Neonatal Exposure to the Brominated Flame Retardant 2,2',4,4',5-Pentabromodiphenyl Ether Causes Altered Susceptibility in the Cholinergic Transmitter System in the Adult Mouse

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Received August 22, 2001; accepted January 8, 2002

Polybrominated diphenyl ethers (PBDEs) are used as flame retardants and have recently been shown to be increasing in the environment and in human mother’s milk. We have recently reported that neonatal exposure to 2,2',4,4',5-pentaBDE (PBDE 99) can induce persistent aberrations in spontaneous behavior and also affect learning and memory functions in the adult animal. The present study indicates that the cholinergic system, in its developing stage, may be a target of and sensitive to PBDEs. Neonatal exposure of male NMRI mice on postnatal day 10, to 2,2',4,4',5-pentaBDE (8 mg/kg bw) was shown to alter the response to a cholinergic agent, nicotine, at an adult age. The nicotine-induced behavior test revealed a hypoactive response to nicotine in PBDE 99-treated animals, whereas the response of controls was an increased activity. These findings show similarities to observations made from neonatal exposure to PCBs and nicotine, compounds shown to affect cholinergic nicotinic receptors. This indicates that PBDE 99 can affect the cholinergic system and might thereby interact with other environmental toxins.

Key Words: behavior, cholinergic system, developmental neurotoxicity, flame retardant (PBDE), neonatal.

In recent studies, we have shown that neonatal exposure to certain PBDEs and PCBs during the period of rapid brain development (Davison and Dobbing, 1968) can cause similar persistent disturbances in spontaneous motor behavior and dysfunctions in learning and memory in adult animals (Eriksson, 1998; Eriksson and Fredriksson, 1996a,b; Eriksson et al., 2001). Earlier studies have shown that neonatal exposure of mice to PCBs affects the cholinergic transmitter system.Effects seen were alterations in nicotinic receptors and also an adult response to the cholinergic agent nicotine that was the opposite of animals neonatally exposed to the vehicle (Eriksson and Fredriksson, 1996b). In view of these findings, the objective of this study was to determine whether recently reported changes in spontaneous behavior in adult mice neonatally exposed to PBDE 99 would include effects on the cholinergic system, and thereby would alter the response in the adult animal to the cholinergic agent nicotine.

MATERIALS AND METHODS

2,2',4,4',5-pentaBDE (purity > 98%) was kindly donated by Eva Jakobsson, Wallenberg Laboratory, Stockholm, Sweden (Eriksson et al., 2001). It was dissolved in a mixture of egg lecithin (Merck, Darmstadt, Germany) and peanut oil (Oleum arachidis) (1:10 w/w) and was sonicated together with water to yield a 20% fat emulsion vehicle and to contain 0.8 mg 2,2',4,4',5-pentaBDE/ml (1.4 μmol/ml).

Sixteen pregnant NMRI mice were obtained from B&K, Sollentuna, Sweden; they were housed individually in plastic cages in a room with an ambient temperature of 22°C and a 12/12-h light/dark cycle. The animals were supplied with standardized pelleted food (Lactamin, Sweden) and tap water ad libitum. The pregnant NMRI mice were checked for births twice a day (0800 and 1800 h). The day of birth was assigned day 0, and pups born during the night were considered to have day 0 as the day they were found. The sizes of the litters were adjusted to 10–12 mice within the first 48 h after birth, and excess pups were killed. The litters contained pups of both sexes during the neonatal period and no separation with regard to sex was made in the preweaning mice. At the age of 4–5 weeks, all females were sacrificed; males were kept in litters (in treatment groups) with their siblings and were placed and raised in groups of 4–7 in a room for male mice only, under the same conditions as detailed above. Only male mice were used.

Ten-day-old mice received, as a single oral dose, 8 mg 2,2',4,4',5-pentaBDE/kg body weight (bw) (14 μmol 2,2',4,4',5-pentaBDE/kg bw) via a metal gastric tube, and the mice serving as controls received vehicle in the same...
manner: 10 ml/kg bw of 20% fat emulsion only. Both the treatment group and the control group contained 3–4 litters, respectively.

The animals were weighed twice during the course of the experiment: first on the day of administration of PBDE 99 (day 10) and then at the time of spontaneous and nicotine-induced behavior testing (age 2 months). Additionally, during the course of the experiment, the animals were checked by visual examination once a day for clinical signs of toxic effects.

At the adult age of 2 months, the mice were subjected to spontaneous behavior testing. The animals were tested between 0800 and 1200 h under the same ambient light and temperature conditions as in their cages. Twelve mice were randomly picked from 3–4 different litters in the different treatment groups. Motor activity was measured for a 60-min period, divided into three 20-min periods, in an automated device consisting of 12 cages (40 × 25 × 15 cm) placed within two series of infrared beams (low and high level; Rat-O-Matic, ADEA Elektronik AB, Uppsala, Sweden) (Eriksson et al., 2001; Fredriksson, 1994). Three variables were measured:

- **Locomotion**: Counting took place when the mouse moved horizontally through the low level grid of infrared beams.
- **Rearing**: Movement in the vertical plane was registered at a rate of four counts per s, when a single high level beam was interrupted, i.e., the number of counts obtained was proportional to time spent rearing.
- **Total activity**: All types of vibration within the cage, i.e., that caused by mouse movements, shaking (tremors), and grooming were registered by a pickup (mounted on a lever with a counterweight) connected to the test cage.

Directly after the spontaneous behavior test, nicotine-induced behavior was studied. The mice were picked up from the test cage and were directly given a single sc injection of 80 μg nicotine base/kg bw [nicotine-bi-(+)-tartrate (Sigma, St. Louis, MO)] or 10 ml 0.9% NaCl/kg bw, sc. This amount of nicotine is known to cause an increased activity in normal adult NMRI mice (Eriksson et al., 2000). Directly after the nicotine injection, the mice were replaced in the test chamber. The nicotine-induced behavior was measured during another 60-min period (60–120 min, in the same way as was the spontaneous behavior) in which motor activity was recorded with regard to the three variables: locomotion, rearing, and total activity. The 60-min period was divided into three 20-min periods.

The statistical data concerning body weight were evaluated by a Student’s t-test. The statistical data obtained from the behavioral tests were evaluated by ANOVA (analysis of variance) using a split-plot design. Pairwise testing between treated and control groups was performed using a Tukey HSD (honestly significant differences) test (p = 0.01) (Kirk, 1968).

**RESULTS**

There were no clinical signs of toxic symptoms in the treated mice at any given time during the experimental period. The mean body weights of the 10-day-old animals did not differ significantly (p > 0.1) between the control (mean bw = 5.13 ± 0.67 g) and PBDE 99-treated animals (mean bw = 5.41 ± 0.79 g), and there was no significant difference in mean body weight (p > 0.1) between the adult animals in the vehicle-treated group and the PBDE 99-treated group (42.38 ± 5.45 and 43.58 ± 4.82, respectively). When comparing the body weight gain during the 6-week period (day 10 to age 2 months) there were no significant differences (p > 0.1) between the vehicle-treated animals (37.25 ± 5.28 g) and the PBDE 99-treated animals (38.17 ± 4.38 g).

Shown in Figure 1 are the results from the spontaneous behavior variables, locomotion, rearing, and total activity in 2-month-old mice exposed to a single oral dose of 8.0 mg PBDE 99/kg bw and from controls receiving 10 ml/kg bw of the 20% fat emulsion vehicle. During the first 60-min period. There were significant group × period interactions [F(6,56) = 14.33; F(6,56) = 16.29; F(6,56) = 15.91] for the variables locomotion, rearing, and total activity, respectively. Pairwise testing between 2,2',4,4',5-pentaBDE-treated animals and controls was performed using Tukey HSD tests. The statistical difference between the control animals and the 2,2',4,4',5-pentaBDE-treated animals is indicated by (A) p < 0.01.

Responses to nicotine in 2-month-old mice treated neona-
tally with 8.0 mg PBDE 99/kg bw or the 20% fat emulsion vehicle are shown in Figure 2. There were significant group \times period interactions \[ F(6,56) = 51.56; F(6,56) = 22.83; F(6,56) = 68.91 \] for the variables locomotion, rearing, and total activity, respectively. Pairwise testing between nicotine-injected and saline-injected animals was performed using Tukey HSD tests. The statistical differences between nicotine-injected and saline-injected animals are indicated by: (A) \( p < 0.01 \); and (a) \( p < 0.05 \). The height of each bar represents mean value \pm SD.

FIG. 2. Nicotine-induced behavior in 2-month-old mice exposed, at a neonatal age of 10 days, to either 8 mg 2,2′,4,4′,5-pentaBDE/kg bw as a single oral dose or the 20% fat emulsion vehicle. The nicotine-induced behavior was studied by using 80 µg nicotine base per kg bw, sc and 10 ml 0.9% NaCl/kg bw, sc. Statistical evaluation was done by an ANOVA with split-plot design. There were significant group \times period interactions \[ F(6,56) = 51.56; F(6,56) = 22.83; F(6,56) = 68.91 \] for the variables locomotion, rearing, and total activity, respectively. Pairwise testing between nicotine-injected animals and saline-injected was performed using Tukey HSD tests. The statistical differences between nicotine-injected and saline-injected animals are indicated by: (A) \( p < 0.01 \); and (a) \( p < 0.05 \). The height of each bar represents mean value \pm SD.

DISCUSSION

We have recently reported that neonatal exposure to various doses of PBDE 99 can induce persistent changes in spontaneous behavior in adult animals (Eriksson et al., 2001). The present study shows that neonatal exposure to PBDE 99 during the critical period of rapid brain development can affect the cholinergic system by causing the opposite response to the cholinergic agent nicotine, compared to mice neonatally exposed to the 20% fat emulsion vehicle.

The mice treated neonatally with PBDE 99 showed the same nonhabituating behavior at the age of 2 months and 4 months as earlier reported (Eriksson et al., 2001). Habituation, here defined as a decrease in the three behavioral variables, locomotion, rearing, and total activity, in response to the diminished novelty of the test chamber over the 60-min period, was observed in the control animals. The PBDE 99-treated animals, on the other hand, were significantly hypoactive during the first 20 min of the 60-min period (0–20 min), but during the last 20-min period (40–60 min) a hyperactive behavior was evident.

In the nicotine-induced behavior test, the response to nicotine was drastically changed and the PBDE 99-treated animals showed a totally opposite response compared to the vehicle-treated mice. In the control animals, hyperactivity was seen after injection of 80 µg nicotine base, while the neonatally treated mice showed hypoactivity. This response to nicotine, after neonatal exposure to PBDE 99, is the same as earlier seen for animals neonatally exposed to PCB 52 (Eriksson and Fredriksson, 1996b) or nicotine (Eriksson et al., 2000; Nordberg et al., 1991). These animals showed hypoactivity after adult exposure to nicotine, in contrast to animals neonatally exposed to 20% fat emulsion vehicle or saline that showed hyperactivity. In these animals it was also shown that the cholinergic nicotinic receptors were affected. The neonatal exposure on postnatal day 10 coincides with the rapid development of the cholinergic system (Falkeborn et al., 1983; Fiedler et al., 1987) and this indicates that PBDE 99 has an effect on the cholinergic system. Furthermore, it is of special interest to note that the hyperactive condition in PBDE 99-treated mice is again observed at the end of the test period (100–120 min.), indicating the persistence of the adverse spontaneous motor behavior defect.

In conclusion, the present study shows that neonatal exposure to PBDE 99 affects the cholinergic system, seen as changes in the adult response to nicotine exposure. It also reproduces the effects on spontaneous behavior in the adult mouse after neonatal exposure to PBDE 99. These findings show similarities to observations made from neonatal exposure
to PCBs and nicotine, compounds shown to affect cholinergic nicotinic receptors. This indicates that PBDE 99 can affect the cholinergic system and might thereby interact with other environmental toxicants.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to Anna Pettersson for superb technical support. This work was supported by grants from the Foundation for Strategic Environmental Research and the European Commission (QLK4-1999-01562).

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