Cortisol Response to Ovine Corticotropin-Releasing Hormone in a Model of Pregnancy and Parturition in Euthymic Women with and without a History of Postpartum Depression

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Hypothalamic-pituitary-adrenal axis abnormalities have been reported in depressed women and those with postpartum blues, compared with nondepressed women. We investigated the effect of gonadal steroids on the hormonal response to ovine CRH in women with (n = 5) and without (n = 7) a past history of postpartum depression (PPD) by creating an endocrine model of pregnancy and the postpartum. Ovine CRH (1 µg/kg) stimulation tests were performed in the baseline follicular phase, during hormone add-back (leuprolide acetate plus supraphysiologic doses of estradiol and progesterone-mimicking pregnancy), and after precipitous withdrawal of hormone replacement (mimicking the puerperium). Significant phase by time (P < 0.005) and phase by diagnosis (P < 0.05) interactions were observed, reflecting increased stimulated cortisol during the supraphysiologic phase, particularly in subjects with a history of PPD. Cortisol area under the curve also showed a significant phase by diagnosis effect (P < 0.05). Significant increases during the supraphysiologic phase were also seen for urinary free cortisol (P < 0.05), cortisol area under the curve (P < 0.001), and plasma corticosterone-binding globulin (P < 0.05).

Our data show that in humans, as in animals, supraphysiologic gonadal steroid levels enhance pituitary-adrenal axis activity, and, further, that women with a history of PPD have an enhanced sensitivity of the pituitary-adrenal axis to gonadal steroids. (J Clin Endocrinol Metab 90: 695–699, 2005)
least 1 yr past their most recent childbirth. A complete psychiatric diagnostic evaluation was performed using the Structured Clinical Interview for DSM-IV (9) and the Schedule for Affective Disorders and Schizophrenia-Lifetime (10). Subjects with past or present psychiatric illness, with the exception of past PPD for the study group, were excluded. Subjects with premenstrual dysphoric disorder were also excluded based on prospective screening performed over two menstrual cycles.

Two experimental groups were studied: women with a history of at least one episode of PPD who were euthymic for the past year or longer (subjects, n = 5), and age-matched (within 3 yr) controls with no history of past or present psychiatric illness (controls, n = 7). The age and body mass index (BMI) of subjects and controls were as follows: age 32.2 ± 7.6 and 30.1 ± 6.2 yr, respectively, and BMI 27 ± 5.8 and 26.8 ± 4.9, respectively.

The study was approved by the NIMH intramural research panel, and all subjects gave oral and written informed consent before their participation.

Study design

The study comprised three distinct, consecutive phases with a total duration of 4 months: a hypogonadal phase (4 wk), a supraphysiologic phase (8 wk), and a withdrawal phase (4 wk). The first phase (hypogonadal) consisted of the induction of a hypogonadotropic hypogonadal state by using an open-label injection of depot leuprolide acetate (Lupron, 5.75 mg im; TAP Pharmaceuticals, Deerfield, IL). This agent is a long-acting GnRH agonist known to suppress the endogenous production of gonadal steroids, achieving a state of medical gonadectomy. A total of four injections were administered during the study, maintaining the suppression of endogenous production of gonadal steroids. During the hypogonadal phase, subjects also received daily oral placebo estrogen and progesterone tablets in a blind fashion. In the second (supraphysiologic) phase, micronized progesterone (Women's Health Pharmacal, Princeton, NJ) were substituted for placebo. The number of tablets prescribed was adjusted biweekly on a random basis during baseline (placebo) and according to plasma hormone levels during active treatment. Estradiol was started at a dose of 4 mg/d and was progressively increased up to 10 mg/d in three divided doses. Progesterone was started at 400 mg/d in three divided doses and increased progressively according to plasma levels. Plasma levels were monitored after 1 wk, 2 wk, and every 2 wk thereafter, in an effort to reach maximal levels after 2–4 wk. Target plasma levels for estradiol and progesterone were approximately 300 pg/ml and 50 ng/ml, respectively. In the third (withdrawal) phase, the active medications were switched in a blinded fashion to placebo, inducing a sharp drop in plasma estradiol and progesterone levels. During this phase subjects continued to take the same number of tablets (placebo) they had received at the end of the supraphysiologic phase. After the withdrawal phase of 4 wk, subjects were followed up for an additional 8 wk while unmedicated (follow-up).

To ensure that the subjects were kept blind to gonadal steroid treatment, several measures were taken. First, active and placebo medication were packaged to look identical. Second, whether on placebo or active medication, the number of tablets prescribed was the same. Third, subjects were not informed of the onset of active treatment. Fourth, to prevent the subject from identifying the study phase by the occurrence of any spotting/bleeding, subjects were told that such a phenomenon could occur at any time throughout the study. Fifth, the research nurse administering study drug and rating scales (and monitoring adverse events) was blind to treatment phase and the presence or lack of any spotting/bleeding, subjects were told that such a phenomenon could occur at any time throughout the study. Fifth, the research nurse administering study drug and rating scales (and monitoring adverse events) was blind to treatment phase and the presence or lack of any spotting/bleeding, subjects were told that such a phenomenon could occur at any time throughout the study.

Hormone assays

Plasma levels of cortisol were measured by RIA on the samples collected during the oCRH test. In addition, corticosteroid-binding globulin (CBG) was measured by competitive assay and plasma leptin by RIA on the 15-min sample of the CRH test, as previously described (12). Plasma estradiol and progesterone were measured by RIA biweekly starting at baseline on samples collected in the morning. Finally, 24-h urine collection was performed once at each phase of the study, before the oCRH test, for further determinations of urinary free cortisol (UFC), norepinephrine, epinephrine, and dopamine. Assay characteristics were previously reported (12).

Statistical analyses

All data are expressed as mean ± sd. The effect of oCRH injection on cortisol response was analyzed by repeated-measures ANOVA, with diagnosis (PPD−, PPD+) as the between-subjects factor and treatment (baseline, supraphysiologic, withdrawal) and time of sampling (−15 to 180 min) as within-subjects factors.

The area under the curve (AUC) was then calculated using the trap-zoidal integration method by subtracting the time-integrated basal hormone levels from the total time-integrated levels throughout the sampling period. The AUC cortisol and UFC data were analyzed using 2 × 3 repeated-measures ANOVAs, with a between-subjects factor of diagnosis and a within-subjects factor of treatment phase. Significant findings were decomposed by Duncan post hoc tests (where permitted by the results of the ANOVA). Significance was accepted at a P < 0.05 level. Correlations between UFC and CBG levels were performed with Pearson product moment correlation coefficients with Bonferroni corrections for multiple correlations. Diagnostic differences in age and BMI were determined with Student's t tests. Estradiol and progesterone levels were compared in the two subject groups across the three treatment phases with repeated-measures ANOVA.

Results

From the series of 16 participants reported in the psychological study (8), we performed the oCRH stimulation test and the other endocrine measurements reported here on 12 consecutive euthymic women, five with (PPD+) and seven without (PPD−) a history of PPD. Subjects in the two groups did not significantly differ in age or BMI. Our pharmacological model, as described previously, was overall well tolerated. Elevated plasma levels of estradiol and progesterone were sustained during the addback phase in the two subject groups (estradiol, 269 ± 92.2 for 290 ± 77.7 pg/ml; and progesterone, 46.6 ± 19.6 for 73.1 ± 32.2 ng/ml mean ± sd in women with a history of PPD for comparison group, respectively), with no significant between group differences observed across the three study phases (baseline, supraphysiologic, withdrawal). The symptoms attributable to the hormonal manipulations performed in this study included mood symptoms (in the PPD+ subjects only) during the supraphysiologic and withdrawal phases, hot flushes in the hypogonadal and withdrawal phases, and sporadic bleeding observed throughout the study. The incidence of the somatic symptoms did not differ between groups; hot flushes did not precede the mood symptoms (observed only in the PPD+...
subjects), and spotting was not phase specific and thus did not compromise the blind.

A significant phase effect was observed for UFC levels ($F_{2,9} = 5.8, P < 0.01$) (Fig. 1A), largely reflecting a 2-fold (but insignificant) increase during the supraphysiologic phase, compared with the baseline phase. A significant phase effect was observed for plasma CBG levels ($F_{2,9} = 3.9, P < 0.05$) (Fig. 1C), reflecting approximately 36% higher levels during the supraphysiologic phase, compared with the baseline phase.

No differences were observed in UFC, plasma CBG, or net cortisol AUC between baseline and withdrawal phases. Similarly, no differences across the three study phases were observed for plasma leptin and urinary norepinephrine, epinephrine, and dopamine (data not shown). UFC and CBG levels were not significantly correlated during any of the three study phases.

Significant phase by time ($F_{8,126} = 2.3, P < 0.005$) and phase by diagnosis ($F_{2,14} = 5.0, P < 0.05$) interactions were seen for oCRH stimulated cortisol, reflecting increased stimulated cortisol during the supraphysiologic phase, with greater increases seen in the PPD+ subjects (Fig. 2). A significant phase (baseline, supraphysiologic, withdrawal) by diagnosis (PPD+ vs. PPD−) effect also was seen for cortisol AUC ($F_{2,8} = 7.6, P < 0.05$) (Fig. 1B). Post hoc analysis within PPD+ subjects revealed a significant increase of cortisol at the supraphysiologic phase, compared with both baseline ($P < 0.01$) and withdrawal ($P < 0.01$) phases. No significant changes in cortisol levels were present in the PPD− group (all $P > 0.10$). Comparison between phases in PPD+ subjects revealed a significant difference in cortisol AUC at the supraphysiologic phase compared with baseline ($P < 0.05$). No group by phase interactions were observed for UFC or CBG.

**FIG. 1.** UFC (A), cortisol AUC (B), and cortisol binding globulin (C) (means ± sd) in women with (PPD+) and without (PPD−) a history of PPD.

**FIG. 2.** Cortisol plasma level responses (mean ± se) to an oCRH stimulation test in women with (PPD+) and without (PPD−) a history of PPD.
Discussion

These results clearly demonstrate in a unique, in vivo model that high concentrations of gonadal steroids in euthyemic premenopausal women for a period of 8 wk have two effects: first, they result in elevated basal cortisol levels as seen by UFC excretion, and second, they increased the reactivity of the HPA axis such that stimulation by oCRH results in higher plasma cortisol levels. This finding is consistent with recently published (1, 13) data showing that exercise-stimulated HPA axis activity in women was enhanced by exogenously administered progesterone relative to a hypogonadal state and during the luteal phase of the menstrual cycle relative to the follicular phase (1, 13). During the third trimester of pregnancy, such an enhanced reactivity of the axis has been described and is attributed to progressively increasing levels of circulating CRH of placental origin and decreasing levels of CRH-binding protein, both phenomena contributing to elevated levels of bioactive free CRH and, thus, hypersecretion of ACTH and cortisol (14). In the present model, the placental CRH component is not present, and thus the increase in HPA axis activation results from the increase in gonadal steroid concentrations. Whereas increases in HPA axis activation have been reported to accompany administration of gonadal steroids (4), the increase in basal, unstimulated cortisol levels (increased UFC) that we observed may be attributable to the observed increase in CBG concentrations accompanying elevated estradiol levels. No significant correlations appeared, however, between UFC and CBG levels in any of the three study phases. As an alternative mechanism, rising levels of gonadal steroids may modulate the HPA axis through regulation (for example) of transcription at hormone response elements on the CRH gene (15, 16).

The second finding of this study is that stimulated cortisol concentrations increased to a greater extent in the pregnancy-like phase of the study in women with a history of PPD (PPD+), compared with those without (PPD−). This difference cannot be attributed to emotional stress elicited in PPD+ women, because such mood symptoms were observed to a greater extent in the withdrawal phase of the study in which, contrary to the supraphysiologic phase, no difference in cortisol level was observed between the two groups. The fact that levels of stimulated cortisol rose to a larger extent in PPD+ women, compared with controls, while receiving supraphysiologic doses of gonadal steroids may have potential consequences in subjects with such history who use oral contraceptives or take hormone replacement therapy. It should be noted, however, that because neither the oral contraceptive nor hormone replacement therapy preparations commonly used translate into supraphysiologic estrogen or progesterone levels (but rather mimic physiologic levels), the relevance of our findings for the hormonal therapy of PPD+ subjects is uncertain. Supraphysiologic doses of estrogens or progestins in PPD+ subjects may, however, induce an exaggerated stress-related increase in cortisol levels. Such increased levels of cortisol have been associated with several adverse consequences, including osteoporosis, in women with major depression (17).

Our observation of differential response in women with a history of PPD is consistent with several possible explanations. The experience of a prior depression may alter the subsequent modulatory effect of gonadal steroids on stimulated pituitary-adrenal response. Abnormal cortisol response to the dexamethasone-CRH test, for example, has been described in remitted depressed patients (18). Whether antecedent puerperal and nonpuerperal depressions would have similar subsequent effects on the pituitary-adrenal axis is unclear.

Alternatively, gonadal steroids may have a differential modulatory effect on the HPA axis (i.e., enhanced reactivity) in women who are also vulnerable to the development of PPD. It is of interest that many women who eventually develop PPD become partially symptomatic during the third trimester (19, 20). From the present data, one can speculate that these early depressive symptoms may be a consequence or concomitant of dysregulation of the HPA axis secondary to the elevation in gonadal steroids that occurs during pregnancy. Whereas we have previously shown that progesterone increases exercise-stimulated HPA axis function (1), progesterone levels in the PPD+ subjects were, if anything, lower than those obtained in the control subjects and hence cannot explain the enhanced reactivity seen in the PPD+ subjects.

Because maternal leptin is elevated during pregnancy (21), we measured this hormone during the different phases of the study. No changes were observed in leptin levels resulting from pharmacological manipulation of the gonadal axis, suggesting that the increase in leptin observed during pregnancy is mainly due to the production of leptin from the placenta rather than the increased estrogen levels observed during pregnancy. Furthermore, no differences in leptin were observed in this small sample between subjects with a history of PPD and controls. Some reports indicate that leptin may be increased, especially at night, in subjects with major depression (11). Because leptin secretion is pulsatile, the observation that single-time determinations of leptin did not differ between subjects and controls does not necessarily rule out the possibility that leptin may differ between patients and controls (22).

The differential response in the HPA axis was not observed in the withdrawal phase, which mimics the postpartum period when, clinically, most cases of PPD begin. Furthermore, in her study Magiakou et al. (7) found prolonged blunting of the HPA axis in women with the blues or depression in the hypogonadal postpartum period, a finding we did not replicate in our model. This discrepancy may reflect the obvious limitations of a pharmacological model of pregnancy. In our model we were able to attain supraphysiologic levels of gonadal steroids, but these levels are much lower than those reached during pregnancy and further are maintained for a relatively short duration (8 wk). As such, the withdrawal phase induced in our model is certainly not identical with the postpartum period. Furthermore, pregnancy is characterized by the production of very high concentrations of CRH by the placenta, causing the down-regulation of the hypothalamic CRH neuron, which results in a blunting of the HPA axis in the postpartum period (23). The absence of this blunting effect in our model may explain our inability to detect a
differential effect of the (hypogonadal) withdrawal phase on the HPA axis in the group of women with a history of PPD. The underlying biological mechanisms responsible for cortisol hyperresponsiveness to oCRH in the presence of increased gonadal steroids need to be elucidated. Such mechanisms could include increased arginine vasopressin secretion, up-regulation of pituitary CRH receptors, or increased steroidogenesis through effects on adrenal steroid synthetic enzymes (24). Similarly, the exaggerated responsivity in women with a history of PPD, although of unknown cause, may represent the effect of polymorphic variants in steroid synthetic genes or other genes in the pituitary-adrenal regulatory pathway (25, 26). As a caveat, the fixed rather than randomized order of the conditions in this study precludes detection of possible order effects. Other limitations of the current study included the following: the sample size was small; ACTH levels were not determined; the model simulated only endocrine change and did not mimic environmental factors (e.g. maternal stressors) (27) that affect pregnancy and parturition; given the uniform time of pituitary-adrenal testing, possible differences in diurnal modulation of the axis could not be determined; and the oCRH test is only one measure of pituitary-adrenal axis activity, the results of which have uncertain relationships to behavior. Despite these limitations, this study demonstrated by using a novel pharmacological model of pregnancy and parturition that women with a history of PPD, although euthymic, exhibit increased cortisol responses to a pharmacological challenge, oCRH, in the context of increased levels of gonadal steroids. Such a trait may cause behavioral adaptation or predispose to the long-term consequences of hypercortisolism.

Acknowledgments

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References


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