently allowed to fall, as occurs in the normal luteal-follicular transition. However, this study differs from the aforementioned studies [24–27], in which mifepristone was started in the early follicular phase, in two important ways. Firstly, exogenous P and E₂ were administered after the LH surge to standardize serum sex steroid concentrations for 10 days. Secondly, elevated E₂ levels were maintained during mifepristone administration.

The reported effects of mifepristone on gonadotropins have been variable and seemingly influenced by factors such as dose, length of administration, and cycle stage. Several studies have addressed the effect of mifepristone on LH patterns during the luteal phase, when both P and E₂ levels are high. When mifepristone (either 50 mg twice daily [28] or 3 mg/kg daily [29]) was given for 3 days during the midluteal phase, mean LH and LH amplitude decreased (the latter by about 35–40%), but there was no significant change of LH frequency. These changes of LH amplitude contrast with the absence of such effects of short-term mifepristone given in the early [24] or mid [30] follicular phase. Another study using 50 mg mifepristone twice daily for 4 days in the midluteal phase suggested a decrease of LH with an increase of LH frequency, but the analysis of pulse frequency was limited by a short sampling period (4 hours) and the use of cosinor analysis [31]. After a single dose of 600 mg mifepristone in the late luteal phase—when P and E₂ levels were falling—LH frequency and amplitude decreased within 22 hours by approximately 78 and 54%, respectively [29]. The reduction of LH amplitude and mean LH in these luteal studies is consistent with the marked reduction of the same parameters in our study.

A number of confounding factors related to the pharmacodynamics of mifepristone may have contributed to an apparent inability of mifepristone to increase LH frequency in this study. For example, it is possible that marked suppression of pituitary LH secretion resulted in difficulties with pulse detection. Mifepristone has been reported to suppress LH concentrations (as described above), to decrease the LH response to exogenous GnRH [29,32], and to decrease GnRH self-priming [33]. Nonetheless, our results were not materially changed when using deconvolution analysis, which is a highly sensitive method of pulse detection [34,35]. Another potential confounder is that mifepristone can exhibit partial P-receptor agonist effects in some contexts [36,37]. For example, in E₂-replete castrate monkeys [38,39] and E₂-replete postmenopausal women [40], mifepristone exerts agonist effects on the endometrium when P is virtually absent. In our study, P concentrations fell to very low levels by the second admission. Thus, it is possible that mifepristone acted as a partial agonist to suppress GnRH frequency in the near absence of P. Lastly, although mifepristone did not clearly increase cortisol in this study, mifepristone is known to be a glucocorticoid-receptor antagonist [36, 41], and this could theoretically be associated with GnRH pulse frequency suppression via central mechanisms (e.g., increased corticotrophin-releasing hormone [CRH] drive). Despite these pharmacodynamic considerations, mifepristone is currently the only P-receptor antagonist available for human studies.

It is also possible that inadequate hypothalamic exposure to mifepristone in part accounts for these results. In rats mifepristone concentrations were approximately 70% lower in the brain than in serum [42]. However, animal studies show that LH frequency (reflecting GnRH frequency) is altered by mifepristone given systemically [9,43], even at doses of approximately 1.6 mg/kg daily [43]. In our study, participants received an average of 0.8, 1.6, and 2.9 mg mifepristone/kg/day with 50, 100, and 200 mg doses, respectively. Also, increases of ACTH observed with mifepristone [36,41] are presumably related to corticosteroid-receptor blockade in the hypothalamus, at least with higher mifepristone doses.

Increases of prolactin with mifepristone have been previously observed in some [44,45] but not all studies [26,27,46,47]. We observed a modest increase of prolactin, but we doubt this was sufficient to markedly reduce GnRH pulse frequency.

In summary, these data suggest that P-receptor blockade with mifepristone does not reverse a suppressed (luteal) LH pulse frequency within 7 days when E₂ concentrations remain elevated. This suggests that removal of P negative feedback alone may be insufficient to raise LH frequency in the luteal-follicular transition.

MATERIALS AND METHODS

Subjects

Seven healthy women (age 25.6 ± 5.9 years [mean $\pm$ SD], range 19–35 years) with no evidence of hyperandrogenism were studied. All subjects reported regular and predictable menstrual cycles (every 28.2 ± 1.1 days, range 27–30 days), and none exercised excessively. None of the women was obese (body mass index 25.3 ± 3.3 kg/m², range 20.1–29.3 kg/m²), none reported recent weight changes, and weight remained stable throughout the study. Reported race was white for 6 participants and black for one participant, with none being of Hispanic ethnicity. Three subjects reported previous pregnancies (gravida 1–3), while four denied previous pregnancy. All participants had normal screening laboratory tests (see Study procedures below). In particular, total testosterone (T) was 34.7 ± 16.1 ng/dl, sex hormone binding globulin (SHBG) 50 ± 18.6 nmol/liter, and calculated free T 4.9 ± 2.5 pg/ml [48]. Study participants took no hormonal medications for at least 90 days prior to study, and none was taking medications known to affect the reproductive axis.

Study procedures

Study procedures were approved by the Institutional Review Board at the University of Virginia. Informed consent was obtained from study participants. Each volunteer underwent a detailed history and physical exam. All subjects were screened for hormonal and health-related abnormalities with determinations of LH, FSH, P, E₂, total T, SHBG, 17-hydroxyprogesterone, dehydroepiandrosterone sulfate (DHEAS), beta-hCG, TSH, prolactin, cortisol, CBC, chemistry and liver panels. A morning cortisol of at least 10 µg/dl was required for further study participation.

All volunteers underwent a control cycle during which evidence for ovulation was obtained (Figure 2). Ten and six days before the beginning of an anticipated menstrual period, a blood sample for plasma P and E₂ was obtained, with a P level > 3 ng/ml being required as presumptive evidence of previous ovulation. During the subsequent menstrual cycle (i.e., the study cycle), the timing of the LH surge was determined using urinary LH detection kits. On the day of initial urinary LH test positivity, a blood sample for LH, FSH, P, and E₂ was obtained. Transdermal E₂ patches (2 patches, each patch delivering 0.1 mg/d, for a total of 0.2 mg/day, applied to the lower abdomen, changed every 3 days) and oral micronized P (100 mg taken at 0700, 1500, and 2300 h) was started within 24 hours of initial LH test kit positivity. Approximately 5 days after starting exogenous E₂ and P, a blood sample for E₂ and P was obtained (around 2–3 hours after a P dose).

After 8–11 days (9.3 ± 1.4 days) of exogenous E₂ and P administration, subjects were admitted to the General Clinical Research Center (GCRC) for assessment of LH pulse frequency and other hormone parameters. Subjects were admitted at 1800 h, and a beta-hCG and hematocrit were obtained to exclude pregnancy and anemia, respectively. Beginning at 2000 h, blood for later hormone measurement was obtained through an indwelling intravenous heparin lock catheter over a 13-hour period as follows: LH every 10 minutes; FSH every 60 minutes; E₂,

P, T, androstenedione, cortisol every 2 hours; and prolactin and DHEAS every 4 hours. After completion of this first GCRC admission, oral P was discontinued and oral mifepristone (50 mg/day [n = 2]; 100 mg/day [n = 3]; 200 mg/day [n = 2]) and transdermal E₂ (0.2 mg/day) were administered for 7 days. Oral iron supplementation (325 mg twice daily) was also started.

After seven days of mifepristone and E₂ use, subjects were admitted to the GCRC for a second blood sampling protocol identical to the first, with the exception that a liver panel and basic metabolic panel (safety tests) were obtained on admission. When the second GCRC admission was complete, E₂ and mifepristone were discontinued, while oral iron supplementation was continued for an additional 4 weeks.

Hormonal measurements

Hormone concentrations were measured in serum. Blood was withdrawn via an indwelling intravenous catheter into serum separator tubes and allowed to clot at room temperature prior to centrifugation. Serum was removed and stored at -20 degrees Celsius prior to analysis, which occurred within several days. Assays were performed by the Ligand Core Laboratory of the Center for Research in Reproduction at the University of Virginia Health System. All samples from an individual woman were analyzed in duplicate in the same assay for each hormone. The pulse analysis programs (see “Data analysis” below) utilize both LH values for each time point; otherwise, the mean of the duplicates was used for data analysis. LH and FSH were measured by chemiluminescence (Diagnostic Products Corporation, Los Angeles, CA; sensitivities 0.1 and 0.05 IU/liter; intraassay coefficients of variation [CVs] 2.3–3.2% and 1.9–2.6%; and interassay CVs 5.3–6.6% and 4.8–6.3%, respectively). Total T, E₂, and P were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA; sensitivities 10 ng/dl, 10 pg/ml, and 0.1 ng/ml, respectively; intraassay CVs 3.5–5.0%, 5.2–6.8%, and 4.0–5.4%, respectively; and interassay CVs 8.2–10.6%, 5.8–15.8%, and 6.2–7.3%, respectively). Cortisol, prolactin, and DHEAS were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA; sensitivities 1.0 µg/dl, 1.0 ng/ml, 7.0 µg/dl, respectively; intraassay CVs 4.2–4.7%, 1.4–2.7%, and 3.9–6.2%, respectively; and interassay CVs 6.3–8.1%, 1.8–4.0%, and 6.8–7.8%, respectively). Androstenedione was measured by radioimmunoassay (Diagnostics Systems Laboratories, Inc., Webster, TX; sensitivity 0.1 ng/ml, intraassay CV 3.3–7.6%, interassay CV 8.7–13.1%). Samples with measured values below assay sensitivity were assigned the value of the assay’s sensitivity. To convert from conventional to SI units: P × 3.18 (pmol/L); total T × 3.47 (pmol/L); E₂ × 3.671 (pmol/L); cortisol × 27.59 (nmol/liter); prolactin × 1 (µg/liter); DHEAS × 0.002714 (µmol/liter); androstenedione × 3.492 (nmol/liter).

Data analysis

LH pulses were identified using the computer algorithm Cluster 7 (37). The parameters used for analysis were a test nadir and peak size of 2 × 2 with a t statistic of 2.45 for both the upstroke and downstroke. Missing values represented less than 0.1% of the total and were ignored. If the amplitude of an LH pulse detected by the Cluster 7 program was within the range of intraassay variability for the LH chemiluminescence method, it was not considered a pulse in subsequent analysis, as previously described [22]. Specifically, the following pulses detected by Cluster 7 were excluded from further analysis: pulses with a peak < 0.5 IU/liter and an amplitude < 0.1 IU/liter; pulses with a peak ≥ 0.5 and < 1 IU/liter and an amplitude < 0.25 IU/liter; pulses with a peak ≥ 1 and < 5 IU/liter and an amplitude < 0.5 IU/liter; and pulses with a peak ≥ 5 IU/liter and an amplitude < 1.0 IU/liter.

Given concerns that the above procedures may be an insensitive method to detect pulses of very low amplitude (especially during the second GCRC admission when LH concentrations were very low), we secondarily applied the multiparameter deconvolution analysis program

AutoDecon [34]. This pulse detection algorithm is based on the fact that serum LH concentrations over time reflect two primary factors—underlying LH secretory events and metabolic clearance (i.e., plasma half-life) of LH [34,35,49]. This method allows determination of the temporal location of each secretory event. In this secondary analysis, we did not exclude pulses that fell within the range of intraassay variability (see above).

Statistical Methods

All data are presented as mean $\pm$ SD unless otherwise stated. The primary endpoint was LH pulse frequency. Results with the three mifepristone doses were not materially different (Table 1) and were therefore pooled for statistical analysis. Secondary endpoints included LH amplitude and mean LH in addition to mean FSH, P, E₂, cortisol, prolactin, androstenedione, testosterone, and DHEAS.

We employed non-parametric statistical tests, which are based on ranks of observations and require no assumptions about the underlying distribution of data. Specifically, Wilcoxon sign rank tests were used to examine hormone parameters between the first and second GCRC admissions. All hypothesis tests were two-sided and conducted at the 0.05 level of significance. Statistical analyses were performed using SAS 9.1 (SAS Institute Inc., Cary, NC).

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Figure 1. Representative example of LH concentration time series in a single subject
 Admission one occurred after 10 days of progesterone and estradiol administration.
 Progesterone was then exchanged for mifepristone (estradiol continued), and admission two
 occurred 7 days thereafter. LH data are shown as the mean $\pm$ SEM of duplicate measurements.
 Asterisks denote detected LH pulses (by Cluster 7).

Control cycle

Study cycle

Figure 2.
Schematic of research protocol.

Table 1

Luteinizing hormone pulse characteristics and other hormone concentrations.

	Admission 1	Admission 2
Progesterone (ng/ml)	14.9 ± 7.2	0.6 ± 0.3 [*]
Estradiol (pg/ml)	271 ± 96	221 ± 169
LH pulses/13 h ^a	3.3 ± 1.5	2.4 ± 1.5
LH pulses/13 h ^b	6.4 ± 2.5	3.6 ± 2.4
LH amplitude (IU/L) ^a	2.7 ± 1.8	0.3 ± 0.3 [*]
LH burst mass (IU) ^b	2.1 ± 1.7	0.3 ± 0.3 [*]
Mean LH (IU/L)	1.1 ± 0.6	0.2 ± 0.1 [*]
Mean FSH (IU/L)	0.6 ± 0.3	0.1 ± 0.1 [*]
Cortisol (μg/dl)	6.9 ± 2.9	7.8 ± 3.2 [*]
Prolactin (ng/ml)	21.3 ± 8.2	36.1 ± 16.8 [*]
Androstenedione (ng/ml)	1.2 ± 0.5	2.3 ± 0.5 [*]
Testosterone (ng/dl)	14.4 ± 6.2	17.6 ± 4.4
DHEAS (μg/dl)	122 ± 93	129 ± 86

Data are presented as mean ± SD (median).

^a pulses detected by Cluster 7,^b pulses detected by AutoDecon.

* P < 0.05 vs. admission 1 values. To convert from conventional to SI units: P × 3.18 (pmol/L); total T × 3.47 (pmol/L); E2 × 3.671 (pmol/L); cortisol × 27.59 (nmol/liter); prolactin × 1 (μg/liter); DHEAS × 0.002714 (μmol/liter); androstenedione × 3.492 (nmol/liter).