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Perinatal exposure to bisphenol-A inhibits synaptogenesis and affects the synaptic morphological development in offspring male mice

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HIGHLIGHTS

- Perinatal exposure to BPA reduced the numeric synaptic density.
- BPA altered the synaptic structural modification in hippocampus.
- BPA reduced the levels of synapsin I and PSD-95 in hippocampus.
- BPA reduced the levels of NMDA and AMPA receptors in hippocampus.

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ABSTRACT

Our previous study indicated that perinatal exposure to low-dose BPA, one of the most common environmental endocrine disrupters, alters behavioral development in offspring mice. Given that synaptic structure of the hippocampus is closely related to behaviors, in the present study, we examined the effects of perinatal exposure to BPA (0.04, 0.4, and 4.0 mg kg^{-1} day^{-1}) on the synaptic density and the synaptic structural modification of pyramidal cells in hippocampus region CA1 and the expressions of synaptic proteins such as synapsin I and PSD-95 and glutamate NMDA and AMPA receptors in male offspring mice on postnatal day (PND) 14, 21, and 56. The results of electron microscope measurement showed that BPA significantly reduced the numeric synaptic density and altered the structural modification of synaptic interface of pyramidal cells with the enlarged synaptic cleft, the shortened active zone, and the thinned postsynaptic density (PSD) on PND 14, 21, and 56 and the increased curvature of synaptic interface on PND 14 and 21. Further analyses of Western blot indicated that BPA markedly reduced the levels of synapsin I and PSD-95 on PND 14, 21, and 56 and down-regulated NMDA receptor subunit NR1 and AMPA receptor subunit GluR1 during development and young adulthood. These results suggest that perinatal exposure to low level of BPA inhibits synaptogenesis and affects synaptic structural modification after birth. The reduced expressions of synaptic proteins synapsin I and PSD-95 and glutamate NMDA and AMPA receptors may be involved in the negative changes in the synaptic plasticity.

1. Introduction

Bispenol-A (BPA), one of the most common environmental endocrine disrupters, is widely used to manufacture polycarbonate plastic food and beverage containers, the resin lining of metal cans, dental sealants, and as additives in a wide array of other products (Vandenberg et al., 2007; Erler and Novak, 2010). Despite its low affinity, BPA acts as estrogen receptors (ERs) agonist or antagonist that can bind to ER-α and β (Welshons et al., 2006) and as an androgen receptor (AR) antagonist that affects multiple steps in the activation and function of the AR (Bonefeld-Jorgensen et al., 2007; Xu et al., 2008; Wolstenholme et al., 2011). Gonadal hormones play an important role in the sexual differentiation of brain and behavior patterns during a critical period early in development. The developing brain is exquisitely sensitive to estrogens and androgens during this time and therefore is particularly vulnerable to BPA. Recently, BPA has become a compelling compound for its adverse effects on brain developmental process (Richter et al., 2007; Chen et al., 2009a; Xu et al., 2011b). For example, perinatal exposure to low-dose BPA (<50 mg kg^{-1} day^{-1}) alters behaviors later in life, including anxiety and memory, in both female and male rodents (Dessi-Fulgheri et al., 2002; Carr et al., 2003; Fujimoto et al., 2006; Ryan and Vandenbergh, 2006; Xu

Abbreviations: AR, androgen receptor; AZ, active zones; BPA, bisphenol-A; DAB, diaminobenzidine; ERs, estrogen receptors; GD, gestational day; LTD, long term depression; LTP, long term potentiation; PBS, phosphate buffer solution; PND, postnatal day; PSD, postsynaptic density; SV, synaptic vesicle.

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et al., 2010c). However, the underlying mechanisms of BPA on brain development remain unclear.

Synaptic plasticity in the hippocampus is considered to be important for processes such as learning and memory. Estrogen enhancement of plasticity is evidenced by increases in neurogenesis, neural network connectivity and synaptic transmission (Brinton, 2009). Morphological studies demonstrated estradiol-induced changes in the number and density of dendritic spines and synapses, and electrophysiological studies shed light on the consequences of these structural changes for the physiology (Woolley, 1998). Recently, BPA, as an environmental endocrine disrupter, was found its interference with synaptic remodeling (Hajszan and Leranth, 2010). BPA exposure for days enhanced dendritic growth in cerebellar Purkinje cell and the synaptic density in hypothalamic neurons of rats during neonatal life (Shikimi et al., 2004; Yokosuka et al., 2008). A rapid enhancement of LTD and an increase of the spine density in hippocampus CA1 were induced by exposed to BPA (Tanabe et al., 2005; Ogiue-Ikeda et al., 2008). Perinatal exposure to BPA leads to deficits in development of LTP and LTD at dorsal striatum and an abnormal cortical-basolateral amygdala synaptic transmission and plasticity, which may be responsible for the hyperactivity and attention-deficit in male rats (Zhou et al., 2009, 2011). Our previous study showed that exposure to BPA rapidly increased the motility and the density of dendritic filopodia in the cultured hippocampal neurons and enhanced passive avoidance memory and phosphorylation of NMDA receptor subunits in hippocampus of young rats (Xu et al., 2010b, 2011a).

Synaptic interface structural modification is sensitive to behavioral training and chemicals. Qiang et al. found that the process of learning and memory significantly induced an increase in the thickness of the postsynaptic density (PSD) and a decrease in the width of the synaptic cleft and the number of local vesicles (Qiang and Wang, 2000). PSD is located in postsynaptic membranes at the synaptic contact zone and has long been considered to be made up of a mixture of proteins, such as cytoskeletal and scaffold proteins, glutamate receptors, calmodulin binding protein, ion channels, and signaling molecules (Trinidad et al., 2005). The changes of protein–protein interaction between the receptors and submembranous scaffolding/signaling proteins of the PSD are reflected in the variable postsynaptic activity and plasticity. Our previous study demonstrated that estrogen improved spatial memory of ovariectomized female mice by concomitant reduction of synaptic cleft width and enlargement of PSD thickness in frontal cortex and hippocampus regions (Xu and Zhang, 2006). According to the knowledge, we consider the effects of perinatal exposure to BPA on behaviors of adult mice (Xu et al., 2010c) may be associated with the synaptic structural development. In the present study, we examined the synaptic density and the synaptic interface structure of pyramidal cells in hippocampus region CA1 by electron microscope. Since estrogens are involved in the formation of excitatory NMDA synapses in the hippocampus (Boon et al., 2005) and the most representative scaffold protein PSD-95 (postsynaptic density protein of 95 kDa) plays a central role in the regulation of synaptic function and is also important as regards linking of the glutamate receptors to downstream signaling pathways in PSD, the expressions of synaptic proteins such as synapsin I and PSD-95, and glutamate NMDA and AMPA receptors were examined to reveal possible underlying mechanisms of BPA on memory and synaptic plasticity in the present study.

2. Materials and methods

2.1. Animals

Male (30–35 g) and female (25–30 g) ICR mice were purchased from Academy of Medical Science of Zhejiang (China) and maintained in standard cages under reversed light:dark 12:12 cycle with free access to food and water. All experiments in the present study were conducted in accordance with the Care and Use Standard of the Laboratory Animal (China Ministry of Health publication, 1998). After acclimatization for 1 w, female mice were housed with males (female:male = 1:1) and vaginal plugs and vaginal smears were checked daily. A sperm-positive smear and a plug determine gestational day (GD) 0. After detection, the pregnant dams were placed individually and assigned to an exposure condition randomly (n = 11 for each condition).

2.2. Treatment

Dams were orally exposed (oral injection) to BPA (99.8%, Shanghai Chemical Reagent Research Institute, Shanghai, China) dissolved in sesame oil (4, 0.4 or 0.04 mg kg^{-1} day^{-1}) or only sesame oil (Jinlongyu, China) as a vehicle control at 8:00 am per day from GD 7 through postnatal day (PND) 21. The volume of the sesame oil was 0.1 mL/30 g body weight which was checked every week to adjust the dosage of BPA as the mice gained weight. The oral route of BPA administration was chosen to mimic the most likely route of exposure to the compound in humans and wildlife. After parturition (PND 0), the pups were counted, weighed, and culled to 10 pups if possible. The pups were identified individually and maintained equivalent sex distributions on PND 7, weaned from its mother on PND 21, and separated into same-sex littermates and housed on PND 28. The male pups were used in the present study.

2.3. Electron microscopic preparations

The male pups from 6 L (n = 6 for each group) were sacrificed on PND 14, PND 21 and PND 56, respectively. Brains were dissected out and fixed overnight with 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (PBS, pH 7.4). The samples were taken from the left side of the hippocampal CA1 pyramidal cell layer, according to the atlas of Franklin (1997). Tissues were processed for transmission electron microscopy routinely (Qiang and Wang, 2000). The thickness of the consecutive, serial ultrathin sections was 70 nm. Electromicrographs of synapses for synapse counting and synaptic interface measurement were taken at magnification of 10000 × and 100000 × using H-7650 TEM (Hitachi, Japan), respectively. Synapses are classified into asymmetric and symmetric synapses, or Gray I type and Gray II type synaptses, which are considered to mediate excitatory and inhibitory transmission respectively (Cuillery, 2000). Asymmetric synapses are with prominent post-synaptic densities and relatively wide synaptic clefts while symmetric synapses are with pre- and post-synaptic densities of equal thickness and narrower synaptic clefts. In the present study, asymmetric synapses were examined for synaptic measurement.

2.4. Morphometric measurement and analysis

The dissector technique proposed by Gundersen et al. (1988) was adopted in the present study for estimation of numeric synaptic densities. Briefly, 15–25 pairs of adjacent ultrathin sections within the pyramidal layers were analyzed from hippocamcpal CA1 of each animal. In each pair of sections, two unbiased dissector frames (5–6 μm) were arranged in a pseudo-random fashion. The first section was considered the "reference" and the second, the "look-up" section. The frame was superimposed such that the pyramidal layer was at the top of the frame. Within the frame, the number of synapses that were present in the "reference" section and absent in the "look-up" section (Q^-) was counted. Each section was used as both "reference" and "look-up" sections in a