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Stress-induced increases in progesterone and cortisol in naturally cycling women

Alexandra Ycaza Herrera*, Shawn E. Nielsen, Mara Mather

Davis School of Gerontology, University of Southern California, 3715 McClintock Ave, Los Angeles, CA 90089, United States

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ABSTRACT

Studies with animals of both sexes show that the adrenal glands release progesterone in addition to cortisol in response to stress. However, little is known about the progesterone response to stress in naturally cycling women. We investigated the effect of stress on estradiol, progesterone, and cortisol levels in women during the follicular phase of the menstrual cycle. We found that physical stress (the cold pressor test) had no effect on estradiol levels, but increased progesterone and cortisol. We also found positive correlations between baseline progesterone and cortisol levels, as well as between the change in progesterone and cortisol before and after water exposure in both the stress and control sessions. Mediation analyses revealed during the stress session, the change in progesterone from baseline to 42-min post-stress onset was mediated by the magnitude of change in cortisol levels across the same time span. Overall, these findings reveal that progesterone released in response to stress as observed in animals and men extends to women during the low ovarian output follicular phase of the menstrual cycle, and that the mechanism of release may be similar to the mechanism of cortisol release.

1. Introduction

The levels of bioavailable, salivary cortisol observed in response to a stressor varies between sexes and across the menstrual cycle in women (Kirschbaum et al., 1992 Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005). For example, women in the luteal phase of the menstrual cycle (moderate estradiol and high progesterone levels) and men exhibit comparable salivary cortisol increases to social stress, while women in the follicular phase (low estradiol and low progesterone) and women on oral contraceptives (low ovarian output of estradiol and progesterone) exhibit significantly smaller salivary cortisol responses to the same social stressor (Kirschbaum et al., 1999). More recent studies show that hormonal contraceptives also attenuate the salivary cortisol response to physical stress compared to naturally cycling women (Nielsen et al., 2013b) and more specifically, luteal women (Nielsen et al., 2014).

One interpretation of these findings is that higher progesterone (P) levels during certain phases the menstrual cycle leads to greater free cortisol levels in response to stress. Other work supports such an interpretation. For example, at least one group of women (those with induced hypogonadism via administration of the gonadotropin releasing hormone agonist lupron) exhibited amplified cortisol responses to exercise stress when also administered progesterone but not estradiol (Roca et al., 2003). However, the interpretation that P amplifies cortisol response to stress fails to acknowledge that the adrenal glands also secrete P and that the influence of menstrual cycle fluctuations of P on cortisol response to stress may be masking whether and how adrenal P may be responding to stress. The effect of stress on adrenal output in animals and men has shown that the adrenal glands secrete not only cortisol, but also P, in response to stress (Fajer et al., 1971 Brown et al., 1976b; Deis et al., 1989 Breier and Buchanan, 1992; Cooper et al., 1995 Elman and Breier, 1997; Duncan et al., 1998 Romeo et al., 2004; Romeo et al., 2006), with limited work examining the effect in women (Childs et al., 2010; Gaffey and Wirth, 2014). This P release during stress is of importance for studies examining menstrual cycle influences on the stress response, as many studies average P values across multiple time points in order to determine average cycle-related P levels during an experimental session (Nielsen et al., 2013a; Nielsen et al., 2014; Petersen et al., 2014). If women also experience adrenal release of P in response to stress, then this adrenal release of P in response to stress may contribute to the pattern of greater bioavailability of cortisol in response to stress during high progesterone phases of the menstrual cycle

* Corresponding author.
E-mail address: ycaza@usc.edu (A.Y. Herrera).

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(Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005). Despite this possibility, little work has tested the relationship between estradiol (E2), P, and the cortisol response to stress in young, naturally cycling women.

In the present study, we aimed to investigate the influence of baseline P on cortisol responses and to test the effect of stress exposure on E2, P, and cortisol levels in response to a physical stressor (Cold Pressor Test; CPT) in naturally cycling women during the early and late follicular phase of the menstrual cycle. Another neglected factor when drawing conclusions regarding the relationship between P and cortisol during the luteal phase of the menstrual cycle is the concomitant increase in E2 also experienced during the luteal phase. We thus elected to test women during the low-P follicular phase of the menstrual cycle, which abolished the concerns for accompanying changes in E2 and the ability to investigate whether P and cortisol shared a similar relationship when P fluctuations are much smaller as observed during the follicular phase than when P levels are much higher as during the luteal phase (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005).

By investigating the P and cortisol relationship during the low-P follicular phase of the menstrual cycle, we made the following hypotheses. First, we hypothesized that both salivary cortisol and salivary P would increase in response to CPT exposure. Second, we hypothesized that baseline salivary cortisol and baseline salivary P would be positively correlated. Finally, based on the aforementioned observed associations between high P and higher levels of stress-induced bioavailable cortisol during the luteal phase of the menstrual cycle (Kirschbaum et al., 1999; Kudielka and Kirschbaum, 2005), we hypothesized that baseline P levels would mediate the cortisol response to CPT exposure, such that higher baseline salivary P levels would account for larger cortisol responses to CPT.

2. Materials and methods

2.1. Participants

Thirty-three naturally cycling undergraduate females from the University of Southern California (18–24 years) participated in this study. Participants attended four sessions after first providing informed consent. Two sessions occurred during the Early Follicular phase (EF; days 1–5, with day 1 being the first day of menses) and two occurred during the Late Follicular phase (LF; days 8–12), order counterbalanced. Twenty-seven women completed all four sessions. Cycle regularity was defined as menses regularly occurring between 25 and 31 days. Women were determined to be regular if they self-reported their prior two cycles as falling within the range of 25–31 days during a phone interview that occurred prior to their participation. During the phone interview, women also reported the expected start date of their next menses. Women were then seen for their first of four sessions upon confirming the start of that post-phone interview menses. The average age of the participants was 20.8 ± 1.8 years (range: 18–24 years) and the average years of education was 14.8 ± 1.8 years (range: 12–18); 77.8% were of non-Hispanic ethnicity and 22.2% of Hispanic ethnicity, and race breakdown was 55.6% Asian, 18.5% Caucasian, 7.4% biracial, 14.8% other, while 3.7% declined to state.

Participants were free from heart disease, peripheral vascular disease, diabetes, Reynaud's phenomenon, cryoglobulinemia, vasculitis, lupus, tingling or numbness in the hands and/or feet, and any other serious chronic illness. They were non-smokers, not using beta-blocker or corticosteroid-based medications, or psychoactive drugs, and had never been pregnant. Former hormonal contraception users had stopped using hormonal contraception at least 6 months before participation.

Participants completed one stress and one control session in both the EF and LF phases, order counterbalanced. Most women first seen during the EF phase completed all 4 sessions within the same menstrual cycle, whereas women first seen during the LF phase completed their 4 sessions across two consecutive menstrual cycles. Three women were seen across more than 2 menstrual cycles due to schedule conflicts.

2.2. Salivary hormone measurements

All sessions were conducted in the afternoons between 1200 and 1900 h, with no session starting later than 1730 h. To ensure stable hormone levels prior to collection of the baseline saliva samples, participants were asked to refrain from exercise and food/drink (except water) within one hour, sleep within two hours, and caffeine and alcohol within three hours of their session start time. The general protocol for all sessions was (see Fig. 1): arrive, drink 8 oz. of water, saliva sample 1 (baseline; minimum of 10 min after finishing water), CPT, saliva sample 2 fifteen minutes after CPT onset (15m-post-stress), behavioral tasks, and saliva sample 3 after all behavioral tasks had been completed, or an average of forty-two minutes after CPT onset (42m-post-stress). While part of a larger behavioral study examining the effects of stress on working memory and emotional memory processes, this study focused only on cortisol, P, and E2 responses to CPT stress, thus behavioral data are not reported here (although timing of tasks is also displayed in Fig. 1).

Salivary samples are a reliable source for determining biologically available, unbound, levels of hormones (Vining et al., 1983; Tunn et al., 1992). Participants passively drooled saliva into a collection tube for each sample. Cortisol levels were measured in all three saliva samples, and P and E2 in the first and last samples. Due to the common practice of determining E2 and P levels by averaging two sample measurements in menstrual cycle studies (Nielsen et al., 2013a, 2014; Petersen et al., 2014), we wanted to test the first and last samples to see whether and how stress affects P and E2. Samples were stored at 0 °C until all data collection was completed, at which time saliva was assayed to determine hormone levels.

Salivary levels of cortisol, 17β-estradiol, and progesterone were measured using Salimetrics, LLC (State College, PA) ELISA kits and measured optically using Molecular Devices, LLC SpectraMax M3 Multi-mode Microplate Reader (Sunnyvale, CA). The inter- and intra-assay variations for cortisol (8.16%; 12.3%), 17β-estradiol (4.12%; 16.2%), and progesterone (11.7%; 19.9%) were within the expected ranges from our lab.

2.3. Stress manipulation

The CPT was used to induce a stress response and has been shown to reliably induce cortisol secretion (Lighthall et al., 2009, 2012; Mather et al., 2010). Participants immersed their non-dominant hand, up to the wrist, in ice water (0–5 °C at time of immersion) for up to three minutes. Participants completed a minimum of one minute with their hand immersed in ice water. Participants unable to complete at least 60 consecutive seconds in the ice water were allowed to remove and re-immerse their hand until they accumulated at least 60 s with their hand in the water. Eighteen participants successfully kept their hands immersed in the stressful ice water for 3 min. Of the nine remaining participants, 4 kept their hands immersed for at least one minute, but fewer than 3 min, and 5 participants removed and re-immersed their hands until accumulating at least 1 min in the ice water. The control condition replaced the ice water with warm water