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Perinatal exposure to di-(2-ethylhexyl) phthalate affects anxiety- and depression-like behaviors in mice

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HIGHLIGHTS

- Perinatal DEHP exposure increased anxiety-like status of pubertal mice and adult females.
- Perinatal DEHP exposure increased depressive-like status of pubertal and adult mice.
- DEHP reduced the level of androgen receptor or ERβ of hippocampus in males or females.
- DEHP inhibited phosphorylation of ERK1/2 of hippocampus in mice.

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ABSTRACT

Di-(2-ethylhexyl) phthalate (DEHP) is an environmental endocrine disrupter. The present study investigated the effect of DEHP on emotional behavior of mice following perinatal exposure (10, 50, and 200 mg kg^{-1} d^{-1}) from gestation day 7 through postnatal day 21. The results showed that, in pubertal males (6-w-old), DEHP decreased the time spent in the open arms and the number of entries into them in elevated plus maze and decreased the time in the mirrored chamber and in the light-box; in pubertal females, DEHP decreased the time spent in the open arms and the number of entries into them, suggesting that DEHP exposure made a anxiogenic effect in pubertal offspring regardless of sex. While DEHP effect on anxiety of adult (12-w-old) displayed sex differences, with decreased time spent in the open arms in the adult females. Perinatal exposure to DEPH significantly extended the time of immobility in forced swim task of pubertal offspring and adulthood regardless of sex. Furthermore, DEHP down-regulated the expressions of androgen receptor (AR) in pubertal male hippocampus and of estrogen receptor (ER) β in pubertal female and adult hippocampus of both sexes and inhibited the phosphorylation of ERK1/2 of hippocampus in pubertal mice and adult males. These results suggest that exposure to DEHP early in life affected the anxiety- and depressive-like behaviors of pubertal offspring and even adult. The disruption of gonadal hormones' modulation of behaviors due to down-regulation of AR or ERβ in the hippocampus may be associated with the aggravated anxiety- and depression-like status induced by DEHP.

1. Introduction

Phthalates are a family of industrial chemicals used primarily to increase the flexibility and durability of plastic products. The worldwide consumption of phthalates exceeds five million metric tons annually (Singh and Li, 2012). Di-(2-ethylhexyl) phthalate (DEHP) is one of the most commonly phthalates, with majorly being used in plastics and many other products including food storage containers, toys, pharmaceuticals, cosmetics, and personal care products (Smith et al., 2011). Given the widespread use of these products, it is unavoidable for wildlife and human being exposed to DEHP. As an environmental endocrine disruptor (EED), DEHP interferes with the normal behavior of estrogen and androgen, especially in the critical periods of organ or systems development stages (in utero and lactational), and thus affects the development and long-term function of hormone-sensitive tissues. Currently, the developmental and reproductive toxicity of DEHP in wildlife and human has caused widespread concern.

The developing brain is highly regulated by endogenous hormones, and hormonally mediated events play critical roles at early stage of the development (McEwen and Alves, 1999). Although the effects of DEHP have been studied in the reproductive and endocrine organs, much less is known about the effects of

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phthalates on brain development. Since maternal DEHP and its primary metabolite, mono-(2-ethylhexyl) phthalate (MEHP), can be transferred either to the fetus across the placenta during pregnancy or to the nursing infant through breast milk during lactation, the brain developmental toxicity of DEHP may begin during gestation and lactation periods (Xu et al., 2007). It was reported that utero and lactational exposure to DEHP had non-monotonic dose–response and a low dose effects on rat brain aromatase activity which catalyzes the final, rate-limiting step in the conversion of androgens to estrogens (Andrade et al., 2006). In males on PND 1, aromatase activity in hypothalamic/preoptic area was inhibited at low doses (0.135, 0.405 mg kg^{-1} d^{-1}) and increased at high doses (15, 45, 405 mg kg^{-1} d^{-1}). In contrast to findings on PND 1, aromatase activity at weaning (PND 22) was more affected in females than in males, and an increased activity was observed at all doses in the females (Andrade et al., 2006). Therefore, it is possible that developmental exposure to DEHP alters brain sexual differentiation and affects the cognitive function and the behavior. Epidemiological studies have associated the concentrations of phthalates in maternal urine with attention deficit disorder and learning disability in U.S. children, 6–15 years (Chopra et al., 2014). A strong positive association between phthalate metabolites in urine and symptoms of attention deficit hyperactivity disorder and an inverse relationship between DEHP and the intelligence were found among school-age children (Cho et al., 2010; Engel et al., 2009; Kim et al., 2009). Yolton et al. found a significant positive association between the concentration of maternal urinary DEHP metabolites at 26 w in pregnancy and nonoptimal reflexes of infant (Yolton et al., 2011). In rodents, single exposure to DEHP in postnatal day (PND) 5 induced significant hyperactivity of pubertal male rats in the dark phase (Ishido et al., 2005).

Gonadal hormones participate in modulation of emotional behavior including anxiety and depression (ter Horst, 2010). Anxiety and depression are increased in postmenopausal women due to the decreased estrogen level (Palanza, 2001). Physiological E_2 reduces anxiety- and depression-like behavior of aged female mice (Walf and Frye, 2010). Androgens participate in modulation of anxiety and depressive behaviors in males. It was reported that testosterone (T) and its 5α reduced metabolite, dihydrotestosterone, as well as the systemic administration of anabolic steroids reduce anxiety-like behavior in rodents (Osborne et al., 2009). Administration of the androgen receptor (AR) antagonist flutamide after birth resulted in an increased depressive-like behavior of pubertal male rat in the forced swim and sucrose preference tests by concomitant of decreased neurogenesis and dendritic spines in the hippocampus (Zhang et al., 2010).

The hippocampus is not only related to cognitive function but directly involved in emotional responses also. Symptoms of cognitive dysfunction are often accompanied with anxiety and depressive disorders (Fan et al., 2010; Engin and Treit, 2007). Rats with cytotoxic hippocampal lesions shown to be marked anxiolytic effects in the social interaction and hyponeophagia tests (Deacon et al., 2002). Recent study found that ventral hippocampal lesions reduced anxiety (Barkus et al., 2010). The anxiolytic properties of estrogens have been reported to be mediated by ERβ (Oyola et al., 2012), and AR is believed to be involved in the anxiolytic effect of testosterone in rat (Hodosy et al., 2012). Carbone's group recently found that exposure to DEHP at 30 mg kg^{-1} d^{-1} from PND 1 to the day of behavior test at age of day 45 or 60 significantly increased anxiety-like behavior in male but not in female rats (Carbone et al., 2013). However little information concerning the effects of developmental exposure to DEHP on emotional behavior is presently available. Considering that early development stage (2 weeks before and after birth) is a critical period of organ or systems development stage during which gonadal hormones play an important role in the sexual differentiation of brain and behavior patterns, the purpose of the present study was to investigate the effect of utero and lactational exposure to DEHP on anxiety- and depression-like behaviors in pubertal offspring mice and after adult.

2. Materials and methods

2.1. Animals and treatment

Male (30–35 g) and female (25–30 g) ICR mice were purchased from the Experimental Animal Center, Jinhua Institute for Drug Control (Zhejiang, China). Mice were housed under standard conditions (temperature 24 ± 1 °C; humidity 50–60%; 12:12 h light/dark cycle) with free access to food and water. To minimize background exposure to DEHP beyond treatment regimen, mice were housed in white poly-propylene cages with ad libitum access to DEHP-free water provided in glass bottles and diet. After acclimatization for 1 week, female mice were housed with males (female:male = 1:1), and vaginal plugs and vaginal smears were checked daily. The presence of sperm-positive smear and vaginal plug determined the gestation day 0 (GD 0), if detected, the pregnant dams were each placed in an individual cage. All experiments in the present study were conducted in accordance to the Care and Use Standard of the Laboratory Animal (China Ministry of Health).

Dams were randomly assigned to five experimental groups (n = 12 L for each condition), orally administered DEHP at dose of 10, 50, or 200 mg kg^{-1} d^{-1} (1 g mL^{-1}, pure > 99%, AMRESCO, USA) dissolved in 0.56% Tween 80 (AMRESCO LLC, Solon, OH, USA) (Kessler et al., 2004), Tween 80 (<0.56%) as the vehicle control, or water as the blank control, from GD7 to weaning (PND 21). All of the dams were allowed to feed their offspring until weaning. The doses of DEHP in the present study were below the no observed adverse effect level (NOAEL, 48 mg kg^{-1} d^{-1}) of DEHP (10, 50, and 200 mg kg^{-1} d^{-1}, converted to human equivalent dose based on the body surface area was approximately 0.81, 4.05, 16.2 mg kg^{-1} d^{-1}) (Huang et al., 2004) and were previously found no reproductive toxicology (Smith et al., 2011).

2.2. Body length, body weight, reproductive organs weight, and hormone levels of pups

Three days after born, the body length of pups was recorded. Body weight of pups was recorded every week. Pups from controls and DEHP groups were weaned at 21 d of age. On PND35, offspring from each litter were housed on a same-sex basis. On PND42, half of both sex pups from each litter were received behaviors test (n = 12 pups from 12 L) or used for proteins analyses (n = 4 pups from 4 L) and reproductive organs weight measurement, and the remain pups were used on PND 84, to investigate the effects of perinatal exposure to DEHP on anxiety- and depression-like behavior of offspring after adult. For the examination of the serum hormone (estradiol (E_2) or testosterone) levels, blood samples (n = 8) were collected from the orbital after the behaviors test, and the samples centrifuged at 3000 rpm for 10 min, the serum separated and the hormone (E_2 in females or testosterone in males) levels were measured using radioimmunoassay (Dorgan et al., 2010).

2.3. Tissue preparation and Western blotting analyses

On PND 42 or 84, the protein expressions in hippocampus were analyzed (4 L for each group, one male and one female from each litter, n = 4). Mice were sacrificed and hippocampus was dissected under 4 °C and then stored at −80 °C until use. Tissue was homogenized in ice-cold RIPA buffer (Beyotime) containing 1% Triton X-100, 1% deoxycholate, 0.1% SDS, and 1 mM PMSF (Beyotime), and