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Ovarian hormones, but not fluoxetine, impart resilience within a chronic unpredictable stress model in middle-aged female rats

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ABSTRACT

Depression is more prevalent in women than in men, and women are at a heightened risk for depression during the postpartum and perimenopause. There is also evidence to suggest that the ovarian hormone milieu may dictate antidepressant efficacy. Thus, it is important to investigate the role of ovarian hormones in the pathogenesis of depression and in the mechanisms that may underlie antidepressant efficacy. In the present study, we used 10-month-old female Sprague-Dawley rats to examine the effects of long-term ovarian hormone deprivation on the development of depressive-like endophenotypes after chronic stress, and on antidepressant efficacy. Four months following ovariectomy (OVX) or sham surgery, all rats were subjected to 6 weeks of chronic unpredictable stress (CUS). During the last 3 weeks of CUS, rats received daily injections of fluoxetine (5 mg/kg) or vehicle. All rats were assessed on measures of anxiety- and depressive-like behavior, hypothalamic-pituitary-adrenal (HPA) negative feedback inhibition, and on markers of neurogenesis and microglia in the dentate gyrus. Our findings demonstrate that long-term ovarian hormone deprivation increased anxiety and depressive-like behavior, as seen by increased immobility in the forced swim test and latency to feed in the novelty suppressed feeding test, and decreased sucrose preference. Further, long-term OVX resulted in impaired HPA negative feedback inhibition, as seen in the dexamethasone suppression test. Fluoxetine treatment showed limited behavioral and neuroendocrine efficacy, however it reduced microglial (Iba-1) expression, and increased cell proliferation, neurogenesis (via cell survival), and the expression of the polysialylated neuronal cell adhesion molecule (PSA-NCAM) in the dentate gyrus, although these effects varied by region (dorsal, ventral) and ovarian status. Taken together, our findings demonstrate that ovarian hormones may impart resilience against the behavioral and neuroendocrine consequences of chronic unpredictable stress, and may modulate the effects of fluoxetine on cell proliferation, neurogenesis, and PSA-NCAM in the middle-aged female.

1. Introduction

Women are more than twice as likely as men to develop depression (Gutiérrez-Lobos et al., 2002); a disparity that is particularly striking during the reproductive years of women (i.e. 25–50 years; Gutiérrez-Lobos et al., 2002). Notably, periods that involve dramatic fluctuations and/or reductions in ovarian hormones, such as the postpartum and perimenopause, carry the highest risk of developing depression in women (Cohen et al., 2006; Hendrick et al., 1998; Soares, 2014). Together, these data provide compelling evidence for a role of ovarian hormones in the pathoetiology of depression.

Indeed, in healthy women, reductions in ovarian hormones triggered sub-clinical depression scores, and estradiol levels were negatively associated with depressive symptoms (Frokjaer et al., 2015). In addition, withdrawal from a hormone simulated pregnancy in women with a history of postpartum depression increased depressive symptoms relative to women without a history of postpartum depression (Bloch et al., 2000). Thus, ovarian hormone

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fluctuations contribute to the development of depressive symptoms in women. Similarly in rats, estradiol withdrawal after hormone simulated pregnancy leads to depressive-like phenotypes (Galea et al., 2001; Green et al., 2009; Green and Galea, 2008). Importantly, in this model, treatment with estradiol or an estrogen receptor (ER) β agonist prevented the development of depressive-like behaviors (Galea et al., 2001; Green et al., 2009). Ovariectomy itself can increase depressive- and anxiety-like behaviors, while treatment with estradiol or selective estrogen receptor modulators (SERMs) can restore this effect (Bekku and Yoshimura, 2005; Li et al., 2014; Okada et al., 1997; Walf et al., 2004). Taken together, these findings corroborate the notion that reductions in ovarian hormones may increase the risk for developing a depressive-like endophenotype. However, most of these studies using females did not utilize a model of depression, and thus the face and construct validity of such studies is uncertain. The present study aimed to fill this gap by investigating the role of ovarian hormones in the development of a depressive-like endophenotype within an animal model of depression.

Ovarian hormones are also implicated in antidepressant efficacy (Thase et al., 2005). For example, in postmenopausal women with depression, antidepressant treatment results in superior outcomes when prescribed alongside hormone therapy than when given alone (Thase et al., 2005), suggesting that ovarian hormones may enhance antidepressant efficacy. Furthermore, in women diagnosed with depression, estradiol has been given as either an adjunct therapy or a stand-alone antidepressant, with some success (Ahokas et al., 2001; Moses-Kolko et al., 2009; Rasgon et al., 2007). Similarly, 17β-estradiol treatment in ovariectomized rats augments the effects of the SSRI, sertraline, to reduce immobility in the forced swim test (Sell et al., 2008). However, this latter study was not conducted in conjunction with an animal model of depression, and thus it is unclear whether similar findings would be obtained from animals with a depressive-like phenotype. Our current study aimed to determine whether ovarian hormones influence antidepressant efficacy in an animal model of depression in middle-aged rodents.

The hippocampus exhibits compromised structural plasticity in depressed patients, as a meta-analysis showed that untreated depression is associated with reduced hippocampal volume, which is seen two years after diagnosis (McKinnon et al., 2009). Decreased hippocampal neurogenesis is observed in post-mortem tissue of depressed individuals (Boldrini et al., 2012) and in animal models of depression (Bessa et al., 2008; Green and Galea, 2008; Wainwright et al., 2011). Conversely, antidepressant treatment restores the reductions in neurogenesis in clinical depression and animal models (Bessa et al., 2008; Boldrini et al., 2012; Green and Galea, 2008). Notably, antidepressant use results in a greater increase in hippocampal volume in depressed women than depressed men (Vakili et al., 2000), and the pro-neurogenic effects of antidepressants are seen in the post-mortem tissue of women, but not men (Epp et al., 2013). Thus, ovarian hormones may modulate the effects of antidepressants on neurogenesis in the hippocampus.

There is also a growing recognition for the role of inflammation in depression and chronic stress (Miller et al., 2009). Remarkably, microglial activation is increased in the prefrontal cortex, insula, and anterior cingulate cortex by approximately 30% in depressed patients relative to controls (Setiawan et al., 2015). Increased inflammation and microglial activation in the hippocampus are also evident in animal models of chronic stress (Frank et al., 2007; Kreisel et al., 2013). Despite the well-established anti-inflammatory effects of ovarian hormones in a variety of disease models (see Habib and Beyer, 2015 for review), to our knowledge, the influence of ovarian hormones on microglia in relation to chronic stress or SSRI exposure has not been investigated, and thus the current study aimed to fill this gap.

Disturbances in the hypothalamic-pituitary-adrenal (HPA) axis are arguably the best characterized endocrine markers of depression. Specifically, a meta-analysis showed that depressed individuals display elevated levels of serum cortisol (Stetler and Miller, 2011), and at least a subset of depressed individuals show impairments in the HPA negative feedback system (Ising et al., 2007; Stetler and Miller, 2011). Antidepressant efficacy is more tightly linked to the restoration of HPA function in women compared to men (Binder et al., 2009), and hypogonadal women have impaired HPA negative feedback (Maes et al., 1992; Young et al., 1993). Therefore, similar to the effects of ovarian hormones on the behavioral efficacy of antidepressants, ovarian hormones may potentiate the efficacy of antidepressants to restore HPA dysfunction.

In this study, we investigated whether ovarian hormones impart resilience against the behavioral and HPA outcomes of chronic unpredictable stress in middle-aged female rats, and whether ovarian hormones influence antidepressant efficacy using fluoxetine. A depressive-like phenotype was examined at the behavioral (forced swim test, novelty suppressed feeding test, and sucrose preference test), endocrine (corticosterone levels and HPA negative feedback inhibition), and neural levels; examining both neuroplasticity (neurogenesis, PSA-NCAM), and microglial expression in the dentate gyrus. We hypothesized that ovarian hormones would be associated with resilience to the development of a depressive-like phenotype under chronic unpredictable stress, and that antidepressant efficacy would be modulated by ovarian hormone status in middle-aged female rats.

2. Materials and methods

2.1. Animals

Forty female Sprague-Dawley rats (Charles River Laboratories, Montreal, Canada), weighing 200–250 g, were used in this study. Animals were pair-housed in a female-only colony room and given *ad-libitum* access to food (Purina rat chow) and water. Colony rooms were temperature and humidity controlled (21 ± 1 °C; 50 ± 10%, respectively), and maintained on a 12-h light/dark cycle (lights on at 07:00). All procedures were approved by the Animal Care Committee at the University of British Columbia, and performed in agreement with ethical guidelines set by the Canadian Council on Animal Care. All efforts were made to reduce the number of animals used and to minimize their suffering.

2.2. Surgery

Animals were randomly assigned to receive bilateral ovariectomy (OVX, n = 20) or sham surgery (Sham, n = 20), which were performed at approximately five months of age, as we have done previously (Barha et al., 2010). Ovarian hormone deprivation by ovariectomy was chosen as a model of surgical menopause (Barha and Galea, 2013). Surgeries were performed under isoflurane anesthesia, with ketamine (30 mg/kg, Bimeda-MTC, Cambridge, ON), xylazine (2 mg/kg, Bayer HealthCare, Toronto, ON) and bupivacaine (applied locally; 4 mg/kg, Hospira Healthcare Corporation, Montreal, QC). Following recovery, animals were handled only during cage cleaning procedures, and left otherwise undisturbed until the beginning of the chronic unpredictable stress (CUS) procedure at approximately 9 months of age.

2.3. BrdU administration

To label dividing progenitor cells and their progeny in the dentate gyrus, all rats received two intraperitoneal injections of 5-