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Postpartum estrogen withdrawal impairs hippocampal neurogenesis and causes depression- and anxiety-like behaviors in mice

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ABSTRACT

Postpartum estrogen withdrawal is known to be a particularly vulnerable time for depressive symptoms. Ovariectomized adult mice (OVX-mice) treated with hormone-simulated pregnancy (HSP mice) followed by a subsequent estradiol benzoate (EB) withdrawal (EW mice) exhibited depression- and anxiety-like behaviors, as assessed by forced swim, tail suspension and elevated plus-maze, while HSP mice, OVX mice or EB-treated OVX mice (OVX/EB mice) did not. The survival and neurite growth of newborn neurons in hippocampal dentate gyrus were examined on day 5 after EW. Compared with controls, the numbers of 28-day-old $BrdU^+$ and $BrdU^+/NeuN^+$ cells were increased in HSP mice but significantly decreased in EW mice; the numbers of 10-day-old $BrdU^+$ cells were increased in HSP mice and OVX/EB mice; and the density of DCX^+ fibers was reduced in EW mice and OVX mice. The phosphorylation of hippocampal NMDA receptor (NMDAr) NR2B subunit or Src was increased in HSP mice but decreased in EW mice. NMDAr agonist NMDA prevented the loss of 28-day-old $BrdU^+$ cells and the depression- and anxiety-like behaviors in EW mice. NR2B inhibitor Ro25-6981 or Src inhibitor dasatinib caused depression- and anxiety-like behaviors in HSP mice with the reduction of 28-day-old $BrdU^+$ cells. The hippocampal BDNF levels were reduced in EW mice and OVX mice. TrkB receptor inhibitor K252a reduced the density of DCX^+ fibers in HSP mice without the reduction of 28-day-old $BrdU^+$ cells, or the production of affective disorder. Collectively, these results indicate that postpartum estrogen withdrawal impairs hippocampal neurogenesis in mice that show depression- and anxiety-like behaviors.

1. Introduction

Postpartum depression (PPD) is a serious medical condition that affects approximately 10–20% of mothers during the first 4 weeks after delivery (Gaynes, 2005). The levels of estrogen (E2) and progesterone (P4) during pregnancy increase 100-fold and 10-fold, respectively, compared with the menstrual cycle levels (Hendrick et al., 1998). When the placenta is expelled during delivery, the levels of E2 and P4 drop off rapidly, and remain low (hypogonadal) for a prolonged postpartum period (McNeilly, 2001). Women tend to exhibit more symptoms of depression during times of large hormonal changes (Parry et al., 2003). Thus, the onset of PPD is thought to arise, at least in part, from the dramatic fluctuations in the levels of the gonadal hormones during the postpartum period. For example, postpartum E2 treatments can rapidly reduce the depressive symptoms in women with PPD who have documented E2 deficiency (Ahokas et al., 2001; Gregoire et al., 1996). The E2 therapy is associated with a greater improvement in depression scores than placebo among patients with severe PPD (Karuppaswamy and Vlies, 2003; Dennis et al., 2008). Experimentally induced E2 withdrawal can precipitate depressive symptoms in women with a history of PPD but not in women without a PPD history (Bloch et al., 2000). In addition, the withdrawal from chronic high levels of pregnancy-associated hormones can produce depression-like behaviors in rodents, which can be prevented by prolonging exposure to high levels of E2 (Galea et al., 2001). However, the neurobiological mechanisms underlying E2 withdrawal-induced PPD still remain unclear.

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The hippocampus plays a role in the etiology of depressive disorders (McEwen, 2005). Depressed patients showed a 10–18% reduction in their hippocampal volumes (Campbell et al., 2004), which can be reversed/prevented by antidepressant treatments (Tendolkar et al., 2013). A large body of evidence has established that the mammalian brain continuously produces newborn neurons in the hippocampal dentate gyrus (DG) throughout adulthood, with a comparative rate as that of mature granular cells death (Gage, 2002). These newly generated neurons can migrate into the granular cell layer of the DG and integrate into the hippocampal synaptic circuits (Toni et al., 2008). Treatments that selectively impair adult neurogenesis could cause depression-like behaviors in mice (Zhou et al., 2011). Several lines of evidence suggest that the number of newborn neurons in the subgranular zone (SGZ) of the DG and the subventricular zone (SVZ) significantly increases during pregnancy (Furuta and Bridges, 2005; Shingo et al., 2003) and then declines immediately after parturition (Pawluski and Galea, 2007). The activation of the estrogen receptor (ER) can enhance hippocampal neurogenesis (Brannvall et al., 2002). The process of adult neurogenesis in the hippocampal DG includes 4 main stages in rats and mice: the mitosis of progenitor cells (<24 h), the migration and neurite growth of the newborn neurons (1–2 weeks after birth), the maturation of the newborn neurons and synaptic connections within the new circuits (3 weeks after birth) and the formation of functional synapses (>3 weeks) (Zhao et al., 2006). Green and Galea (2008) reported that cell proliferation was reduced on day 4 of estradiol benzoate (EB) withdrawal after a hormone-simulated pregnancy (HSP). However, the influence of EB withdrawal after HSP on survival and neurite growth of newborn neurons has not yet been reported.

The morphological and functional connections between the hippocampus and amygdala support the involvement of adult-generated neurons in affective behaviors. E2 has been reported to enhance the phosphorylation of the NR2B subunit of the *N*-methyl-D-aspartate receptor (NMDAr) *via* Src-mediated signal transduction (Bi et al., 2000; Sha et al., 2015). The density of the NMDAr has been reported to be reduced in the hippocampus of depressed patients compared with the controls (Nudmamud-Thanoi and Reynolds, 2004). The activation of NMDAr is important for the survival and/or circuit integration of the newborn neurons in the hippocampal DG (Tashiro et al., 2006). This temporal correlation between the regulation of survival and the circuital integration of the newborn neurons suggests that NMDAr-mediated survival regulation of mature newborn neurons is closely related to synapse formation (Zhao et al., 2006). Therefore, it is proposed that the EB withdrawal after HSP down-regulates NMDAr to affect the survival of newborn neurons.

Because the E2 levels are sustained at elevated levels throughout the third trimester, followed by a dramatic decrease post-parturition, an "E2 withdrawal state" is produced during the first few weeks after parturition. The administration of EB and P4 to ovariectomized rats daily creates a hormone-simulated pregnancy (HSP), and then the hormones were abruptly withdrawn to mimic the early postpartum reduction of these hormones (Galea et al., 2001). Two to four days after the EB withdrawal of HSP, the rats exhibited the depression-like behaviors (Galea et al., 2001; Stoffel and Craft, 2004). In the present study, we examined the depression- and anxiety-like behaviors in mice on days 2–5 of EB withdrawal after HSP, as well as the survival of immature or mature newborn neurons, and neurite growth of the newborn neurons in the DG on day 5 of EB withdrawal. To explore the underlying mechanisms of the EB withdrawal-affected hippocampal neurogenesis and affective behaviors, we examined the phosphorylation of hippocampal NMDAr NR2B and Src or the hippocampal BDNF level. Our results indicate that the EB withdrawal after HSP through down-regulating NMDAr can reduce the survival of the hippocampal mature newborn neurons in mice, which is accompanied by the production of depression- and anxiety-like behaviors.

2. Materials and methods

The present study was approved by Animal Care and Ethical Committee of Nanjing Medical University. All animal handling procedures followed the guidelines of Institute for Laboratory Animal Research of the Nanjing Medical University.

2.1. Animals

Female mice (ICR, Oriental Bio Service Inc., Nanjing), weighing 32–35 g (15–16 weeks) at beginning of experiment, were used. The animals were maintained in a constant environmental condition (temperature 23 ± 2 °C, humidity 55 ± 5%, 12:12 h light/dark cycle) in the Animal Research Center of Nanjing University. They had free access to food and water before and after all procedures.

2.2. EB withdrawal after hormone-simulated pregnancy

The mice were bilaterally ovariectomized (OVX mice) under isoflurane anesthesia. The sham operation (control mice) groups underwent the same procedure as the OVX mice, except that the bilateral ovaries were not excised. After 7 days of recovery, the OVX mice were subcutaneously (SC) injected with EB (0.5 μg/day) and P4 (0.8 mg/day) dissolved in 0.1 ml sesame oil at 0830 h daily for 16 days, and then treated with EB (10 μg/day) alone for 7–11 consecutive days (Fig. 1). The EB and P4 doses were chosen based on previous studies in OVX rats, which were treated with an injection (SC) of EB (2.5 μg/day) and P4 (4 mg/day) for 16 days and then with a high dose of EB (50 μg/day) for 7 days (Galea et al., 2001). According to the calculation of the equivalent doses based on the surface area (Voisin et al., 1990), the dose used in the mice was approximately twice that of the rats. The control mice received the vehicle (0.1 ml sesame oil) treatment on the same schedule. In this study, all mice were randomly assigned to 5 experimental groups: control mice (the sham OVX mice were treated with vehicle); HSP mice (the OVX mice were treated with EB and P4 for 16 days, and then with a high dose of EB alone for 12 days); EW mice (the OVX mice were administered EB/P4 for 16 days, and then a high dose of EB alone for 7 days); OVX mice (the mice were ovariectomized on the same day as the EB withdrawal after HSP) and OVX/EB mice (the OVX mice were treated with a high dose of EB for 5 days).

2.3. Behavioral examinations

The behavioral tests were performed on days 2–5 after EB withdrawal (days 25–28 of HSP; Fig. 1). All behavioral data were captured by a video monitor (Winfast PVR; Leadtek Research Inc., Fremont, CA) and analyzed using TopScan Lite 2.0 (Clever Sys., Reston, VA).

2.3.1. Open-field test

Open field test (OFT) was used to examine spontaneous locomotor activity in a cuboid plexiglass box (60 cm × 60 cm × 40 cm) with a gray floor divided into 16 equal squares and two areas: a peripheral area and a square center. Lighting in the maze was 15 lux (Hartmann et al., 2012). The test was conducted between 1200 and 1600 h (Stoffel and Craft, 2004). The central zone was defined as the 4 central squares (30 cm × 30 cm). Each mouse was placed in one corner of the arena and allowed to freely explore the apparatus for 6 min. The following parameters were evaluated using the computer software (EthoVision; Noldus, Alexandria, Virginia):