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Research Report

Sex differences in the adult HPA axis and affective behaviors are altered by perinatal exposure to a low dose of bisphenol A

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ABSTRACT

Bisphenol A (BPA), an estrogen-mimicking endocrine disrupter, when administered perinatally can affect affective behaviors in adult rodents, however the underlying mechanisms remain largely unclear. Postnatal day (PND) 80 vehicle-injected control female rats showed more obvious depression- and anxiety-like behaviors than males, indicative of sexually dimorphic affective behaviors. When female breeders were subcutaneously injected with BPA (2 μg/kg) from gestation day 10 to lactation day 7, sex difference of affective behaviors was impaired in their offspring (PND80 BPA-rats), as results that female BPA-rats showed a visible "antianxiety-like" behavior, and male BPA-rats increased depression-like behavior compared to vehicle-injected controls. Notably, basal levels of serum corticosterone and adrenocorticotropin (ACTH), and *corticotropin-releasing hormone* mRNA were increased in male BPA-rats, but not in female BPA-rats, in comparison with vehicle-injected controls. Following mild-stressor the elevation of corticosterone or ACTH levels was higher in male BPA-rats, whereas it was lower in female BPA-rats than vehicle-injected controls. In comparison with vehicle-injected controls, the level of *glucocorticoid receptor* (*GR*) mRNA in hippocampus or hypothalamic paraventricular nucleus was increased in female BPA-rats, while decreased in male BPA-rats. In addition, the levels of hippocampal *mineralocorticoid receptor* (*MR*) mRNA, neuronal nitric oxide synthase (nNOS) and phospho-cAMP response element binding protein (*p*-CREB) were increased in female BPA-rats, but were decreased in male BPA-rats. Furthermore, the testosterone level was reduced in male BPA-rats. The results indicate that the perinatal exposure to BPA through altering the GR and MR expression disrupts the

Abbreviations: ACTH, adrenocorticotropin; BPA, bisphenol A; CREB, cAMP response element binding protein; CRH, corticotropin-releasing hormone; DLT, dark light test; ER, estrogen receptor; ERRγ, estrogen-related receptor γ; FST, forced swimming test; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; MR, mineralocorticoid receptor; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; OFT, open field test; PND, postnatal day; PVN, paraventricular nucleus

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GR-mediated feedback of hypothalamic–pituitary–adrenal (HPA) axis and MR-induced nNOS–CREB signaling, which alters sex difference in affective behaviors.

1. Introduction

Bisphenol A (BPA), an environmental estrogenic chemical, has been attracting increased attention because of its high potential for human exposure *via* BPA-containing products included in certain baby bottles, food containers, resin-based food can linings and dental sealants. Accumulating studies have indicated that depression and anxiety are more frequent in the sons and daughters of women who had been treated with environmental endocrine disrupters during pregnancy (Vessey et al., 1983). Clinical investigations show that the prevalence of depression and anxiety in women is approximately twice of that in men (Essau et al., 2010). The perinatal exposure to low-dose BPA can alter sexually dimorphic brain structure (Funabashi et al., 2004), sex difference of affective behaviors (Kubo et al., 2001) or cognitive skills (Carr et al., 2003). Our recent studies showed that male rats in locomotor activity and affective behaviors are more sensitive to perinatal BPA action than female rats (Zhou et al., 2009, 2011b). However, the underlying mechanisms remain largely unknown.

hypothalamic–pituitary–adrenal (HPA) system is considered to be the 'final common pathway' for controlling affective behaviors in the mediation of the stress response. A high percentage of patients with depression or anxiety disorder exhibit the increasing insensitivity of the HPA-axis to the feedback of cortisol (Goncharova and Lapin, 2002), which led to the increased corticosteroid levels (Vreeburg et al., 2010). In addition, the number of corticotropin-releasing hormone (CRH) expressing neurons shows a gender difference in the human hypothalamic paraventricular nucleus (PVN) (Bao and Swaab, 2007). Ovarian steroids have been found to increase HPA-axis activity, and enhance the HPA-axis response to psychological stress (Roy et al., 1999). The up-regulation of CRH and nuclear estrogen receptor α (ERα) was observed in mood disorders, both in males and females (Bao et al., 2005). Conversely, testosterone attenuates HPA-axis activity (Bagatell et al., 1994; Viau and Meaney, 1991). Limbic system in particular hippocampus provides corticosteroid-mediated feedback to the HPA-axis through low affinity glucocorticoid receptors (GR) and high affinity mineralocorticoid receptors (MR). Abnormalities in the hippocampal GR impairs corticosteroid-mediated negative feedback on the HPA-axis (Holsboer, 2000) leading to the hyperactivity of HPA-axis in depression (Zhou et al., 2011a). During early development the impaired feedback control of the HPA-axis may persist into adulthood to acquire GR resistance in some specific feedback areas (De Bellis et al., 1999) and GR hypersensitivity in other brain regions (Nemeroff, 1996). Additionally, hippocampal neuronal nitric oxide synthase (nNOS) which depends on MR activation (Zhou et al., 2011a) is also involved in the stress-related behaviors and is implicated in depression and anxiety (Zhang et al., 2010). Recently, it is found that nNOS-derived nitric oxide (NO) through affecting cAMP response element binding protein (CREB) activation contributes to the sex difference of affective behaviors (Hu et al., 2012).

Gonadal hormones play an important role in the perinatal sexual differentiation of brain and patterns of behavior (Farabollini et al., 1999). It has been proved that the "masculinization"/"defeminization" of the brain during the perinatal period depends on the action of estrogen or testosterone, the latter is thought to exert action through its conversion by brain aromatase into estrogen (Lephart, 1996) and binding to androgen receptors (Sato et al., 2004). Neonatal treatment with estradiol in female rats (Patchev et al., 1995) and neonatal castration (Lucion et al., 1996) impair the sexual dimorphism of brain structure and function. Because BPA is found to have both estrogenic and antiandrogenic action in vitro (Sato et al., 2004; Steinmetz et al., 1997), it is proposed that the sex difference in HPA-axis responses can be affected by perinatal exposure to BPA.

In this study, we examined the effects of perinatal exposure to BPA on affective behaviors through open field test (OFT), dark light test (DLT) and forced swimming test (FST). To explore the mechanisms underlying the BPA-altered affective behaviors, we further examined the HPA-axis activity, GR-mediated HPA-axis responses following mild-stress and the MR-induced nNOS–CREB signaling.

2. Results

2.1. *Influence of injections of dams on sex-specific affective behaviors of offspring*

Two-way ANOVA displayed that the factor of sex has a significant main effect on affective behaviors (OFT: $F_{(1,43)}=7.418$, $P=0.009$; DLT: $F_{(1,43)}=50.687$, $P<0.001$; FST: $F_{(1,43)}=6.309$, $P=0.016$; Fig. 1A–C), however, there was no statistic effect of injection in the OFT ($F_{(1,43)}=0.001$, $P=0.975$), DLT ($F_{(1,43)}=0.212$, $P=0.648$), or FST ($F_{(1,43)}=0.38$, $P=0.541$). And no significant interaction was found between injection and sex (OFT: $F_{(1,43)}=0.045$, $P=0.833$; DLT: $F_{(1,43)}=0.799$, $P=0.376$; FST: $F_{(1,43)}=0.003$, $P=0.959$). The statistic results suggest that injections of dams in the present study have no effect on affective behaviors of offspring. Consistently, few studies have reported the once-daily s.c. injection has a detectable effect on dams and offspring during perinatal periods.

2.2. *Influence of the perinatal BPA exposure on sex-specific affective behaviors*

Two-way ANOVA displayed main effects of BPA treatment and sex and their interaction on the time in the center fields of OFT (BPA treatment: $F_{(1,44)}=10.969$, $P=0.002$; sex: $F_{(1,44)}=0.228$,