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Effects of perinatal exposure to low dose of bisphenol A on anxiety like behavior and dopamine metabolites in brain

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ABSTRACT

Bisphenol A (BPA), an endocrine-disrupting chemical, is widely present in the environment. It has been reported that perinatal exposure to low doses of BPA that are less than the tolerable daily intake level (50 μg/kg/day) affects anxiety-like behavior and dopamine levels in the brain. Although the dopaminergic system in the brain is considered to be related to anxiety, no study has reported the effects of low-dose BPA exposure on the dopaminergic system in the brain and on anxiety-like behavior using the same methods of BPA exposure.

To investigate the relationship between alterations in anxiety-like behavior and changes in the dopaminergic system in the brain induced by BPA, we examined the effects of BPA on anxiety-like behavior using an open field test in juvenile and adult mice and measured DA and DOPAC levels and the DOPAC/DA ratio in the dorsal hippocampus (HIP), amygdala (AMY), and medulla oblongata (MED) using high-performance liquid chromatography (HPLC) in adult mice.

In males, BPA decreased the time spent in the center area of the open field in both juveniles and adults. In addition, BPA increased DA levels in the dorsal HIP and MED and decreased the DOPAC/DA ratio in the dorsal HIP, AMY, and MED in adults. The activity of monoamine oxidase (MAO)-B, the enzyme that metabolizes DA into DOPAC, was reduced in the MED. In females, those changes were not observed.

These results suggest that an increase in anxiety-like behavior induced by perinatal exposure to BPA may be related to decreases in DA metabolites in the brain, and there are sex differences in those BPA effects.

1. Introduction

Bisphenol A (BPA), an endocrine-disrupting chemical, affects estrogen, androgen, and thyroid hormone systems (Wetherill et al., 2007) BPA is used primarily in the production of polycarbonate plastics and epoxy resins that are used in most food containers and beverage cans (Kawamura et al., 2001). BPA can be widely detected in the environment, including food (Sajiki et al., 2007) and human biological samples (Sajiki et al., 1999; Vandenberg et al., 2010). Many studies have reported that BPA is transferred from the maternal body to the newborn via maternal blood and breast milk (Schonfelder et al., 2002; Sun et al., 2004; Vandenberg et al., 2007) and that perinatal exposure to BPA affects the brain development of offspring, which leads to alterations in the central nervous system that affect behaviors (Cox et al., 2010; Gioiosa et al., 2007; Patisaul and Bateman, 2008; Rubin et al., 2006; Zhou et al., 2011) and neurotransmitter levels (Honma et al., 2006; Matsuda et al., 2010b; Nakamura et al., 2010).

Cox et al. (2010) reported that perinatal exposure to BPA increased anxiety-like behaviors in both juvenile and adult mice. These authors suggest that an alteration of the dopaminergic system is one of the mechanisms of the anxiogenic effect. Dopamine (DA) is metabolized to 3,4-dihydroxyphenylacetic acid (DOPAC) in the terminal of synapses and mitochondria by monoamine oxidase-A/B (Thorpe et al., 1987). DA levels and DA metabolites, including the DOPAC/DA ratio (DA turnover) and MAO-A/B activity, are related to anxiety behavior (Chen et al., 2004; Chiavegatto et al., 2009; Pandaranandaka et al., 2006; Thiemann et al., 2009).

The medulla oblongata (MED) is an important brain region for DA metabolism (Kitahama et al., 2000; Phillips et al., 2001). DA neurons in the MED project to the limbic regions (Reyes and Van Bockstaele, 2006; Zagon et al., 1994) including the amygdala (AMY) and the hippocampus (HIP), which play important roles in emotional behavior (de la Mora et al., 2005; Matsuda et al., 2010a; Zarrindast et al., 2010). BPA has been reported to induce abnormal development of these regions. BPA

Abbreviations: AMY, Amygdala; BPA, Bisphenol A; CON, Control; DOPAC, 3,4-dihydroxyphenylacetic acid; DA, Dopamine; EPM, Elevated plus maze; E_2, Estradiol; ER, Estrogen receptor; GD, Gestational day; HPLC, high-performance liquid chromatography; HIP, Hippocampus; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; MAO, Monoamine oxidase; MED, Medulla oblongata; OFT, Open field test; PBS, Phosphate buffered saline; PND, postnatal day.

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suppresses the expression of estrogen beta protein and the density of NeuN-positive neurons in the HIP (Kunz et al., 2011; Xu et al., 2010). In addition, BPA causes GABAergic disinhibition and DAergic enhancement in the AMY (Zhou et al., 2011). An alteration of the DAergic system in the limbic regions of BPA-exposed mice may be associated with a change in anxiety-like behavior. To our knowledge, there has been no study investigating the effects of BPA on DA levels and DA turnover in the AMY other than 2 studies of the HIP in which the effects of BPA on behavior were not examined (Honma et al., 2006; Matsuda et al., 2010b). Different methods of BPA exposure may cause different results (Kubo et al., 2003; Matsuda et al., 2010b; Tian et al., 2010; Xu et al., 2010).

To investigate the relationship between the alteration of anxiety-like behavior and BPA-induced changes in the dopaminergic system in the brain, we examined the effect of BPA on anxiety-like behavior and DA and DOPAC levels, the DOPAC/DA ratio, and MAO-A and MAO-B activity levels in the brain (dorsal HIP, AMY, and the whole MED) in both sexes using the same methods of BPA exposure.

2. Materials and methods

2.1. Reagents

Standard DA hydrochloride and DOPAC were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Wako Pure Chemicals (Tokyo, Japan), respectively. DL-Isoproterenol hydrochloride was purchased from Sigma Chemical Co. as an internal standard. Authentic BPA, LC-grade ethanol and acetonitrile were purchased from Wako Pure Chemicals. All other chemicals were of special grade (Wako Pure Chemicals, Osaka, Japan). DA and DOPAC standards were freshly prepared from stock solutions of 1 mg/mL of the same 0.1 M perchloric acid (PCA) solution. The BPA stock solution (10 μg/mL) contained BPA, phosphate buffered saline (PBS; pH 7.4), and 0.01% methanol. The BPA stock solution was diluted with PBS to 0.075 μg/mL for the BPA group. The vehicle contained 0.01% methanol in PBS (pH 7.4). From the preliminary studies, we had confirmed that the vehicle did not have any effect compared to non-treatment. Thereafter, all solutions were sterilized at 120 °C for 20 min. Pure MAO-A and MAO-B were purchased from Sigma-Aldrich Japan, Inc. (Tokyo, Japan).

2.2. Animals and treatments

C57BL/6J female mice at gestational day (GD) 8 were purchased from SLC (Shizuoka, Japan). After the mice were housed in the laboratory for two days, the female mice were injected with the vehicle for the control group (offspring, CON) or with BPA (250 ng/kg, calculated using 30 g of expected female mouse body weight) for the BPA group (offspring, BPA) from GD 10 to postnatal day (PND) 20. The injection volume was 100 μL. The vehicle or BPA was administered daily by subcutaneous injection between 10 a.m. and 12 noon. The dose and injection method were obtained from a previous study (Rubin et al., 2006). The female mice were weighed from GD 11 to PND 21 every three days. The litters were culled to six pups (male:female = 3:3) at PND 2. We determined gender by measuring the distance between the anus and external genitalia. The pups were weaned and housed in same-sex groups (3–5/cage) at PND 21. To eliminate litter effects, sibling pups were not used in the same experiments.

One week after the open field test described below (conducted at 9 weeks of age), the mice were anesthetized with CO_2, and brain samples were collected. The whole MED was dissected out with scissors, and the AMY and dorsal HIP were dissected out from the slices with a sharp scalpel under a stereoscopic microscope according to Matsuda et al. (2010a). The samples were immediately frozen in dry-ice/ethanol and randomly divided into two groups for the measurements of dopamine levels and MAO activity. The samples were stored at −85 °C until use.

The mice were maintained under a controlled temperature (23 ± 1 °C) with a 12/12 h light/dark photoperiod. They had free access to laboratory chow (CE-2, CLEA Japan, Inc., Tokyo) and tap water.

The animal use procedures were approved in advance by the Guide for Animal Experimentation of the Chiba University Graduate School of Medicine.

2.3. The open field test for juvenile and adult mice

The open field apparatus was a square field (50 × 50 × 30 cm) made of white acrylic material. Open field tests were performed on juvenile and adult mice. At 4 and 8 weeks of age, each mouse was placed in the corner of the apparatus at the beginning of the test and allowed to move freely for 10 min. The total distance and total center time were recorded. The center area was 16 × 16 cm. The total distance was evaluated as an index of locomotor activity, and the total center time was evaluated as an index of anxiety. The data analysis was performed using Image J OF4 (O'Hara & Co., Ltd.), a modified software based on the public domain Image J program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/ij).

2.4. The measurement of dopamine levels in adult mice

Dopamine and DOPAC were extracted from each part of the brain according to Matsuda et al. (2010b). An LC/ECD system (LCSS905 series; JASCO International, Tokyo, Japan) equipped with an electrochemical detector (CoulochemII 5200A; ESA) was used for monoamine determinations. The detector conditions were as follows: guard cell (5020) potential, E, 450 mV; analytical cell (5011) potentials, E_1, −50 mV and E_2, 400 mV; and sensitivity, 1 μA. Separation was performed using a 5-μm SunFire C18 (4.6 × 150 mm; Waters) column at 35 °C under isocratic conditions with a mobile phase of acetonitrile, methanol and 0.04 M phosphate buffer adjusted to pH 3.0 with phosphoric acid containing 0.04 M citrate, 7.5 mM sodium 1-heptasulfonate and 0.08 mM EDTA·2Na (2.4:5.8:91.8, v/v/v). The flow rate and injection volume were 1.0 mL/min and 10 μL, respectively.

2.5. The measurement of MAO activity in adult male mice

We measured MAO-A/B activity in males, because DA turnover was altered only in males as mentioned in the Results section. MAO-A and MAO-B were extracted from the dorsal HIP, AMY, and whole MED of the adult mice. Each brain sample was added to 0.5 mL of buffer containing 0.23 M mannitol, 0.07 M sucrose, 10 mM Tris–HCl, and 1 mM EDTA (pH 7.4). After homogenization, the samples were centrifuged at 700 × *g* for 10 min at 0 °C. The supernatants were centrifuged at 5000 × *g* for 10 min at 0 °C. The pellets were suspended with 0.5 mL buffer and centrifuged at 24,000 × *g* for 10 min at 0 °C. The pellets were also suspended with 200 μL of either MAO reaction buffer consisting of 100 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES; pH 7.5) and 5% glycerol (for measuring MAO-A activity) or MAO-B reaction buffer consisting of 100 mM HEPES (pH 7.5), 5% glycerol, and 10% dimethyl sulfoxide (for measuring MAO-B activity). Then, we measured MAO activity by the MAO-Glo Assay that was purchased from Promega KK (Tokyo, Japan). The luminescent signal was measured and recorded using Gene Light GL-200 (Microtec Co., Ltd., Tokyo, Japan). The signal integration time was three seconds.

2.6. Statistical analyses

For behavioral tests, DA levels, DOPAC levels, and the DOPAC/DA ratio, a two-way (sex × group) ANOVA and multiple analyses according to Bonferroni were used to test the significance of differences. For MAO activity, a one-way (group) ANOVA was used to test the significance of differences. Correlations between anxiety-like