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Short Communication

Paroxetine blunts the corticosterone response to swim-induced stress and increases depressive-like behavior in a rat model of postpartum depression

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ABSTRACT

Perinatal depression (PND) affects 15% of women. During the perinatal period both stress- and gonadal hormones fluctuate widely. Putatively, these fluctuations are involved in PND disease mechanisms. The serotonin system is sensitive to such hormone fluctuations, and serotonin reuptake inhibitors (SSRIs) are used to treat PND, although treatment is suboptimal and it is not known at which peripartum time-point SSRI treatment may be most efficacious. In this study, we investigate the effect of the SSRI paroxetine (5 mg/kg s.c.) on swim stress-induced corticosterone in a rat model of postpartum depression. In the rat model corticosterone (CORT; 40 mg/kg s.c.) was administered in Sprague Dawley rats across postpartum day (PD)2 to PD14. Stress response was measured during the first exposure to the forced swim test (FST1), and depressive-like behavior was measured in both FST1 and FST2. We found that paroxetine completely blunted the swim stress-induced CORT response and increased depressive-like behavior in both FST1 and FST2. Our findings suggest that in the postpartum context, SSRIs compromise stress axis dynamics, which are needed for a healthy stress response. This is likely unfavorable for reversing depressive-like behavior and may provide a rationale for augmentation strategies beyond SSRIs alone to optimize the clinical management of PND.

1. Introduction

One in seven women will experience perinatal depression (PND), with the highest risk occurring during the second week postpartum (Munk-Olsen et al., 2006). The peripartum period is associated with dramatic changes in the hypothalamic-pituitary-adrenal (HPA) and −gonadal (HPG) axes. The rapid decline in estradiol (Bloch et al., 2000; Guintivano et al., 2013), and abnormal glucocorticoid dynamics in the postpartum (Seth et al., 2016), contribute to risk mechanisms for PND in susceptible women. Disturbances in the serotonin system are involved in the pathophysiology and treatment mechanisms of major depressive disorder, and this same system is sensitive to hormone fluctuations, and may be a critical factor in PND susceptibility (Brunton and Russell, 2008). Estradiol supplementation in the postpartum has beneficial effects in PND, and serotonin reuptake inhibitors (SSRIs) are a key treatment strategy for PND (di Scalea and Wisner, 2009). However, SSRIs work differently under different hormonal settings; e.g., short-term (cyclic) SSRI treatment works efficiently in women with premenstrual dysphoric disorder (Steinberg et al., 2012), whereas SSRI treatment for PND shows mixed results (di Scalea and Wisner, 2009). Notably, in ovariectomized rats, the antidepressant-like effect of acute sertraline treatment depends on concomitant estradiol-replacement and is not seen in ovariectomized controls (Sell et al., 2008).

During major depressive episodes, the HPA axis is normally hyperactivated, often with blunted dynamics, and when SSRI treatment results in remission, the HPA axis dynamics are typically restored (Ruhé et al., 2015). During the postpartum, the HPA axis is suppressed (by negative feedback mechanisms from high corticosteroid levels during pregnancy) in healthy individuals (Brunton and Russell, 2008). In women with PND, the HPA axis dynamics are blunted for an extended period relative to healthy women postpartum (Seth et al., 2016). In addition, whereas cortisol levels are elevated in early onset PND, they are typically decreased in later postpartum PND, which emphasizes critical phase-specific mechanisms in PND and heterogeneity of the disease (Seth et al., 2016). The effects of SSRIs on HPA dynamics in PND are, therefore, difficult to predict, likely to be phase-specific, and have not been carefully studied to date.

In this study, we investigate the effect of paroxetine on

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corticosterone response to swim stress and on depressive-like behavior in a modified rat model of postpartum depression, induced by chronic high corticosterone (CORT) exposure postpartum (Brummelte et al., 2006). We were interested in evaluating phasic changes across the early to mid-postpartum period, and we hypothesized that paroxetine would remediate any CORT-induced increase in depressive-like behavior and would alter HPA dynamics in response to stress.

2. Material and methods

2.1. Animals

One hundred and twelve female (200–225 g) and ten male Sprague-Dawley rats (250–275 g) from Charles River were initially housed in same-sex pairs, under 12 h:12 h cycle (lights on 0700) and given rat chow (Jamieson's Pet Food Distributors) and tap water *ad libitum*. For breeding, one male was housed with two females overnight, and the presentation of sperm in vaginal lavages indicated pregnancy. Rats were single housed, with paper towel and enrichment tube, and were weighed weekly. Protocols were in accordance with ethical guidelines set by Canada Council for Animal Care and were approved by the University of British Columbia Animal Care Committee.

One day after birth (day of birth = postnatal day [PD] 0), all litters were culled to 5 females and 5 males, and dams were assigned to treatment groups, balancing for sucrose preference (not part of this study) and bodyweight.

2.2. Experimental design

For an experimental overview, see Fig. 1A. Beginning PD2, dams received daily injections (0830-1030) of either vehicle (10% ethanol in sesame oil, 1 ml/kg s.c.) or CORT (Sigma-Aldrich; 40 mg/kg), as a model of postpartum depression, adapted from Brummelte et al. (2006), which gave CORT postpartum for 22 days. CORT was suspended in 100% ethanol for a few hours before adding sesame oil, and fresh suspension was made every three days. To treat PND, dams also received daily injections of the SSRI paroxetine HCl (PXT; Sequoia Research Products; 5 mg/kg) or vehicle (5% dextrose in sterile water, 1 ml/kg s.c.) starting on PD2. PXT was brought into solution using an ultrasonicator. A low dose of PXT which increases 5-HT levels (Hajós-Korcsok et al., 2000) was chosen, in part to reduce the risk of side effects caused by higher dose. Dams were split into early postpartum (stress response measured PD2), and mid-postpartum (stress response measured PD12), resulting in n = 14/group. Dams underwent two exposures in the forced swim test (FST), 24 h apart, with the first exposure (FST1) used to measure the CORT response and behavior, and the second exposure (FST2) given to examine behavior. The day after, animals were tested in the open field test (OFT), and the day after OFT, 24–28 h after their last injection, rats were euthanized, and blood was collected for estradiol determination. Euthanasia method differed with six animals per group given an overdose of Euthanyl, and heart blood collected, and eight animals per group decapitated, and trunk blood collected.

2.3. Forced swim test

The FST is used to assess depressive-like behavior. A glass cylindrical tank (45 × 28 cm) was filled with water (25 ± 0.1° C) to a depth of 30 cm, and light was dimmed during the test. FST1 was a 15 min test performed at 1200–1430, and was used as an acute stressor. FST2, a 5 min test the day after, assessed the behavioral response. Video recordings were scored (BEST Collection Software) for time spent immobile, swimming, climbing, and latency to immobility, by an observer unaware of treatment conditions.

2.4. Swim stress-induced HPA reactivity

Basal CORT levels were measured at 1130–1330, stress CORT levels were measured immediately after FST1, and recovery CORT levels were measured after 2 h recovery in the home cage. Blood (max 100 µl) was collected via tail vein puncture within 3 min of moving the cage or immediately following removal from the FST. Some samples were not successfully collected, but a minimum group size of 11 was maintained.

2.5. Open field test (OFT)

Locomotor activity was measured in the OFT. The 120 × 120cm2 arena (divided into 16 equal-sized squares) with 40 cm high walls was placed in a dimly lit room with a video camera installed above. Dams were placed in the arena facing a corner and activity was video recorded for 10 min. The videos were analyzed with Anymaze (Stoelting Co., USA) to assess the total distance travelled and time spent mobile.

2.6. Radioimmunoassay

Blood was stored overnight at 4° C to allow blood to clot completely. Following centrifugation at 10000*g* for 15 min, serum was collected and stored at −20° C.

Total CORT was measured using ImmuChem Double Antibody ^{125}I Radioimmunoassay Kit (MP Biomedicals). The cross-reactivity with other steroids is less than 0.4%.

Ultra-Sensitive Estradiol RIA Kit (DSL4800, Beckman Coulter) was used to measure estradiol (E_2). All samples were run in duplicate. Intra-assay coefficient of variation (CV) was 17% for estradiol and 6% for CORT. Samples with CV > 20% were discarded from analysis.

2.7. Data analyses

To determine whether a swim stress-induced CORT response was elicited, serum CORT was analyzed separately for each group, using repeated measures ANOVA with time (baseline, stress, recovery) as a within-subject factor. As a follow-up analysis, we tested whether the swim stress-induced CORT increase from baseline (Δ stress response) differed between control and PXT groups, using an unpaired student's *t*-test for each postpartum timepoint.

OFT, FST behavior and estradiol were each analyzed using factorial ANOVA with condition (CORT, OIL), treatment (PXT, vehicle), and postpartum time (early, mid postpartum) as categorical factors. Because euthanasia method may affect serum estradiol we included euthanasia method as a variable in the initial analysis, but it was not significant ($p = 0.79$), and we continued the analysis without this variable. Furthermore, when time spent mobile or distance travelled in the OFT were included as covariates in the FST analyses, they did not contribute significantly ($p > 0.22$, and $p > 0.15$, respectively), and did not significantly alter any main or interaction effects, and hence were left out of the model. Post-hoc tests used the Newman-Keul's test. Effects were considered significant when $p < 0.05$. Data were analyzed using Statistica 64 (StatSoft Inc.) or GraphPad Prism6 (GraphPad Software Inc.). All bar graphs are presented with data points, mean, and 95% confidence intervals (CI).

3. Results

3.1. Paroxetine blunts swim stress-induced corticosterone response

We found no significant swim stress-induced CORT change from baseline in the PXT groups regardless of CORT treatment, either in early (after first injection; Fig. 1E and F) or mid (after 11 daily injections; Fig. 1I and J) postpartum (early, Fig. 1E; $F_{2,35} = 1.30$, $p = 0.29$; mid, Fig. 1I; $F_{2,35} = 2.97$, $p = 0.08$). Control dams elicited a significant stress response and a full recovery of CORT, both in early (fig. 1C; main