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Letter

Motor activities of newborns prenatally exposed to low-dose bisphenol A in diverse mouse strains

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ABSTRACT — Studies on the low-dose effects of xenoestrogens have yielded conflicting results that may have resulted from differences in estrogen sensitivity between the mouse strains used. We developed a mouse newborn behavioral testing method for evaluating the risk of neurotoxicity of environmental chemicals, by means of determining a newborn's motor activity through applying the tare function of an analytical balance. Motor activities including crawling, pivoting, and tremors of C57BL/6J and ICR mouse newborns exposed to bisphenol A (BPA) at 200 μg/kg/day on embryonic days 6 through 18 were evaluated for 5 min on postnatal day 1 by the testing method. Motor activities of mature male offspring exposed prenatally to BPA were also evaluated in wheel cage and open field tests. Maternal BPA oral dosing increased the motor activity in newborns of both strains and mature offspring of the C57BL/6J strain. The findings indicate that both mouse strains provide adequate models for the newborn neurobehavioral study of prenatal exposure to environmentally relevant levels of estrogen-mimicking chemicals.

Key words: Bisphenol A, Motor activity, Newborn, Environmental estrogen, Strain difference

INTRODUCTION

To evaluate the neurotoxic effects of chemicals on newborns in rodents, we recently developed a mouse newborn neurobehavioral testing method, involving the quantitative determination of a newborn animal's activity automatically using the tare function of an analytical balance (Nagao *et al.*, 2013). We demonstrated that the developmentally estrogenic compounds treated ICR mice showed an increase in motor behavior when we estimated the activity using this testing method on postnatal day 1 (Komada *et al.*, 2014; Nagao *et al.*, 2014; Ozaki *et al.*, 2015).

Our research focuses on the neurobehavioral effects of prenatal exposure to environmentally relevant levels of the xenoestrogen bisphenol A (BPA). BPA is one of the environmental endocrine disruptors released by plastics and resin known to interfere with hormonal responses. Nuclear estrogen receptor binding assays indicate that BPA has at least a 10,000-fold lower affinity for the two estrogen nuclear receptors than 17β-estradiol. Low doses of BPA administered perinatally can modify exploratory behavior and anxiety in rats (Fujimoto *et al.*, 2013). It was shown that perinatal BPA exposure disrupted sexually dimorphic behavior in the postnatal developmental period and adult mice, when evaluated by the elevated plus maze and open field tests (Gioiosa *et al.*, 2013; Nakamura *et al.*, 2012). Additionally, Palanza *et al.* (2008) described greater activity in male mice in the open field test following prenatal low-dose BPA exposure.

The NTP Low-Dose Peer Review Panel published a final report (NTP, 2001), which stated that there was "credible evidence for low-dose effects" and suggested that different experimental animal strains may account for reports of both positive and negative effects for the same parameters. Environmental estrogens can affect spermatogenesis, and some mouse strains such as C57BL/6J are much more sensitive to estrogenic compounds than other strains such as CD-1 (Spearow *et al.*, 1999). In the present study, therefore, we studied the neurobehavioral effects of prenatal exposure to low-dose BPA on newborn motor activities in two mouse strains, inbred C57BL/6J mice and outbred ICR mice.

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MATERIALS AND METHODS

Animals and BPA exposure

C57BL/6J (B6, CLEA Japan, Inc., Osaka, Japan) and ICR (Japan Slc, Inc., Osaka, Japan) mice were used. The experimental protocols were approved by the Animal Care and Use Committee of Kinki University. Food (CE-2, CLEA Japan, Inc.) and distilled water were available *ad libitum.* Pregnant mice were administered bisphenol A (BPA, Sigma-Aldrich, St. Louis, MO, USA) at 0 (0.5% CMC-Na) and 200 μg/kg/day by oral gavage on gestational days 6 through 18, and allowed to give birth. The day of birth was designated as postnatal day (PND) 0.

Neurobehavioral procedures

Newborn motor activity test on PND 1

Seven pregnant females per group, and 3 male and 3 female newborns per dam were used. Recently, we developed a mouse newborn behavioral testing method for evaluating the neurotoxic risk of chemicals (Komada *et al.*, 2014; Nagao *et al.*, 2013, 2014). Briefly, an electric balance (Tuning-fork analytical balance, HTR-80, SHINKO DENSHI CO., LTD., Tokyo, Japan, capacity 80 g, readability 0.0001 g, repeatability (σ) 0.0001 g, interface RS232C, D-SUB9P) on a shock-proof stage was used to evaluate the absolute values obtained from the range of fluctuations between weighing values resulting from the movement (crawling, pivoting, tremors) of newborns on PND 1 from 12:00 pm to 1:00 pm. Weighing values resulting from "righting" were excluded from the evaluation. The absolute value was defined as the activity of a newborn, and the total motor activity of a newborn was the sum total of the absolute values for 5 min. The movements (crawling, pivoting, tremors) were defined as motor activities showing an absolute value of 0.0001 or more. The changing weight values caused by the movement of the newborn on a plastic dish (94/16, Greiner Bio-One GmbH, Kremsmünster, Austria) on the balance pan were recorded by a personal computer every 0.1 sec via WinCT (Windows Communication Tools) software (version 4.01, A&D Company Ltd., Tokyo, Japan), and the activity of individual newborns was determined. Please see the movie and figures detailing the procedure of the behavioral test (Komada *et al.*, 2014).

Wheel cage activity test and open field test of mature offspring

Subsequently, 9 male offspring at 9 weeks of age per group of both strains were used for evaluating the motor activity in a wheel cage (KN-78-M, Natsume Seisakusho Co., Ltd., Tokyo, Japan) for 24 hr and a round open-field area, which comprised a floor of 100 cm in diameter, and a 30-cm-high wall around the perimeter. Both the floor and interior surface of the wall were painted black. The floor was divided into 19 equally sized grids (in surface area). Ambulation was recorded by a computer during a 3-min trial between 10:00 am and 12:00 pm. The light and noise levels averaged 500 lx and 45 dB, respectively, at the center of the area.

Statistical analysis

The data on activity of newborn and mature mice were analyzed via two-way analysis of variance (ANOVA) with treatment as a factor. Regardless of whether or not the repeated measure ANOVA detected significant interactions, one-way ANOVA was followed by tests for simple main effects, and detailed multiple comparisons were made using Tukey's honestly significant difference post-hoc tests, given corresponding significant F-values. Statistical evaluation of data was performed using JMP (version 9.0; SAS Institute Inc., Cary, NC, USA). All data on newborns used the litter average as the statistical unit, and significance was assumed for probability levels of 0.05 or less.

RESULTS AND DISCUSSION

In the two mouse strains, B6 and ICR, there were no adverse changes in general conditions or spontaneous delivery of dams exposed to BPA, and the numbers of live newborns on PND 0 in the BPA-treated groups were comparable to those in the controls (data not shown).

As for the behavioral changes of the newborns on PND1, the activities in the BPA-treated group of B6 and ICR strains were significantly higher than those in the respective controls in both sexes. The activities in the ICR strain showed an increasing tendency compared to those in the B6 strain (Fig. 1). ANOVA on motor activity revealed main effects of treatment, $F(1,54) = 81.376$, $p = 0.0001$, and strain, $F(1,54) = 3.960$, $p = 0.052$. ANOVA also revealed a main effect of sex, $F(1,54) = 1.296$, $p = 0.260$, but no interactions between treatment and strain or sex. There was no significant correlation between the body weight and activity.

During fetal life, the intrauterine environment is critical for normal development, and even small changes in the levels of hormones, such as estradiol or estradiol-mimicking chemicals, can lead to changes in brain structure and function, and consequently in behavior (Palanza *et al.*, 2008). The motor behaviors of newborns are regulatory behaviors that are orchestrated and coordinated by the neocortex. It is considered that the increase

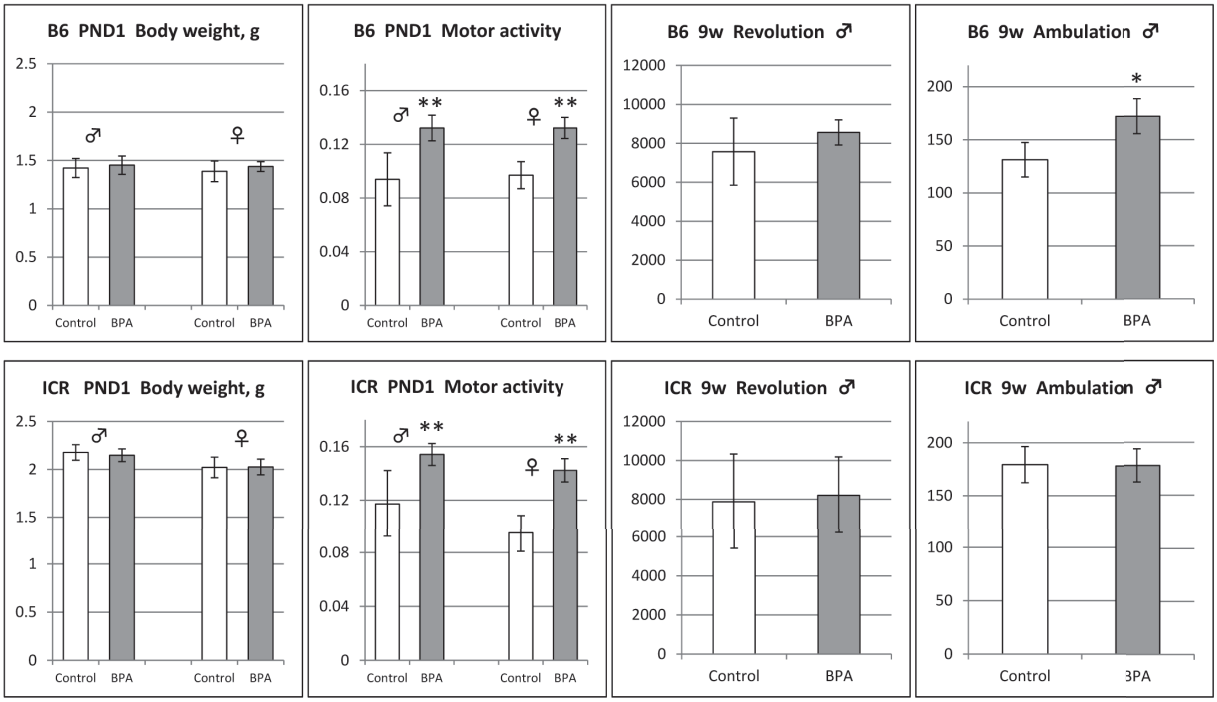

Fig. 1. Activities of PND 1 newborns and mature offspring of B6 and ICR mouse strains exposed prenatally to BPA at 200 µg/kg/day. Activities of newborns consisted of gross movements (crawling and pivoting) and small movements (tremors) for 5 min (Komada *et al.*, 2014; Nagao *et al.*, 2013, 2014; Ozaki *et al.*, 2015). Weighing values resulting from gross movement (righting) were excluded from further evaluation. Spontaneous activities of mature offspring were evaluated in the wheel cage test (revolutions/24 hr) and open-field test (ambulation/3 min). Vertical lines represent standard deviations. *Significantly different from the respective control, $p < 0.05$. **Significantly different from the respective control, $p < 0.01$.

in motor behaviors was caused by neocortex anomalies, layer formation, neural positioning, and neural projection. Previously, we showed that maternal BPA oral dosing was related to hyperplasia of the cortical plate during the development of the B6 mouse telencephalon (Komada *et al.*, 2012). In addition, we reported that the layer anomalies of the neocortex were identified in developmentally BPA-treated ICR mouse newborns, suggesting that these anomalies might be related to the abnormal motor behaviors of newborns (Komada *et al.*, 2014). Taken together, it is reasonable to suggest that the increase in the motor activities of B6 and ICR newborns exposed prenatally to BPA may be associated with the change in brain structure induced by this compound.

Prenatal and lactational BPA exposure disturbed behavior in adult mice (Nakamura *et al*., 2012; Palanza *et al*., 2008). In the present study, adult male mice exposed prenatally to BPA in both strains were tested for spontaneous activity to examine the continuation of hyperactivity detected in the newborns on PND 1. In the wheel cage activity test, the number of revolutions in the BPA-treated group in both strains was comparable to that of the respective controls (Fig. 1). ANOVA revealed main effects of strain, $F(1,34) = 0.003$, $p = 0.960$, and treatment, $F(1,34) = 1.239$, $p = 0.274$. The strain x treatment interaction was not significant. In the open field test, ambulation in the BPA-treated group of the B6 strain was significantly increased compared to that in the B6 control group, and there was no significant difference in ambulation between the BPA-treated group of ICR strain and controls. ANOVA revealed main effects of strain, $F(1,34) = 13.927$, $p = 0.001$, and treatment, $F(1,34) = 6.292$, $p = 0.017$, and significant effects of strain x treatment, $F(1,34) = 14.928$, $p = 0.001$.

In conclusion, prenatal exposure to low-dose BPA led to early-stage motor hyperactivity in mouse newborns of B6 and ICR strains, indicating that both mouse strains provide adequate models for the newborn neurobehavioral study of prenatal exposure to environmentally relevant levels of estrogen-mimicking chemicals. Although the mechanisms of hyperactivity in mouse newborns exposed to BPA *in utero* remain unknown, it is tempting

to speculate that BPA might underlie the recent increase in the number of children with neurobehavioral disorders including ADHD and autism, which is based on organic functional disorder of the central nervous system. Adult B6 mice exposed prenatally to BPA showed hyperactivity when evaluated for the ambulation in the open field test. The relationship between the hyperactivity of B6 newborns on PND 1 and the increased ambulation of B6 mature male offspring following prenatal exposure to low-dose BPA should be clarified for the early detection of neurobehavioral abnormalities and early treatment of patients with neurobehavioral disorders.

Conflict of interest---- The authors declare that there is no conflict of interest.

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