Fundamental Toxicological Sciences (Fund. Toxicol. Sci.)
Vol.2, No.1, 37-39, 2015

Letter

Effects of prenatal exposure to low doses of diethylstilbestrol on motor activity in newborn mice

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(Received December 26, 2014; Accepted January 7, 2015)

ABSTRACT — We developed a newborn mouse behavioral testing method to evaluate the risk of neurotoxicity of environmental toxicants, based on determining a newborn's motor activity by applying the tare function of an analytical balance. Motor activities of newborn ICR mice exposed prenatally to diethylstilbestrol (DES) at 0.005-0.5 μg/kg/day on days 5 through 18 of gestation were evaluated on postnatal day 1. The activities of male newborns in the 0.05 μg/kg/day group were significantly increased compared to those of the controls, and the increasing tendencies were observed in both sexes of the highest group. The findings indicate that prenatal exposure to low doses of DES causes hyperactivity in newborn mice.

Key words: Diethylstilbestrol, Motor activity, Neurobehavior, Newborn mouse

INTRODUCTION

Studies on rodents have shown that prenatal exposure to some neurotoxicants adversely affects neonatal orientation, attention, and motor function maturity, as well as the activity level, executive function, response inhibition, and sensory processing later in life (Schneider *et al.,* 2011). Although there have been a large number of animal toxicological studies carried out on pregnant animals, including embryos/fetuses and mature animals, there is a paucity of reports on animal toxicology studies utilizing newborn animals a few days after birth. To evaluate the neurotoxic effects of chemicals on newborn rodents, we recently developed a newborn mouse neurobehavioral testing method, based on the quantitative determination of a newborn animal's activity using the tare function of an analytical balance. We demonstrated that newborn mice exposed prenatally to low-dose bisphenol A (BPA) showed hyperactivity (Nagao *et al.,* 2013, 2014; Komada *et al.,* 2014). Neurobehavioral development is modified by prenatal exposure to hormone-mimicking drugs or chemicals (Bignami, 1996). Diethylstilbestrol (DES) is a potent estrogenic drug that has been extensively studied regarding its developmental effects on experimental animals. In addition, DES is commonly used as a positive control chemical in toxicological studies of putative estrogenic chemicals (vom Saal and Welshons, 2006). Thus, the purpose of this study was to determine whether prenatal exposure to low doses of DES result in neurobehavioral changes of newborn mice.

MATERIALS AND METHODS

ICR mice (CLEA, Osaka, Japan) were used. The experimental protocols were approved by the Animal Care and Use Committee of Kinki University. Food (CE-2, CLEA, Osaka, Japan) and distilled water were available ad libitum. Four to 6 pregnant mice were administered diethylstilbestrol (DES, Sigma Chemicals, St. Louis, MO, USA) at 0 (corn oil), 0.005, 0.05, or 0.5 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 (P) 0. Two male and 2 female newborns from each dam were used for neurobehavioral evaluation on P1.

We recently developed a newborn mouse behavioral testing method to evaluate chemical neurotoxicity based on the determination of newborn motor activity using an electric balance (Nagao *et al*., 2013, 2014; Komada *et al*., 2014). Briefly, an electric balance (HTR-80, SHINKO DENSHI CO., LTD., Tokyo, Japan, capaci-

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ty: 80 g, readability: 0.0001 g, repeatability(σ): 0.0001 g) on a shock-proof stage was used to evaluate the absolute values obtained from the range of fluctuations between weight values resulting from movement (crawling, pivoting, tremors) of newborns on P1 from 12:00 pm to 1:00 pm. The absolute value of the change in weight was defined as the activity of a newborn, and the total activity of a newborn was the sum total of the absolute values of the change in weight for 5 min. The changing weight values obtained by movement of the newborn in a plastic dish (94/16, Greiner Bio-One GmbH, Frickenhausen, Germany) placed on the balance pan were recorded by a personal computer every 0.1s via WinCT (Windows Communication Tools) software (version 3.00, A&D Company Ltd., Tokyo, Japan) and the activities of individual newborns were determined. Gross movements (crawling and pivoting) showing an absolute value of 0.0002 or more and small movements (tremors) showing an absolute value of 0.0001 were defined as activity. Please see the movie and figures detailing the procedure of the behavioral test (Komada *et al.,* 2014).

The data on newborn activity were analyzed by two-way 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 test, given corresponding significant F-values. For all data, the litter average was used as the statistical unit.

RESULTS AND DISCUSSION

Body weights of male and female newborns on P1 in the DES-treated groups were comparable to those in the controls (data not shown). Concerning the behavioral changes of newborns on P1, the total activity (sum total of the absolute values) in the males of the 0.05 μg/kg/day DES-treated group was significantly increased compared to those in the control group. ANOVA revealed main effects of treatment [$F(3, 17) = 4.821$, $p = 0.013$]. The total activities in the females of the DES-treated groups were not significantly different from those in the control group, whereas an increasing tendency was observed in the 0.5 μg/kg/day DES-treated group. ANOVA revealed main effects of treatment [$F(3, 17) = 2.834$, $p = 0.069$]. Total absolute values of 0.0001 (tremors) in males and females in the DES-treated groups were comparable to those of the controls. The ANOVA for males and females revealed main effects of treatment [$F(3, 17) = 0.133$, $p = 0.939$, and $F(3, 17) = 0.198$, $p = 0.897$, respectively]. Therefore, the present study demonstrated increased motor activities in newborns prenatally exposed to DES.

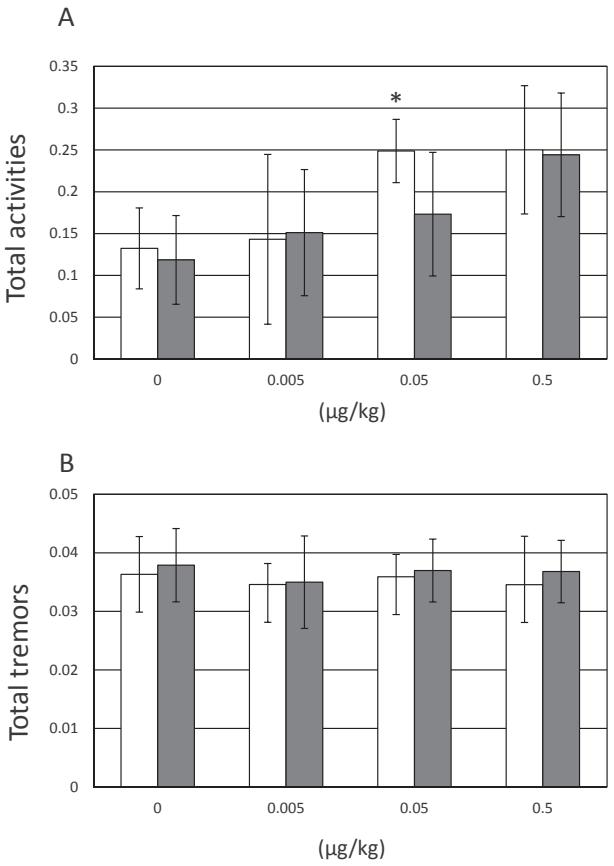

Fig. 1. Total activities (A) from crawling, pivoting and tremor, and total tremors (B) of P1 newborns from dams in the DES-treated and control groups. Vertical lines represent standard deviations. *Significantly different from the control, $p < 0.05$. Total activities (crawling, pivoting, tremor) of a newborn refer to the sum total of the absolute values for 5 min, and total tremors is the sum total of absolute values of 0.0001 (see Nagao *et al.*, 2014; Komada *et al.*, 2014). The numbers of dams used for the neurobehavioral evaluation of newborns were 5 (DES 0.005 μg/kg/day), 6 (DES 0.05 μg/kg/day), 4 (DES 0.5 μg/kg/day), and 6 (control group), respectively. The numbers of male and female newborns were 10 and 10 (DES 0.005 μg/kg/day), 12 and 12 (DES 0.05 μg/kg/day), 8 and 8 (DES 0.5 μg/kg/day), and 12 and 12 (control group), respectively. □: male newborns, ■: female newborns.

The motor behaviors of newborns are regulatory behaviors initiated and coordinated by the neocortex. It is considered that the increase in motor behaviors was caused by neocortex anomalies, layer formation, neural positioning, and neural projection. We demonstrated that

prenatal exposure to BPA led to hyperactivity in newborn mice, with impaired neocortical lamination and abnormalities of dopaminergic neuronal projections to the neocortex, suggesting that histologic abnormalities of the neocortex are associated with hyperactivity (Komada *et al.,* 2012, 2014).

Prenatal exposure to low doses of DES led to motor hyperactivity in newborn mice in the present study. Although the mechanisms of hyperactivity in newborns exposed to DES *in utero* remain unknown, we speculate that endocrine-disrupting chemicals such as DES and BPA may 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. As the next step in our neurobehavioral studies of newborn animals exposed prenatally to hormone-mimicking compounds, the relationship between brain damage, including layer abnormalities of the neocortex, and the behavioral abnormalities of newborns, and that between newborn behavioral abnormalities and such abnormalities of mature animals exposed prenatally to those compounds should be clarified. However, it should be taken into account that the early stage of postnatal brain development in the mouse is congruent with brain development of human fetus in the latter part of gestation, when newborn mouse behavior was evaluated.

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

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