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Research report

Increased symptoms of illness following prenatal stress: Can it be prevented by fluoxetine?

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HIGHLIGHTS

- Prenatal stress augmented endotoxin-induced symptoms of illness in mice.
- Prenatal fluoxetine reversed the effect of prenatal stress on sucrose consumption.
- Prenatal fluoxetine augmented the effect of prenatal stress on body weight loss.
- The effects of prenatal stress and prenatal fluoxetine were sex-dependent.

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ABSTRACT

Stress during pregnancy is associated with lifetime negative consequences for the offspring. The present study examined the effects of prenatal stress on symptoms of illness following an immune challenge in mice. Additionally, this study examined whether pretreatment with fluoxetine (FLX) could prevent the effects of maternal stress on illness symptoms. Mice prenatally exposed to stress, with or without FLX were administered with saline or endotoxin. In males, prenatal stress significantly augmented endotoxin-induced body-weight loss and reduced food consumption; prenatal FLX did not prevent these responses, and, in many cases, augmented them. In females, prenatal stress worsened endotoxin-induced suppression of sucrose intake, and prenatal FLX reversed this effect. These findings provide the first indication of altered response to an immune challenge following prenatal stress and selective serotonin reuptake inhibitor (SSRI) treatment. These results may have implications for health and well-being of offspring exposed to stress during pregnancy.

1. Introduction

Maternal stress during pregnancy is associated with unfavorable outcomes for the offspring. Human and animal studies demonstrated a link between prenatal negative life events and slower fetal growth, increased risk for premature delivery and greater incidence of low birth weight, as well as persistent changes in neuroendocrine, behavioral, and metabolic systems [2,19,23,36,55,54]. Prenatal psychosocial adversities were also associated with increased risk for illness later in life, probably due to changes in host response to immune challenge [31,39,48]. Maternal stress during pregnancy was associated with bias for Th2 over Th1 cytokine secretion in adult offspring [16], and with altered innate and adaptive immune responses in cultured umbilical cord blood mononuclear cells [56]. In infant monkeys, prenatal stress decreased proliferative and cytokine responses of lymphocytes [13,12] and reduced bacterial colonization of the gut [7]. Additionally, in rodents prenatal stress reduced lymphocyte count, decreased in vitro proliferation of lymphocytes, suppressed NK cytotoxicity and modulated cytokine secretion [14,25,24,30,33,35,52].

Increasing body of evidence suggest that prenatal stress may serve as a model for maternal depression. In rats, prenatal stress induced postnatal maternal depressive-like behavior [47]. In addition, negative life events are a key risk factor for major depression [11,18,19], particularly in pregnant women [27,38]. Furthermore, recent studies showed that perinatal exposure to selective serotonin reuptake inhibitor (SSRI) antidepressants moderated some of the effects of maternal stress. For example, perinatal fluoxetine

Abbreviations: FLX, fluoxetine; FWD, food and water deprivation; GD, gestational day; LPS, lipopolysaccharide; PND, post-natal day; RST, restraint; Sal, saline; SSRI, selective serotonin reuptake inhibitor.

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(FLX) diminished the effects of prenatal stress on depressive- and anxiety-like behaviors [47] abolished the decrease in circulating corticosteroid binding globulin [32], and suppressed the changes in hippocampal cell proliferation and neurogenesis [47].

Pregnant women suffering from mood disturbances commonly use SSRI antidepressants. The beneficial effects of SSRI on the core symptoms of depression are well-documented [57]. However, increasing body of evidence suggest that SSRIs readily cross the placental barrier and impact the developing fetal brain [1,10,28,58]. Recent studies from our lab indicated that prenatal SSRI exposure had long-lasting implications for host response to an immune challenge. In these studies, offspring mice, prenatally exposed to FLX, were administered with endotoxin (lipopolysaccharide, LPS). Prenatal FLX diminished LPS-induced secretion of the proinflammatory cytokines interleukin-6 and Tumor Necrosis Factor $-\alpha$ and augmented LPS-induced interleukin-1β secretion. Moreover, prenatal FLX diminished LPS-induced body-weight loss and food consumption [3]. These results provided the first indication that prenatal SSRI exposure may result in long-lasting changes in the development of the response to an immune challenge in the offspring [3].

Taken together, the above-mentioned studies showed that prenatal stress altered the development of the response to an immune challenge [2]. Prenatal stress served as a model for maternal depression, and several of its effects were moderated by perinatal exposure to SSRIs antidepressants [32,47]. In our studies, prenatal fluoxetine exposure diminished host response to an endotoxin challenge [3]. Thus, the goal of the present study was to examine the effects of prenatal stress on the response to an immune challenge, and the possible moderating effect of prenatal SSRIs on this effect.

2. Materials and methods

2.1. Animals

Subjects were offspring (females and males) 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-h light/dark cycle (lights on at 7:00 P.M.). 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, when available. Different animals were used for the different experiments listed below as well as for experiments published elsewhere [51].

2.2. Breeding and prenatal fluoxetine 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, 10 mg/kg/day, s.c. in a volume of 10 ml/kg, MedChem Express), or with equal volumes of sterile saline (Sal). The dose used in this study was chosen based on our previous studies [51]. In humans, FLX prescription ranges from 20 to 80 mg/day, which would correspond to approximately 0.29–1.14 mg/kg. Considering that the precautionary principle considers animals more resistant than humans do, higher doses are tested in animals. Thus, in the present work we could have used at least 30 mg/kg of FLX [34,17]. FLX administration continued until parturition. 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).

2.3. Prenatal stress

On GD 14–18, dams were restraint (RST) individually in transparent plastic cylinders three times daily for 45 min (at 9:00; 12:00; 15:00) during the dark phase of the diurnal cycle [41–43,46,47,50,53]. This time period during pregnancy is when stress can result in postpartum depressive-like behavior in the dam [50] and, thus was used as a model of maternal stress/depression, as previously described [32,47,46,45]. 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 [29,44,49].

2.4. Procedure

2.4.1. Experiment 1: effect of prenatal fluoxetine exposure on litter size and offspring body weight

Dams were treated with saline or FLX as described. The number of pups per litter was noted within 24 h following parturition in control ($n = 70$) and FLX ($n = 66$) litters. In a separate study, body weight of offspring was recorded daily from post-natal day (PND) 1 until PND 60. Prior to weaning, all pups from the same litter were weighed together, and pup average weight-per-litter was calculated. Following weaning, same-sex littermates were weighed together, and average pup weight was calculated for males and females separately. This study included 15 Sal and 13 FLX exposed litters, producing 78 Sal females, 54 Sal males, 57 FLX females and 79 FLX males.

2.4.2. Experiment 2: effect of prenatal stress and fluoxetine treatment on litter size, offspring body weight and LPS-induced symptoms of illness

Pregnant dams were randomly assigned into three groups. The first group was administered with saline throughout pregnancy and underwent FWD procedure as described (Sal-FWD). The second group was administered with saline throughout pregnancy and underwent RST stress procedure (Sal-RST). The third group was administered FLX throughout pregnancy and underwent RST stress procedure (FLX-RST). An additional group administered with FLX and exposed to FWD was not included, as our lab has already assessed the relevant effects of prenatal FLX (Experiment 1 and [3]. Repeating these measurements would have required a large number of animals, with such a design not conforming to ethical guidelines.

The number of pups per litter was noted within 24 h following parturition in Sal-FWD ($n = 7$) Sal-RST ($n = 7$) and FLX-RST ($n = 17$) litters. Body weight of offspring was recorded every third day from post-natal day (PND) 1 until PND 80. Different litters were weighed on different days to provide a more thorough assessment of daily body-weight changes. Prior to weaning, all pups from the same litter were weighed together, and pup average weight per litter was calculated for each 3-day interval. Following weaning, same-sex littermates were weighed together, and average pup weight was calculated for males and females separately for each 5-day interval.

To assess the response to an immune challenge, changes in body weight, food and sucrose consumption following LPS administration were examined. Male and female offspring at the age of 30–35 days (young) or 60–80 days (adult) were housed individually at least 48 h before the beginning of measurements. Water bottles in each cage were replaced with a bottle of sucrose solution (2% in dH2O, Sigma) for a period of 24 h, followed by a period of at least 24 h with no exposure to sucrose (water only). For baseline measurements, animals were weighed and supplied with weighed food and two drinking bottles: water and sucrose. Body