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Prenatal SSRI alters the hormonal and behavioral responses to stress in female mice: Possible role for glucocorticoid resistance

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ABSTRACT

Life time prevalence of major depression disorder (MDD) is higher in women compared to men especially during the period surrounding childbirth. Women suffering from MDD during pregnancy use antidepressant medications, particularly Selective Serotonin Reuptake Inhibitors (SSRI). These drugs readily cross the placental barrier and impact the developing fetal brain. The present study assessed the effects of prenatal exposure to fluoxetine (FLX), an SSRI antidepressant drug, on corticosterone and behavioral responses to stress in female mice. In young females, prenatal FLX significantly elevated corticosterone response to continuous stress. In adults, prenatal FLX augmented corticosterone response to acute stress and suppressed the response to continuous stress. Additionally, prenatal FLX significantly augmented stress-induced increase in locomotion and reduced anxiety- and depressive-like behaviors in adult, but not young mice. The dexamethasone suppression test revealed that prenatal FLX induced a state of glucocorticoid resistance in adult females, indicating that the negative feedback control of the hypothalamic-pituitary-adrenal axis response to stress was disrupted. These findings provide the first indication of altered hormonal and behavioral responses to continuous stress and suggest a role for the development of glucocorticoid resistance in these effects. According to these findings, prenatal environment may have implications for stress sensitivity and responsiveness to life challenges. Furthermore, this study may assist in understanding the limitations and precautions that should be taken in the use of SSRIs during pregnancy.

1. Introduction

Major depression disorder (MDD) is a frequent and disabling psychiatric disorder. Lifetime prevalence of MDD is 2–3 folds higher in women compared to men, with a considerable increase in the incidence in pregnant women (Bromet et al., 2011; Seedat et al., 2009; Nasreen et al., 2011; Hartley et al., 2011). Although the pathogenesis is unknown, hormonal dysregulation, abnormalities in hypothalamic-pituitary-adrenal axis (HPA) axis activity, and the contributions of genetics and epigenetics seems to play key roles in the development of perinatal mood disorders (Meltzer-Brody, 2011). Studies showed that 3–8% of pregnant women suffering from MDD use Selective Serotonin Reuptake Inhibitor (SSRI) antidepressants, which readily cross the placental barrier and impact serotonin transmission in the developing fetal brain (Alwan et al., 2011; Hayes et al., 2012; Hendrick et al., 2003; Olivier et al., 2011a; Yonkers et al., 2009). In the fetus, serotonin acts as a growth factor regulating the development of neural systems and as a trophic factor regulating developmental processes such as cell division, differentiation, migration, myelination, synaptogenesis, and dendritic pruning (Gaspar et al., 2003; Whitaker-Azmitia et al., 1996). Thus, prenatal SSRI treatment may cause long-lasting changes in neuronal development of the fetal brain.

To date, no gross SSRI-related teratogenic effects have been identified. However, human infants born following prenatal exposure to SSRI are at higher risk for various abnormalities, including cardiac defects, persistent pulmonary hypertension, and a self-limiting neonatal syndrome (Homberg et al., 2010). Prenatal SSRI exposure was also associated with shorter mean gestational age, lower birth weight and neonatal neurobehavioral disturbances (irritability, weak or absent cry, increased motor activity) or "withdrawal" symptoms in humans (Oberlander et al., 2010). Additionally, human and animal studies demonstrated that prenatal SSRI resulted in a variety of behavioral disturbances, such as delayed motor development, altered emotional behavior and increased aggression. Hormonal changes following prenatal SSRI were also observed, including reduced cord blood level of cortisol, increased levels of thyroid stimulating hormone (TSH) and reduced dehydroepiandrosterone (DHEA) levels (Ansorge et al., 2004, 2008; Avitsur et al., 2015b; Bairy et al., 2007; Davidson et al., 2009; Gobbi et al., 2001; Hilli et al., 2009; Laine et al., 2003; Lira et al., 2003; Oberlander et al., 2005, 2009, 2010; Svirsky et al., 2015). Together these findings indicate that in utero modulation of serotonin

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transmission may have lasting effect on the development of neuroendocrine and behavioral systems.

Along these lines, studies have suggested that prenatal exposure to SSRI may have implications for the development of the HPA axis, thus affecting its function later in life. In human infants, prenatal SSRI exposure altered HPA stress response patterns and reduced early evening basal cortisol levels (Oberlander et al., 2008). Prenatal SSRI also increased serum corticosteroid-binding-globulin (CBG) levels in human neonates along with a diurnal change in infant salivary cortisol (Pawluski et al., 2012a) and reduced cord blood level of cortisol in human neonates (Davidson et al., 2009). In rats, 28 days of fluoxetine administration beginning on postnatal day 1 decreases circulating levels of corticosterone and reduces the expression of the glucocorticoid receptor in the hippocampus (Pawluski et al., 2012b). An additional study in rats reported that FLX exposure at a similar time period had no effect on stress-induced corticosterone secretion, however decreased serum CBG levels in adult offspring rats (Knaepen et al., 2013).

Changes in HPA axis function may have wide implications for health and well-being. HPA hormones are crucial for the response to stress aiding the organism in reestablishing homeostasis (Silverman et al., 2005). Changes in HPA activity and reactivity following prenatal exposure to SSRI may therefore affect the offspring's stress resilience, health and well-being (Avitsur et al., 2015a). Thus, the goal of the present study was to examine the long lasting effects of prenatal SSRI exposure on corticosterone and behavioral responses to stress. Additionally, the effect of prenatal SSRI on HPA negative feedback control was examined using the dexamethasone suppression test. As one of the hallmarks of the response to chronic stress is a decrease in spleen and thymus weight (Everds et al., 2013), the effects of prenatal SSRI on these responses were also assessed.

Many of the effects of prenatal SSRI were found to be sex-dependent (e.g., Avitsur et al., 2015b; Knaepen et al., 2013; Pawluski et al., 2012b; Rayen et al., 2011; Rayen et al., 2013; Rayen et al., 2014; Svirsky et al., 2015). These sex differences were associated with sex hormones actions, as well as epigenetic factors affecting the development serotonergic pathways and stress-related processes (Boulle et al., 2015; Boulle et al., 2016; Rayen et al., 2015; Zohar et al., 2015). In addition, the response of the HPA axis to stress is sex-dependent, with females secreting higher levels of hormones (Goel et al., 2014; Panagiotakopoulos and Neigh, 2014). Thus, sex differences in the effects of prenatal SSRI on the response to stress are expected. The present study, therefore, focused on the response of females to prenatal SSRI, and the response of males is described elsewhere (Avitsur, unpublished observations).

2. Materials and methods

2.1. Animals

Subjects were female offspring of ICR (CD1) mice purchased from Harlan Laboratories (Israel). Subjects were born and raised at the Academic College of Tel Aviv-Yaffo animal facility. All subjects were given free access to food and water and were maintained on a reversed 12-hour light/dark cycle (lights on at 7:00 pm). Animal care procedures were approved by the Israel National Committee of Animal Care and Use. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Breeding and prenatal treatment

Male and nulliparous female mice were housed together until pregnancy was determined by the presence of vaginal plug (gestational day (GD) 0). From GD1 throughout pregnancy, females were housed individually and injected daily with fluoxetine (FLX, 1 mg/ml per kg/day, s.c., Santa Cruz Biotechnology, Inc.), or with equal volumes of sterile saline (Sal). The dose used in this study was chosen based on previous reports from our lab and others (e.g., Avitsur et al., 2015b; Bairy et al., 2007; da-Silva et al., 1999; Forcelli and Heinrichs, 2008; Svirsky et al., 2015). FLX administration was discontinued at parturition to allow a better understanding of the relative contribution of in utero drug exposure via the placenta from that of neonatal exposure via maternal milk (Avitsur et al., 2015b; Svirsky et al., 2015). Daily observations of dams and pups from birth until weaning indicated that maternal behavior was not altered by the prenatal treatment, all pups survived until weaning and no significant withdrawal symptoms were observed (data not shown). In all studies, up to two animals from the same litter were included in the same experimental group. Offspring mice served as subjects at the age of 30–33 days (young, prepubescent) or 60–70 days (adult). Only female offspring were included in the present report. Male offspring were included in a separate study.

2.3. Restraint stress (RST)

Individual mice were placed in well-ventilated 50-ml centrifuge tubes. For *acute stress*, mice underwent a single restraint session of 2 h. For *continuous stress*, mice underwent 9 restraint sessions of 8 h each over 10 days (6 RST session, one day off, and then 3 more cycles). All stress sessions began at 8:00–9:00 am. While animals are being restraint they are also deprived of food and water. To delineate the effects of the stress from those of hunger and thirst control mice were food and water-deprived (FWD) at the time of RST (Hermann et al., 1994; Quan et al., 2001; Sheridan et al., 2000).

2.4. Blood collection and plasma corticosterone assessment

Blood was collected by facial vein puncture into heparin-coated tubes (Avitsur et al., 2013; Golde et al., 2005). Plasma was separated and stored at −80 °C until assayed. Corticosterone was quantified using radioimmunoassay (RIA) in duplicates (ImmuChem double antibody corticosterone ^{125}I RIA kit, MP Biomedicals, Orangeburg, NY) according to the manufacturer's instructions. Precision: intra-assay 4.4–10.3% (within runs), inter-assay 6.5–7.2% (between runs) according to level; sensitivity: minimal detectable dose 7.7 ng/ml; specificity: cross-reactivity with similar steroids - negligible; range of detection 25–1000 ng/ml (values obtained from manufacturer).

2.5. Procedure

Timeline of the experimental procedure is described in Fig. 1. The study included four different experiments. Each of the following experiments was conducted on a separate group of offspring subjects. Also, separate groups of animals were tested at young and adult ages.

2.5.1. Experiment 1: effect of prenatal fluoxetine exposure on corticosterone response to acute stress

Young and adult female mice, prenatally treated with Sal or FLX underwent acute FWD/RST as described. Blood was collected 30 min before, immediately after (within 2 min), and 2 h after termination of the FWD/RST session for analysis of corticosterone. This experiment included 76 subjects, including 38 Sal- and 38 FLX-subjects. Each experimental group (same age, prenatal and postnatal manipulations) included 7–11 subjects.

2.5.2. Experiment 2: effect of prenatal fluoxetine exposure on corticosterone response to continuous stress

Young and adult female mice, prenatally treated with saline or FLX underwent continuous FWD/RST as described. As the results of a previous experiment demonstrated that prenatal FLX treatment did not affect baseline corticosterone levels (see below Section 3.1), baseline corticosterone was not assessed in this study. Ethical considerations limit the number of times blood can be collected from each animal to a maximum of 1% of body weight per 2 weeks. This limitation allowed