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Effects of early pubertal exposure to di-(2-ethylhexyl) phthalate on social behavior of mice

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ABSTRACT

Di-(2-ethylhexyl) phthalate (DEHP), a main member of phthalates used as plasticizer in PVC plastics, is an environmental endocrine disrupter. The present study investigated the effect of DEHP on social behavior of mice following pubertal exposure (1, 10, 50, and 200 mg/kg/d) from postnatal day 28 through postnatal day 42. The results showed that, in pubertal females, DEHP reduced the time spent in social play and social investigation and inhibited sociability, but a contrary effect was found in pubertal males, suggesting that the effect of DEHP on pubertal social behavior displays sex differences. In adults, DEHP reduced sociability in females and inhibited social play and social investigation in males, suggesting that early pubertal exposure to DEHP not only plays a significant role in puberty but also alters social behavior in adults. In addition, the present study showed that the higher dose of DEHP (50, 200 mg/kg/d) reduced the relative weight of bilateral testis and anogenital distance of pubertal or adult males, suggesting an anti-androgenic activity of DEHP. These results suggest that early pubertal exposure to DEHP sex- and age- specifically affected the social behaviors of pubertal and even adult mice.

Introduction

Di-(2-ethylhexyl) phthalate (DEHP), a member of phthalates, is used as plasticizer in PVC (polyvinyl chloride) plastics. DEHP is found almost ubiquitous in a number of consumer products worldwide such as foodstuff packaging, paints, toys, adhesives, lubricants and building materials, personal care items, electronics, and medical devices (Horn et al., 2004; Shea, 2003). Because the phthalate plasticizers can evaporate, leach, or migrate into the environment, humans and wildlife exposed to DEHP for a long time (Heudorf et al., 2007; Fromme et al., 2002). There is evidence that DEHP displays weak estrogen mimicking potency and anti-androgenic activity (Mankidy et al., 2013; Yolton et al., 2011; Christiansen et al., 2010; Borch et al., 2004). Adverse developmental and reproductive effects in laboratory animals and wildlife, as well as in humans, have raised concerns over the potential health impacts from exposure to DEHP (Zhang et al., 2013; Noriega et al., 2009; Carbone et al., 2013; Akingbemi et al., 2004; Pan et al., 2006; Wirth et al., 2008; Meeker et al., 2009). However, less is known about the effects of DEHP on brain and behavioral development.

Puberty is another critical period for the brain development besides the prenatal and neonatal periods. Actually sexual development is a gradual process, and the organization of neural circuits underlying behavior is completed at the final stage of puberty (Romeo, 2003). In this process, the pubertal rise in gonadal hormones is crucial for masculinization and defeminization of reproductive behavior, as well as other sexually differentiated behaviors such as social interaction (Primus and Kellogg, 1990). During the transitional phase of puberty, sexual hormones can induce structural modifications of the central nervous system (CNS), with consequent effects on the development of behavioral and cognitive functions (Pilgrim and Hutchison, 1994; Sisk and Zehr, 2005). So, it is likely that interference of DEHP with gonadal hormones during this period changes the brain structure and behavior in adulthood. In the present study, we exposed mice to DEHP during early puberty from the postnatal day (PND) 28 to PND 42, just before the peak in gonadal hormones, to test the hypothesis that puberty may represent a sensitive period to DEHP exposure for animal's social behavior.

Social behaviors in rodents are important for forming bonds, foraging, defending territories, avoiding predators, and reproducing successfully. In mammals, initial social interactions are learned very early in life, with their mother and any other care givers (Kiser et al., 2012). Social play behavior has been suggested to be important for an animal's social development which might contribute to develop intraspecific communicative skills including social ties or social behaviors in the suitable contexts (Vanderschuren et al., 1996). As a result, juveniles who played more would have better socially competent in adulthood (Pellis et al., 2010). Social behaviors were reported to have sex differences and were influenced by gonadal hormones (Slamberová et al., 2011). Estrogens induced an increased inter-individual interaction (McCarthy et al., 1997). The lower level of gonadal hormone in puberty makes sexually dimorphic behaviors susceptible to DEHP exposure. It was reported

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that pubertal exposure to bisphenol-A masculinized female social behavior (Yu et al., 2011). To our knowledge, it is unclear whether pubertal DEHP exposure affects social behaviors in mice. Here, we characterized several behaviors in puberty and in adults to examine the response to early pubertal exposure to DEHP.

Material and methods

Animals and treatment

Three-week-old male (13–18 g) and female (9–14 g) ICR mice were purchased from the Experimental Animal Center, Zhejiang Academy of Medical Science. Mice were housed in a temperature-controlled (26 ± 2 °C) and humidity-controlled (50%–60%) barrier facility with a 12-hour light/dark schedule (lights on at 6:00 AM). To minimize background exposure to DEHP beyond treatment regimen, mice were housed in white polypropylene cages with ad libitum access to DEHP- and bisphenol-A-free water provided in glass bottles. All mice were fed a phytoestrogens- and estrogen-free chow (manufactured by the Experimental Animal Center, Jinhua Institute for Drug Control, China) upon arrival and for the duration of the experiment. All studies described here were conducted in accordance with the Care and Use Standard of the Laboratory Animal (China Ministry of Health publication). During the course of the study, adequate measures were taken to minimize the number of animals used and their suffering.

After acclimatization for 1 week, the mice were randomly assigned to male and female treatment groups and were orally exposed (ig, about 0.05 mL) to DEHP (>99%, AMRESCO, America) or to only the vehicle controls once/day. DEHP was dissolved in arachis oil (Jinlongyu, China) and was introduced into the back of the mouth using a gavage needle. The oral route of DEHP administration was chosen to mimic the most likely route of exposure to the compound in humans and wildlife. According to the no-observed-adverse-effect level (NOAEL; 4.8 mg/kg/day) estimated by the Europe and America for oral exposure to DEHP and humans' daily exposure levels, we set the four lower levels of DEHP: 1, 10, 50, or 200 mg/kg/d (pharmacological dose conversion is 0.081, 0.81, 4.05, 16.2 mg/kg/d for human based on the body surface area) (Reagan-Shaw et al., 2007). The body weight of each mouse was weighed every week to adjust the drug volume. Considering early puberty is critical for the organization of behavior (Sisk and Zehr, 2005), early pubertal mice were exposed to DEHP for two weeks from the postnatal day (PND) 28 to PND 42. Mice were allowed 3 days without exposure before behavioral test and protein analyses to remove the acute effect of DEHP. To avoid the influence of estrus cycle in females on various behavioral characteristics, the estrus stage of adult females was checked after behavior test by taking vaginal smears and the stage of the cycle was determined by examining cell type composition of the vaginal smears. The great majority of the mice were in diestrus. A few females (1–2) in estrus or proestrus were found in each treatment and were excluded.

Body weight, reproductive organs weight, anogenital distance and serum hormone levels

The body weight of each mouse was recorded before DEHP exposure (at 4 weeks old) and 3 d after being exposed to DEHP for 2 weeks (at 6.5 weeks old) (n = 30 animals per group). Fifteen mice from each group were used for behavior tests in puberty and in adults (n = 15). The remaining were used for the examinations of reproductive organs, anogenital distance, and serum hormone levels at 45 days old (puberty, n = 7) or at 84 days old (adult, n = 8) respectively (Fig. 1). Uterus in females or bilateral testis and epididymis in males were dissected and weighed due to their high sensitivity to gonadal hormones. Blood samples were collected from the retro orbital sinus and the serum hormone (estradiol or testosterone) levels were measured using radioimmunoassay (performed by Medical Solutions Diagnostics and Assay Core of Jinhua Central Hospital; n = 7 or 8 animals per group). The anogenital distance (AGD, the length from the caudal base of the genital tubercle to the anterior aspect of the anus) was measured using a micrometer lens on a dissecting microscope (Do et al., 2012).

Behavioral testing

Social behavior testing was begun on PND 45 (puberty) and on PND 84 (adult) during the dark phase (between 18:00 and 22:00 h) under red light conditions. All sessions were automatically recorded with a computer-based video tracking system (VideoMot2BWM; TSE System GmbH, Germany). The investigator was not visible by the mice. The apparatus was cleaned with 75% alcohol to remove odors of the previous mice after each test. All behavioral data were collected using software (TSE System GmbH, Germany) by a trained observer, blind to experimental groups until data analysis was completed.

Social play test

Social play behavior was tested in a neutral arena (45 × 35 × 20 cm) with the floor covered with clean sawdust. On the testing day, the animals were socially isolated in cages (22 cm × 18 cm × 15 cm) for 3.5 h prior to the experiment to induce a maximal increase in social play behavior (Niesink and Van Ree, 1989). The test consisted of placing two age-, sex-, and treatment-matched unfamiliar mice into the test cage for 15 min (n = 7 pairs). The total amount of time spent in playful social interactions including pinning (one lays on its back and is pinned by the other mouse), social approach (one animal approaching and soliciting another), chasing, crawling over/under, charging, wrestling, social sniffing, and social grooming were recorded. In adults, aggressive and antagonistic repertoires are considered part of play behaviors. And the time spent in sniffing another animal's body including anogenital area and grooming were recorded as social investigation (Vanderschuren et al., 1997; Bredewold et al., 2014; Porrini et al., 2005).

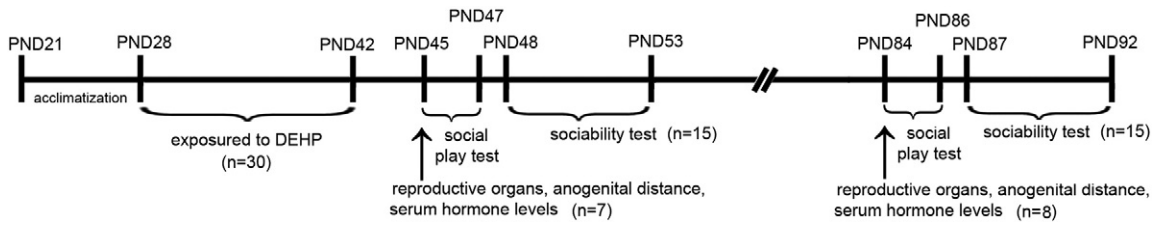

Fig 1. The overall research design and testing timetable. After acclimatization for 1 week, 4-week-old male and female mice were randomly assigned to five experimental groups (n = 30) and were orally administered DEHP (1, 10, 50, or 200 mg/kg/d), or the vehicle control from postnatal day 28 (PND 28) to PND 42. On PND 45 and PND 84, half of mice from females or males submitted to the behavior tests (n = 15). The remaining were for the examinations of reproductive organs, anogenital distance, and serum hormone levels at age of 45-d-old (puberty, n = 7) and at age of 84-d-old (adult, n = 8) respectively.