CORTISOL RESPONSE TO oCRH IN A MODEL OF PREGNANCY AND PARTURITION IN EUTHYMIC WOMEN WITH AND WITHOUT A HISTORY OF POSTPARTUM DEPRESSION

Abbreviated Title: Enhanced stimulated cortisol with history of PPD

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ABSTRACT

Introduction: Hypothalamic-pituitary-adrenal (HPA) axis abnormalities have been reported in depressed women and in women with postpartum "blues" compared to non-depressed women.

Methods: We investigated the effect of gonadal steroids on the hormonal response to oCRH 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. oCRH (1 µg/kg) stimulation tests were performed in the baseline follicular phase, during hormone addback (leuprolide acetate plus supraphysiologic doses of estradiol and progesterone-mimicking pregnancy), and following precipitous withdrawal of hormone replacement (mimicking the puerperium).

Results: Significant phase by time (p < .005) and phase by diagnosis (p < .05) interactions were observed, reflecting increased stimulated cortisol during the supraphysiologic phase, particularly in subjects with a history of PPD (PPD+). Cortisol AUC also showed a significant phase by diagnosis effect (p < .05). Significant increases during the supraphysiologic phase were also seen for UFC (p < .05), cortisol AUC (p < .001), and plasma CBG (p < .05).

Conclusion: 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.
INTRODUCTION

Abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis have been associated with the pathogenesis of depression in women. Studies of the effects of estrogen on the HPA axis have demonstrated extensive interactions and generally show a stimulatory effect of estrogen on the HPA axis (1-4). However, in a prior study, we demonstrated that progesterone rather than estradiol appeared responsible for the increased HPA axis activity observed during the luteal phase (1). The 3rd trimester of pregnancy is characterized by very high estrogen and progesterone plasma levels and by a hyperactive HPA axis with high plasma cortisol levels (5). During this period, total and free cortisol concentrations and 24-h urinary free cortisol excretion increase to levels similar to those seen in a mild form of Cushing’s syndrome (6). Conversely, the postpartum period is associated with suppressed hypothalamic CRH secretion attributed to dysregulation of the hypothalamic CRH neuron together with the postnatal hypoestrogenic state (7).

About 10% of women develop a form of depression during the postpartum period and a much higher percent experience the “blues.” Magiakou et al (7) reported that women affected by the “blues” or depression (PPD) in the postpartum period experienced a more blunted ACTH response to ovine oCRH stimulation compared to nondepressed women. In a pharmacological model which partially attempts to simulate pregnancy, parturition and the postpartum period (8), we demonstrated that in vivo manipulation of gonadal steroid concentrations differentially affects mood in women with and without a predisposition for postpartum depression. In the present study we used a subsample of women from the earlier study to further evaluate the potential involvement of the pituitary-adrenal axis in the pathogenesis of postpartum depression.
METHODS

Subjects

This study was part of a research project exploring the potential effects of gonadal steroids on mood and the susceptibility to postpartum depression (for a detailed description of the study design see Bloch et al (8). The main inclusion criteria were healthy, euthymic, medication-free (including oral contraceptives) women, 22-45 year old, with regular menstrual cycles (n = 12). All women had one or more biological children and were at least one year past their most recent childbirth. A complete psychiatric diagnostic evaluation was performed using the Structured Clinical Interview for DSM-IV (SCID) (9) and the Schedule for Affective Disorders and Schizophrenia - Lifetime (SADS-L) (10). Subjects with past and present psychiatric illness, with the exception of past postpartum depression 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 postpartum depression who were euthymic for the past year or longer (subjects, n = 5), and age-matched (within three years) controls with no history of past or present psychiatric illness (controls, n = 7). The age and BMI of subjects and controls were as follows, age: 32.2 ± 7.6 and 30.1 ± 6.2, 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 prior to their participation.

Study Design

The study comprised three distinct, consecutive phases with a total duration of four months - a hypogonadal phase (four weeks), a supraphysiologic phase (eight weeks), and a withdrawal phase (four weeks). The first phase (hypogonadal) consisted of the induction of a hypogonadotrophic hypogonadal state by using an open-label injection of depot leuprolide acetate
(Lupron, 3.75 mg IM). This agent is a long-acting gonadotropin releasing hormone (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 Pharmacy, Madison, WI) and estradiol (Estrace, Bristol-Myers Squibb, NJ) were substituted for placebo. The number of tablets prescribed was adjusted bi-weekly 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/day and was progressively increased up to 10 mg/day in three divided doses. Progesterone was started at 400 mg/day in three divided doses and increased progressively according to plasma levels. Plasma levels were monitored after one week, two weeks, and every two weeks thereafter, in an effort to reach maximal levels after two to four weeks. 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 four weeks, subjects were followed for eight additional weeks while unmedicated (follow-up).

To ensure that the subjects were kept "blind" to gonadal steroid treatment, the following 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 bi-weekly) was blind to treatment phase and the presence or lack of a history of postpartum depression.

**oCRH stimulation test** – Ovine CRH (oCRH) stimulation tests were performed three times throughout the study using a dose of 1 µg/kg BW, as previously described (11): during the early follicular phase prior to the first leuprolide injection (baseline), between weeks 6 and 8 of active estradiol and progesterone (plus leuprolide) addback (supraphysiologic), and two weeks after the last leuprolide injection and switch of active medication to placebo (withdrawal). The oCRH test was performed starting between 7 and 9 a.m.

Subjects were instructed to fast from midnight before the test and to present to the clinic between 7 and 8 a.m. Subjects were weighed and then placed in a semi-recumbant position. An intravenous catheter was inserted in the antecubital space and the subjects rested for 40 minutes prior to receiving 1 µg/kg oCRH by IV push. Blood samples for ACTH and cortisol were drawn at -15, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes. Blood samples were collected in pre-chilled tubes containing EDTA, centrifuged and aliquoted promptly, and plasma was stored at -70°C until assayed.

**Hormone Assays**

Plasma levels of cortisol were measured by RIA on the samples collected during the oCRH test. In addition, 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 bi-weekly starting at baseline on samples collected in the morning. Finally, 24-h urine collection was performed once at each phase of the study, prior to the oCRH test, for further determinations of urinary free cortisol (UFC), norepinephrine, epinephrine, and dopamine. Assays 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 phase (baseline, supraphysiologic, withdrawal) and time of sampling (-15 to 180 minutes) as within subjects factors.

The area under the curve (AUC) was then calculated using the trapezoidal 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 x 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 postpartum depression. 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 vs 290 ± 77.7 pg/ml; and progesterone: 46.6 ± 19.6 vs 73.1 ± 32.2 ng/ml mean ± SD in women with a history of PPD vs 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 ($F_{2,9} = 5.8, p \leq 0.01$) (Fig 1, panel A), largely reflecting a two fold (but insignificant) increase during the supraphysiologic phase compared to the baseline phase. A significant phase effect was observed for plasma CBG levels ($F_{2,9} = 3.9, p < 0.05$) (Fig 1, panel C), reflecting approximately 36% higher levels during the supraphysiologic phase compared to 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 ($F_{8,126} = 2.3, p < .005$) and phase by diagnosis ($F_{2,14} = 5.0, p < .05$) 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 ($F_{2,8} = 7.6, p < 0.05$). Post-hoc analysis within PPD+ subjects revealed a significant increase of cortisol at the supraphysiologic phase compared to both baseline ($p < 0.01$) and withdrawal ($p < 0.01$) phases. No significant changes in cortisol levels were present in the PPD- group (all $p$'s > 0.13). Comparison between PPD+
and PPD- subjects revealed a significant difference in cortisol AUC at the supraphysiologic phase only (p < 0.05). No group by phase interactions were observed for UFC or CBG.

DISCUSSION

These results clearly demonstrate in a unique, in vivo model, that high concentrations of gonadal steroids in euthymic premenopausal women for a period of eight weeks have two effects; first, they result in elevated basal cortisol levels as seen by urinary free cortisol 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 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 3rd 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. While 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 basal and 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 to 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 where, contrary to the supraphysiologic phase, no difference in cortisol level was observed between the two groups. The fact that levels of urinary free and stimulated cortisol rose to a larger extent in PPD+ women compared to controls while receiving supraphysiologic doses of gonadal steroids may have potential consequences in subjects with such history who use oral contraceptives (OC) or take hormone replacement therapy (HRT). It should be noted, however, that because neither the OC or HRT preparations commonly used translate into supraphysiological estrogen or progesterone levels (but rather mimic physiological 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 dex-CRH test, for example, has been described in remitted depressed patients (18). Whether antecedent puerperal and non-puerperal 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
3rd 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. While 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.

As 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). As 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 supraphysiological levels of gonadal steroids, but these levels are much lower than those reached during pregnancy and further are maintained for a relatively short duration (eight weeks). As such, the withdrawal phase induced in our model is certainly not identical to the postpartum period. Further, pregnancy is characterized by the
production of very high concentrations of CRH by the placenta, causing the downregulation 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 hyper-responsiveness to oCRH in the presence of increased gonadal steroids need to be elucidated. Such mechanisms could include increased AVP secretion, upregulation of pituitary CRH receptors, or increased steroidogenesis through effects on adrenal steroid synthetic enzymes (24). Similarly, the cause of the exaggerated responsivity in women with a history of PPD, while unknown, 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 exhibit, while euthymic, increased cortisol responses to a pharmacological challenge, oCRH, in the context of increased levels of gonadal steroids. Such a trait may compromise behavioral adaptation or predispose to the long-term consequences of hypercortisolism.
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Legends

Figure 1 - Urinary free cortisol (panel A), Cortisol area under the curve (panel B) Cortisol binding globulin (panel C) (means + SD) in women with (PPD+) and without (PPD-) a history of PPD.

Figure 2 – Cortisol plasma level responses (mean ± SE) to an oCRH stimulation test in women with (PPD+) and without (PPD-) a history of PPD.
Conversion factors to SI units are as follows: UFC x 2.759 = nmol/d; CBG x 18.868 nmol/L; cortisol x 27.59 = nmol/L
* Diagnosis by phase effect (p < 0.05), accounted for by increased stimulated cortisol in PPD+ subjects during the supraphysiologic phase.

Conversion factors to SI units are as follows: cortisol x 27.59 = nmol/L