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Newborn mice exposed prenatally to bisphenol A show hyperactivity and defective neocortical development

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ABSTRACT

The central nervous system is especially susceptible to toxic insults during development. Prenatal administration of bisphenol A (BPA) induces histologic anomalies in the dorsal telencephalon of the embryo. Whether these anomalies affect the morphogenesis and maturation of neuronal function of the newborn neocortex, however, is unknown. To evaluate the neurodevelopmental and behavioral effects of prenatal BPA exposure at 20 and 200 μg/kg/day in newborn mice, we performed a detailed histologic analysis of the neocortex and tested for the presence of behavioral abnormalities in newborn mice prenatally exposed to BPA using our newly developed behavioral test. Observations of newborn mice prenatally exposed to BPA revealed abnormal neuronal distribution and layer formation, hypoplasia of layer 6b, and abnormal dopaminergic neuronal projections in the neocortex. Further, the newborn mice exhibited hyperactivity. These findings suggest that prenatal BPA exposure induces neurobehavioral toxicity associated with abnormal dopaminergic neuronal projections, and abnormal corticogenesis and lamination. Histologic and behavioral analyses of newborn mice are considered useful for assessing the neurodevelopmental and behavioral toxicity of chemicals.

1. Introduction

The developing central nervous system (CNS) is especially susceptible to toxic insults, and functional changes are induced at a lower exposure dose than that causing adverse effects in adults (Bearer, 2000; Komada et al., 2010). Fetuses and young children are often more vulnerable to chemical toxicants that alter the structure and/or function of the brain, although the effects of individual neurotoxicants vary. Bisphenol A (BPA) is a compound widely used in the food industry for polycarbonate bottles and can linings, as well as in dentistry as a hardener for sealants and for tooth lacquering. BPA has estrogenic activity (Gould et al., 1998; Krishnan et al., 1993; Steinmetz et al., 1997) and disrupts methylation (Kundakovic et al., 2013). We and others have reported that prenatal BPA exposure induces abnormal neurogenesis and hyperplasia of the cortical plate (CP) in the dorsal telencephalon of the mouse embryo (Itoh et al., 2012; Komada et al., 2012; Nakamura et al., 2006) and morphologic anomalies in the neocortex of the adult mouse (Nakamura et al., 2007). Prenatal BPA exposure is implicated in abnormal behavior, learning deficits, and anxiety in adult rodents (Farabollini et al., 1999; Fujimoto et al., 2006; Gioiosa et al., 2007; Kubo et al., 2003; Miyagawa et al., 2007; Porrini et al., 2005; Rubin et al., 2006; Ryan and Vandenbergh, 2006; Xu et al., 2007). Perinatal BPA exposure disrupts sexually dimorphic behavior in the postnatal development period and in adult mice, assessed using the elevated plus maze and open field tests (Nakamura et al., 2012). In addition, rodents exposed prenatally to BPA exhibit hyperactivity resembling attention-deficit hyperactivity disorder (ADHD) in these behavioral tests and dopaminergic neurons or dopamine neurotransmitter levels are decreased in the midbrain (Ishido et al., 2004; Mizuo et al., 2004; Nakamura et al., 2010). The reduction of dopaminergic neurons in the midbrain may lead to the hyperactivity observed in adult rodents exposed prenatally to BPA (Ishido et al., 2004; Mizuo et al., 2004).

Patients with ADHD and autism exhibit abnormal behavior in terms of primitive reflexes, ability to roll over, crawl, as well as in

Abbreviations: CNS, central nervous system; ADHD, attention-deficit hyperactivity disorder; BPA, bisphenol A; E, embryonic day; P, postnatal day; IF, the length of the interhemispheric fissure; TRC, the length of the rostral–caudal length; CP, cortical plate; TH, tyrosine hydroxylase.

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general movements and kinetic activity as infants (Hadders-Algra and Groothuis, 1999; Teitelbaum et al., 1998). We recently reported a newly developed newborn behavioral testing method to detect abnormal motor behavior and increased motor behavior on postnatal day (P) 1 in mice exposed prenatally to a neurodevelopmental toxicant, methylnitrosourea or diethylstilbestrol (Nagao et al., 2013, 2014). The behavioral testing method sensitively detects hyperactivity in newborn rodents (Nagao et al., 2013, 2014).

In the present study, we examined the effects of prenatal exposure to BPA at 20 and 200 μg/kg/day on the neocortical structures in newborn mice. Hypoplasia of layer 6b, abnormal layer formation, and abnormal neuronal distribution were confirmed to affect dopaminergic neuronal projections in the neocortex. Dopaminergic neurons in the mesocortical dopaminergic pathway control behavior and brain reward system (Hosp and Luft, 2013; Tzschentke, 2001; Volkow et al., 2011). Assessment using the newly developed newborn behavioral testing method revealed hyperactivity. These findings suggest that prenatal BPA exposure induces histologic and behavioral abnormalities in newborn mice.

2. Material and methods

2.1. Animals and housing

Eight-week-old ICR mice purchased from CLEA (Osaka, Japan) were used after two weeks acclimation to the animal facility. Experimental protocols were approved by the Animal Care and Use Committee of Kinki University. The mice were kept under specific pathogen-free conditions and a constant light-dark cycle (dark period from 7:00 pm to 7:00 am) at 24 ± 1 °C and 55 ± 5% relative humidity. Food (Certified Rodent Chow CE-2, CLEA, Osaka, Japan) and drinking water were available ad libitum. Certification analysis of each lot of the diet was performed by the manufacturer. Distilled water was available via glass bottles with Teflon seals during the experimental period. Pregnant mice were housed individually throughout the study in polypropylene plastic tubs with stainless steel lids and corncob bedding. Ten-week-old mice were allowed to copulate overnight at a 1:1 female to male ratio. Females were checked at 12 h intervals for the presence of vaginal plugs, indicating copulation, and were separated from the male if a plug was present. The presence of a plug represented embryonic day (E) 0. Pregnant mice were allowed to give birth and nurse their pups until P1 or P3. The day of birth was designated as P0. In the morning of P1, the number of newborns in a litter was adjusted to 3–5 males and 3–5 females (total number of newborns in a litter after adjustment was 8). Pups were weighed on P1 and P3, the number of pups in each litter during the lactation period was recorded, and viability on P3 after litter size adjustment was determined. The number of mice used in each experiment is shown in Table 1.

2.2. Test substances and treatment regimen

BPA (2, 2-bis(4-hydroxyphenyl)propane 4,4′-isopropyl-lidenediphenol, CAT no. 80-05-7, Sigma–Aldrich, Tokyo, Japan) was suspended in corn oil and administered by oral gavage from E6 to E18. A fresh dose solution was prepared every 5 days and analyzed prior to dosing. The BPA concentration was confirmed to be within ±10% of the targeted concentration. Administration was performed at a defined time (12:00 pm–12:15 pm). In a previous study, we demonstrated hyperplasia of the CP and the promotion of neurogenesis in mouse embryos exposed to BPA at 200 μg/kg/day (Komada et al., 2012). Based on those results, BPA doses of 20 and 200 μg/kg/day were selected for the present study. Twelve pregnant mice in each group were administered BPA at 20 or 200 μg/kg/day. Nineteen pregnant mice treated with corn oil served as controls.

2.3. Tissue preparation and immunohistochemistry

For immunohistochemistry, including neuronal birth-date analysis, we used 9 males newborns of 3 dams in each group. On P3, newborns were weighed and humanely killed by exsanguination under anesthesia with sevoflurane (Maruishi Pharmaceutical. Co., Ltd., Osaka, Japan), and the brains were sampled and weighed. For brain measurements, the length of the interhemispheric fissure (IF) and the length of the rostral–caudal telencephalon (TRC) were measured to calculate their ratio. Subsequently, the brains were fixed in periodate lysine paraformaldehyde for 3 h at 4 °C, and washed in phosphate-buffered saline. Brains from 9 males newborns in each group were embedded in paraffin and sectioned at 5 μm for histologic and immunohistochemical observation. Newborn body and brain weights were analyzed using Student's *t*-test. Immunohistochemistry was performed as described previously (Komada et al., 2008).

We used the following antibodies: anti-Nurr1 (1:200; AF2156; R&D System Inc., MN, USA), anti-Cux1 (1:50; sc-13024; Santa Cruz Biotechnology Inc., Texas, USA), anti-Tle4 (1:50; sc-13377; Santa Cruz Biotechnology Inc., Texas, USA), anti-Foxp2 (1:200; ab16046; abcam, Cambridge, UK), anti-bromodeoxyuridine (cross reacts with chlorodeoxyuridine; CldU; 1:50; BU1/75; AbD Serotec, Kidlington, UK), anti-bromodeoxyuridine (cross reacts with iododeoxyuridine; IdU; 1:50; B44; BD Biosciences, NJ, USA), and anti-tyrosine hydroxylase (TH; 1:500; AB152; Millipore, MA, USA).

2.4. Neuronal birth-date analysis

For neuronal birth-date analysis, pregnant females in each group were injected intraperitoneally with 50 mg/kg CldU (MP Biomedicals, Solon, OH, USA) on E14 and 50 mg/kg IdU (Sigma Chemical Co., St. Louis, MO, USA) on E16 to label the neurons generated at each

Table 1
The number of mice used in each experiment.

Group	Body weight		Brain weight	IF/TRC[c]	Behavioral test	Histologic analysis
	P1	P3	P3	P3	P1	P3
Control	♂: 40[a]/10 dams[b] ♀: 40/10 dams	♂: 36/9 dams[b] ♀: 36/9 dams	♂: 36/9 dams ♀: 36/9 dams	♂: 36/9 dams ♀: 36/9 dams	♂: 27/9 dams ♀: 30/10 dams	♂: 9/3 dams –
BPA 20 ug/kg/day	♂: 24/6 dams ♀: 24/6 dams	♂: 24/6 dams ♀: 24/6 dams	♂: 24/6/ dams ♀: 24/6/ dams	♂: 24/6/ dams ♀: 24/6/ dams	♂: 18/6 dams ♀: 18/6 dams	♂: 9/3 dams –
BPA 200 ug/kg/day	♂: 20/6 dams ♀: 20/5 dams	♂: 24/6 dams ♀: 24/6 dams	♂: 24/6/ dams ♀: 24/6/ dams	♂: 24/6/ dams ♀: 24/6/ dams	♂: 15/6 dams ♀:15/5 dams	♂: 9/3 dams –

[a] The number of newborn mice.
[b] Dams used in the experiment on P1 (behavioral test) were different from dams used in the experiment on P3 (immunohistochemistry) because the later dams were treated with CldU on day 14 of gestation and with IdU on day 16 of gestation.
[c] IF: the length of the interhemispheric fissure; TRC: the length of rostral–caudal telencephalon.