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Brief communication

Pubertal exposure to Bisphenol A increases anxiety-like behavior and decreases acetylcholinesterase activity of hippocampus in adult male mice

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ABSTRACT

The negative effects of Bisphenol A (BPA) on neurodevelopment and behaviors have been well established. Acetylcholinesterase (AChE) is a regulatory enzyme which is involved in anxiety-like behavior. This study investigated behavioral phenotypes and AChE activity in male mice following BPA exposure during puberty. On postnatal day (PND) 35, male mice were exposed to 50 mg BPA/kg diet per day for a period of 35 days. On PND71, a behavioral assay was performed using the elevated plus maze (EPM) and the light/dark test. In addition, AChE activity was measured in the prefrontal cortex, hypothalamus, cerebellum and hippocampus. Results from our behavioral phenotyping indicated that anxiety-like behavior was increased in mice exposed to BPA. AChE activity was significantly decreased in the hippocampus of mice with BPA compared to control mice, whereas no difference was found in the prefrontal cortex, hypothalamus and cerebellum. Our findings showed that pubertal BPA exposure increased anxiety-like behavior, which may be associated with decreased AChE activity of the hippocampus in adult male mice. Further studies are necessary to investigate the cholinergic signaling of the hippocampus in PBE induced anxiety-like behaviors.

1. Introduction

Developmental exposure to Bisphenol A (BPA) has posed a major threat to the physiological health of humans and wildlife since it began being produced on a large scale (Erler and Novak, 2010; Flint et al., 2012; Kang et al., 2006). A number of studies have indicated prenatal BPA exposure disrupts neurodevelopment, causes brain pathologies and produces behavioral problems in children and adults (Nakamura et al., 2007, 2012; Perera et al., 2012). However, few studies are available on the behavioral effects of pubertal BPA exposure (PBE). Puberty is critical to physical development, cognitive abilities and social maturation and animals may be particularly vulnerable to the effects of hormones during puberty because this period is a time of rapid growth (Patton and Viner, 2007). Moreover, disruptions of pubertal neurodevelopment may contribute to the emergence of adult behavioral problems, such as anxiety-like behavior (Korosi et al., 2012). In addition, data from the 2007–2009 Canadian Health Measure Survey suggest that teens have highest BPA levels compared to other age groups (Bushnik et al., 2010). Recent epidemiological investigation suggests that teen children exposed to BPA might be associated with behavioral and learning development (Hong et al., 2013). We predicted that anxiety-like behavior would be particularly sensitive to BPA and might serve as a useful barometer for determining the neurotoxicity of endocrine-disrupting compounds (EDCs).

Anxiety is a behavioral response to potentially threatening stimuli (Blanchard et al., 2001). However, the molecular mechanism of anxiety behavior is still unknown. Recent research suggests that decreased anxiety-like behavior is directly associated with acetylcholinesterase (AChE) activity of the hippocampus, since AChE knockdown in the hippocampus promotes anxiety-like behavior in mice (Mineur et al., 2013). AChE is one of the most crucial and efficient enzymes for nerve response and function (Tsim and Soreq, 2012). Cholinergic neurotransmission has been shown to have a range of different effects on human and animal behavior (Abreu-Villaça et al., 2011; Mineur et al., 2013). AChE acts at the termination of acetylcholine-mediated neurotransmission, rapidly hydrolysing acetylcholine (ACh) into acetate and choline.

We hypothesized that pubertal exposure to BPA would induce excessive levels of anxiety-like behavior. To verify this hypothesis, we exposed pubertal male mice to 50 mg BPA/kg through their diet, a dose reported to cause epigenetic and behavioral alterations following early BPA exposure in rodents (Dolinoy et al., 2007; Jašarević et al., 2011) on anxiety-like behaviors. In addition, we also assayed the AChE activity in various brain regions associated with anxiety behavior.

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2. Materials and methods

2.1. Chemicals

Bisphenol A (BPA > 99% purify) was purchased from Sigma–Aldrich (USA); Modified AIN-93G diet (diet with 7% corn oil substituted for 7% soybean oil) and modified AIN-93G diet supplemented with 50 mg/kg of BPA was purchased from HFK Bioscience co., Ltd. (Beijing, China). Acetylcholinesterase (AChE) assay kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animals and treatment

Forty young (28-day-old) CD-1 mice weighing 16 ± 1.0 g were obtained from the Experimental Animal Center of Shanxi Medical University. Because estrous cycles of adult female mice can affect behavior (Meziane et al., 2007), only male mice were used in these experiments. They were housed under climate controlled conditions with a 12-h light/dark cycle and provided with modified AIN-93G diet and ultrapure water (Milli-Q Water purification system). An acclimation period of 1 week for adaptation of the mice to the new animal housing was provided before initiating the experiments. The mice were randomly divided into the following two groups (20 mice in each group): Control group and BPA group. On postnatal day (PND) 35, male mice were exposed to 50 mg BPA/kg diet per day for a period of 35 days. The estimated daily treatment of BPA in our study was 10 mg/kg body weight (bw). A subset of mice were used in behavioral assay (Control $n = 14$; BPA $n = 14$). The elevated plus maze (EPM) and light/dark test were separated by 1 week. Brains of the remaining mice were collected for AChE activity analysis. All protocols were approved by the Institutional Animal Care and Use Committee of China.

2.3. Elevated plus maze (EPM)

This test measures anxiety-like behaviors, the anxiety induced by open arm and height. The elevated (50 cm) test apparatus consisted of four arms shaping a plus sign. Two of the arms had no walls around them, whereas the other two arms out of the center had 20 cm walls. The mice were transported to a behavior test room. Each mouse was placed in the "crossing zone" of the EPM for a 5 min session. The time spent in the open arm and numbers of entries into the open arm were recorded (Neufeld et al., 2011).

2.4. Light/dark test

The light/dark test measures anxiety, the anxiety induced by light. The test apparatus consists of a large light compartment (two thirds; 30 cm × 30 cm × 30 cm) and a small dark compartment (one third; 15 cm × 30 cm × 30 cm). A dividing wall with a door (5 cm × 5 cm) for the mouse to travel through freely divided the two compartments. Mice were transported to a test room for behavioral testing. Each animal was placed in the dark compartment. The time spent in light chamber and the latency to the light chamber was recorded (Bourin and Hascoët, 2003).

2.5. AChE activity assay

All mice, with the exception of behavioral assays, were sacrificed by decapitation on PND71. Brain samples were collected from the hippocampus, prefrontal cortex, cerebellum and hypothalamus and homogenized in PBS. The homogenate was centrifuged at 12,000 × g for 10 min at 4 °C. The supernatant was removed to determine AChE activity according to the Ellman method (Ellman et al., 1961). The rate of acetylcholine hydrolysis was measured at 412 nm in a spectrophotometer for 6 min. AChE activity is expressed as μmol product per mg of protein (U/mgprot).

2.6. Statistical analysis

Behavioral data were assessed by trained experimenters blinded to group assignment. Statistical analysis was conducted using GraphPad Prism5 software (GraphPad Software Inc., San Diego, USA). All data were analyzed using two tailed t-tests to determine statistical significance between control group and BPA group. Values in graphs were expressed as mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Pubertal BPA exposure: adult anxiety-like behavior

Our data show that the BPA-exposed mice have increased anxiety-like behavior, as measured by the EPM and Light/dark test (Fig. 1). Notably, BPA-exposed mice exhibited a clear decrease ($p = 0.0015$) in time spent in the open arm of the EPM (Fig. 1A). BPA-exposed mice had significantly less entries into the open arm of the EPM maze compared to controls ($p = 0.0171$, Fig. 1B). In the light/dark test, the BPA-exposed mice displayed a reduction ($p = 0.0004$) in time spent in the light chamber (Fig. 1C) and a significantly increased latency ($p = 0.0131$) compared to controls (Fig. 1D).

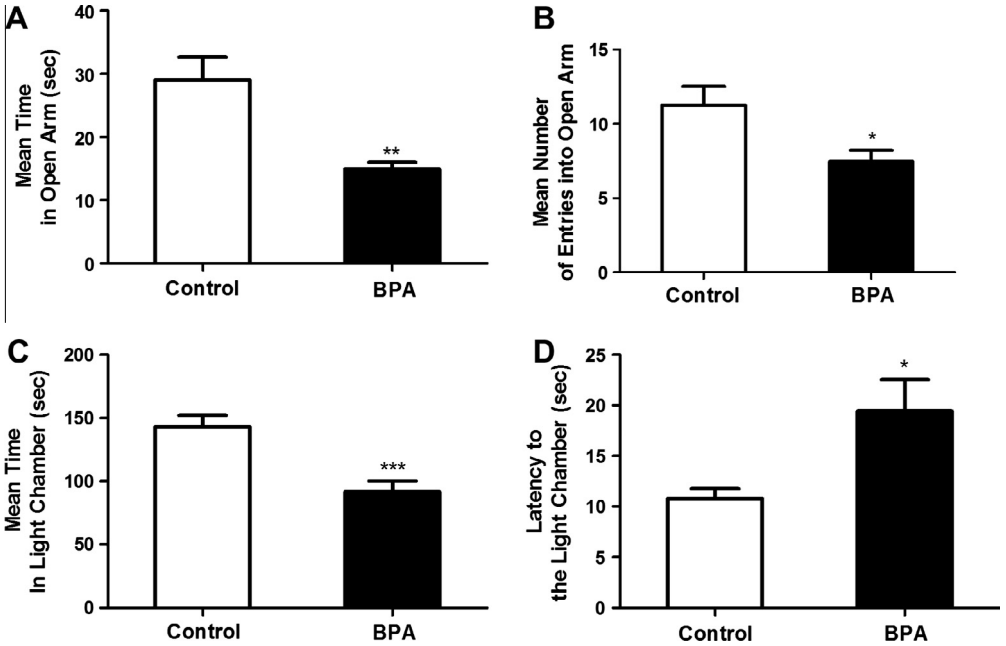

Fig. 1. Results of elevated plus maze (EPM) and light/dark tests. BPA mice spent less time in the open arm (mean ± SEM; Controls: 29.00 ± 3.68 s and BPA: 14.91 ± 1.08 s, $p < 0.01$). (A) had less entries in the open arms (mean ± SEM; Controls: 11.27 ± 1.24 s and BPA: 7.46 ± 0.78, $p < 0.05$), (B) BPA mice spent less time in the light chamber (mean ± SEM; Controls: 143.0 ± 9.19 s and BPA: 91.77 ± 8.47 s, $p < 0.001$), (C) had higher latency to the light chamber (mean ± SEM; Controls: 10.77 ± 0.99 and BPA: 19.46 ± 3.088, $p < 0.05$), and (D) two tailed t-test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.