Prenatal Exposure to a Low Dose of 4-Hydroxy-2′, 3, 3′, 4′, 5′-Pentachlorobiphenyl Increases Emotional Behaviors in Mice

Fumio Soeda, Taku Kaitsuka, Tetsuya Shirasaki, and Kazuo Takahama*

Department of Environmental and Molecular Health Sciences, Graduate School of Pharmaceutical Sciences Kumamoto University, 5–1 Oe-Honmachi, Kumamoto 862–0973, Japan

(Received March 11, 2005; Accepted May 14, 2005; Published online May 20, 2005)

Hydroxylated polychlorinated biphenyl (OH-PCB) is a major metabolite of PCB, which is an endocrine disruptor. In this study, we investigated the neurobehavioral effects of prenatal exposure to a very low dose of OH-PCB (4-hydroxy-2′, 3, 3′, 4′, 5′-pentachlorobiphenyl, 4-OH-pentaCB) in mice. 4-OH-pentaCB, dissolved in corn oil, was orally given at 0.1 μg/30 μl/animal/day to pregnant mice from gestation days 11 to 17. In the open field test, the number of ambulation and rearing drastically increased among 4-OH-pentaCB exposed mice compared with the control. However, 4-OH-pentaCB exposure had no effect on passive avoidance. These results suggest that an extremely low dose of 4-OH-pentaCB may selectively disrupt neurobehavioral functions involved in ambulation and rearing in mice.

Key word —— hydroxylated polychlorinated biphenyl, ambulation, endocrine disruptor

INTRODUCTION

Hydroxylated PCB is a major metabolite of polychlorinated biphenyl (PCB). The level of hydroxylated PCBs (OH-PCBs) in blood has been shown to be 10–20% of the total PCB levels in Canadian Inuit, Latvian and Swedish men.[1,2] Maternal exposure of rats to 5 or 25 mg/kg body weight of PCB mixture (Aroclor 1254) from gestation days 10 to 16 resulted in selective accumulation of OH-PCBs in fetal plasma and the brain.[3]

Perinatal exposure to PCBs, endocrine disruptors, has been reported to produce neurologic impairment in many species, including humans. PCBs cause hyperactivity, stereotypic circling, and decreased motor coordination in mice.[4–7]

Recent studies have shown that endocrine disruptors, such as bisphenol A, have been reported to produce an inverted-U dose-response curve for stimulation of the proliferation of human prostate cancer cells, with maximum stimulation occurring at very low concentrations,[8,9] and no stimulation occurring at high concentrations.

In this study we investigated the neurobehavioral effects of prenatal exposure to a low dose of one of OH-PCBs, 4-hydroxy-2′, 3, 3′, 4′, 5′-pentachlorobiphenyl (4-OH-pentaCB), in mice.

MATERIALS AND METHODS

Animals and Treatments —— Six mated ddY mice (SLC, Shizuoka, Japan) arrived on gestation day (GD) 5 in our laboratory, and were housed individually in cages (340 × 225 × 130 mm) with a bedding of paper chips, in the animal center of the Graduate School of Pharmaceutical Sciences. Throughout the experiment, a 12 : 12-hr light : dark cycle was kept with lights on from 08:00–20:00. The temperature was maintained at 22 ± 2°C. Standard mouse chow and tap water were freely available to the animals. 4-OH-pentaCB, obtained from AccuStandard Chemicals, (New Haven, CT, U.S.A.; purity of 97.7%) was dissolved in corn oil and administered orally at 0.1 μg/30 μl/animal once a day from GD11 to GD17. The control group was given pure corn oil at 30 μl/animal. After weaning on postnatal day (PND) 21, offspring was separated by sex and housed in standard cages until the onset of behavioral testing. Dams were weighed on GD5, 9, 11, 13 and 16. The offspring was weighed on PND 28, 36 and 45.

Open Field Test —— Open field testing was done on 4-week-old offspring and dams. The open field used for measuring ambulation, rearing, defecation, urination, face washing and grooming was a white circle arena with a diameter of 600 mm. The floor was equally divided into 19 segments. It was illuminated by an incandescent lamp (100 W) hanging 800 mm above the floor. The total session duration

*To whom correspondence should be addressed: Department of Environmental and Molecular Health Sciences, Graduate School of Pharmaceutical Sciences Kumamoto University, 5–1 Oe-Honmachi, Kumamoto 862–0973, Japan. Tel. & Fax: +81-96-371-4334; E-mail: takahama@gpo.kumamoto-u.ac.jp

was 5 min, subdivided into five 1-min intervals for analysis. The occurrences of rearing, defecation, urination, face washing, and grooming in each session were counted. Ambulation was determined according to the number of segments crossed on the floor. Thirty minutes after the first session, the second session was performed. This test was carried out during the light phase of the cycle.

Passive Avoidance Test —— Passive avoidance testing was done on 6–7-week-old offspring and dams. The apparatus consisted of two compartments: one was a light chamber ($320 \times 320 \times 270$ mm high), and another one a dark chamber ($155 \times 96 \times 180$ mm high) with a grid floor. As a training session, the mice were gently placed in the light chamber and allowed to escape into the dark chamber through the slit door, and the latency time to enter the dark chamber was measured. Then, they received a 150-volt foot shock for 5 sec and were withdrawn from the chamber. At 24 hr after the training session, as a testing session, the animals were again placed in the same chamber and the latency time required for entry into the dark chamber was measured. As a cut-off time for latency in a testing session, 600 sec was used. This test was carried out during the light phase of the cycle.

Data Analysis —— Data were analyzed using unpaired *t*-tests to assess the statistical significance between control and 4-OH-pentaCB data. $p < 0.05$ was considered statistically significant.

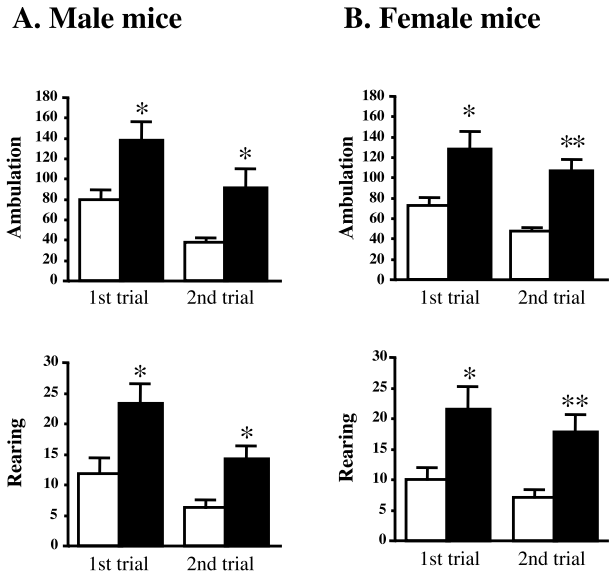

Fig. 1. Effect of Prenatal 4-OH-pentaCB Exposure on Ambulation and Rearing in Offspring

Data show the means (± S.E.M.) of the number of ambulation (top panel) and rearing (bottom panel) in offspring. The number of ambulation and rearing was significantly increased by 4-OH-pentaCB exposure. The 2nd trial was carried out 30 min after the 1st trial. A. □: control ($n = 14$), ■: 4-OH-pentaCB ($n = 14$). B. □: control ($n = 18$), ■: 4-OH-pentaCB ($n = 17$), $*p < 0.05$, $**p < 0.01$.

RESULTS

Reproductive and Developmental Outcomes

The dose of 4-OH-pentaCB used in this study did not affect the gestational weight gain of the dams. Other reproductive and developmental parameters including gestation length, litter size, percentage of live births, birth weight and postnatal growth were also unaffected. There were no effects of 4-OH-pentaCB exposure on the weight gain and footstep of offspring. Further, 4-OH-pentaCB-exposed dams and their offspring performed normally in traction tests and showed a normal pain response to a tail pinch.

Open Field Activity

In 4-OH-pentaCB-exposed male and female offspring, the number of ambulation and rearing increased remarkably in the first and the second trials (Fig. 1). However, other parameters such as defecation, urination, face washing, and grooming were not affected, expect for an increase in defecation and a decrease in face washing in female offspring in the first trial. Dams exposed to 4-OH-pentaCB were also subjected to neurobehavioral examination. No neurobehavioral parameters were affected by the exposure.

Passive Avoidance Learning

4-OH-pentaCB exposure had no effect on passive avoidance response either in male or female of offspring.

DISCUSSION

This is the first documentation showing that prenatal exposure to a very low dose of 4-OH-pentaCB causes remarkable neurobehavioral effects in mice, shown as increases in the number of ambulation and rearing in the open field. The effects were relatively selective, because they were not accompanied by changes in footstep, grip strength and pain response. Further, reproductive and developmental data of the dams and their offspring showed no effects from 4-OH-pentaCB exposure, indicating that 4-OH-

pentaCB was not toxic to the reproductive function and development of the mice.

Although it is well known that both PCBs and their hydroxylated metabolites disrupt thyroid hormone homeostasis by interacting with TTR, it has also been reported that prenatal and perinatal exposure to PCBs produce neurobehavioral effects similar to those observed in the present study. In these reports, however, the selectivity of the effects seems to be lower compared with that of the present study. In about one-half of CD-1 mice exposed to 3,4,3′,4′-tetrachlorobiphenyl (4-CB) at a dose of 32 mg/kg from GD10 to 16, a neurological syndrome consisting of increased locomotor activity occurred.[6] But body weight gain in the offspring with the neurological syndrome was significantly depressed. Further, the offspring of mice exposed to the same dose of 4-CB during gestation also revealed a neurological syndrome consisting of intermittent stereotypic circling and hyperactivity. In addition, the offspring showed other symptoms such as impaired forelimb grip strength, inability to traverse a wire rod, and so forth. Similar effects have been reported in offspring treated with Aroclor 1254, a mixture of PCBs.[3]

In the present study, 4-OH-pentaCB exposure did not impair passive avoidance responses in male and female offspring, although a disruption in another avoidance response has been observed in mice exposed *in utero* to high doses of PCBs.[5,6] This difference seems to come from differences in exposure doses and experimental periods. Previously, we reported that prenatal exposure to diethylstilbestrol (DES) impaired the passive avoidance responses of mice.[10] However, we found that motor and emotional functions were not so disrupted by DES exposure (unpublished data). The present results indicate that the brain disrupting action of OH-PCB, unlike that of DES, occurs on motor and/or emotional functions rather than on learning or memory functions.

The mechanisms underlying the increase in the occurrences of ambulation and rearing are still unknown. The following discussion, however, may have some merit. Among various substances known to change ambulation in mice, methamphetamine is one of the most potent. Direct administration of methamphetamine into the nucleus accumbens possessing dopaminergic neurons causes a prominent increase in the number of ambulation.[11] Further, it has been known that methamphetamine decreased levels of dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) in the striatum.[12,13] Therefore, it seems possible that methamphetamine-induced increase in ambulation might be caused by a decrease in DAT and VMAT2. PCBs also decrease levels of DAT and VMAT2, causing a reduction in striatal dopamine.[14] Further studies are needed on whether 4-OH-pentaCB affects the dopamine system, including DAT and VMAT2 to cause an increase in ambulation.

A recent *in vitro* study revealed that 4-OH-pentaCB at 10^{-10} M suppressed thyroid hormone receptor (TR)-mediated transcriptional activation by T_3 (10^{-7} M) on DR-4 (TRE)-Luc in CV-1 cells.[15] Furthermore, Umezu *et al*. reported that the perinatal administration of propylthiouracil, a thyroid hormone synthesis inhibitor, to pregnant ICR mice caused an increase in ambulation in matured male offspring.[16] This being the case, it seems reasonable that 4-OH-pentaCB may suppress TR-mediated transcription and consequently increase the number of ambulation in mice. Apart from the above, Kester *et al*. demonstrated that various OH-PCBs, including 4-OH-pentaCB, were extremely potent inhibitors of human estrogen sulfotransferases.[17] In addition, some OH-PCBs competitively bind to estrogen receptors and exhibit estrogenic activity in the mouse uterus.[18] Further studies are undoubtedly needed to elucidate mechanisms of brain disrupting actions caused by prenatal exposure to a minute amount of 4-OH-pentaCB. Nevertheless, the present findings reveal that prenatal exposure to an extremely low dose of 4-OH-pentaCB may selectively disrupt neurobehavioral functions involved in ambulation and rearing in mice, raising the possibility that changes in ambulation and rearing in the open field may be a very sensitive marker for assessing the brain disrupting actions of environmental chemicals such as PCBs and their derivatives.

Acknowledgements This work was supported by the Ministry of Education, Culture, Sports, Science and Technology, Grant-in-Aid for Young Scientists (B) and Grant-in-Aid for Scientific Research on Priority Areas (A).

REFERENCES

1) Sandau, C. D., Ayotte, P., Dewailly, E., Duffe, J. and Norstrom, R. J. (2000) Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compounds in whole blood from Canadian inuit. *Environ. Health Perspect*., **108**, 611–616.

2) Sjodin, A., Hagmar, L., Klasson-Wehler, E., Bjork, J. and Bergman, A. (2000) Influence of the consumption of fatty Baltic Sea fish on plasma levels of halogenated environmental contaminants in Latvian and Swedish men. *Environ. Health Perspect*., **108**, 1035–1041.
3) Morse, D. C., Wehler, E. K., Wesseling, W., Koeman, J. H. and Brouwer, A. (1996) Alterations in rat brain thyroid hormone status following pre- and postnatal exposure to polychlorinated biphenyls (Aroclor 1254). *Toxicol. Appl. Pharmacol*., **136**, 269–279.
4) Chou, S. M., Miike, T., Payne, W. M. and Davis, G. J. (1979) Neuropathology of "spinning syndrome" induced by prenatal intoxication with a PCB in mice. *Ann. N.Y. Acad. Sci*., **320**, 373–395.
5) Storm, J. E., Hart, J. L. and Smith, R. F. (1981) Behavior of mice after pre- and postnatal exposure to Arochlor 1254. *Neurobehav. Toxicol. Teratol*., **3**, 5–9.
6) Tilson, H. A., Davis, G. J., McLachlan, J. A. and Lucier, G. W. (1979) The effects of polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. *Environ. Res*., **18**, 466–474.
7) Tilson, H. A., Jacobson, J. L. and Rogan, W. J. (1990) Polychlorinated biphenyls and the developing nervous system: cross-species comparisons. *Neurotoxicol. Teratol*., **12**, 239–248.
8) Nagel, S. C., vom Saal, F. S., Thayer, K. A., Dhar, M. G., Boechler, M. and Welshons, W. V. (1997) Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environ. Health Perspect*., **105**, 70–76.
9) Welshons, W. V., Nagel, S. C., Thayer, K. A., Judy, B. M. and vom Saal, F. S. (1999) Low-dose bioactivity of xenoestrogens in animals: fetal exposure to low doses of methoxychlor and other xenoestrogens increases adult prostate size in mice. *Toxicol. Ind. Health*, **15**, 12–25.
10) Takahama, K., Kaitsuka, T., Shirasaki, T. and Soeda, F. (2003) Brain disrupting actions of prenatal diethylstilbestrol exposure in mice-behavioral, neurochemical and electrophysiological studies. *J. Health Sci*., **49**, 423–428.
11) West, C. H., Boss-Williams, K. A. and Weiss, J. M. (1999) Motor activation by amphetamine infusion into nucleus accumbens core and shell subregions of rats differentially sensitive to dopaminergic drugs. *Behav. Brain Res*., **98**, 155–165.
12) Fleckenstein, A. E., Metzger, R. R., Wilkins, D. G., Gibb, J. W. and Hanson, G. R. (1997) Rapid and reversible effects of methamphetamine on dopamine transporters. *J. Pharmacol. Exp. Ther*., **282**, 834–838.
13) Brown, J. M., Hanson, G. R. and Fleckenstein, A. E. (2001) Regulation of the vesicular monoamine transporter-2: a novel mechanism for cocaine and other psychostimulants. *J. Pharmacol. Exp. Ther*., **296**, 762–767.
14) Richardson, J. R. and Miller, G. W. (2004) Acute exposure to aroclor 1016 or 1260 differentially affects dopamine transporter and vesicular monoamine transporter 2 levels. *Toxicol. Lett*., **148**, 29–40.
15) Iwasaki, T., Miyazaki, W., Takeshita, A., Kuroda, Y. and Koibuchi, N. (2002) Polychlorinated biphenyls suppress thyroid hormone-induced transactivation. *Biochem. Biophys. Res. Commun*., **299**, 384–388.
16) Umezu, T. and Morita, M. (2001) Effects of endocrine disruptors on Central Nervous System (1): Effects of prenatal and neonatal shortage of thyroid hormone on behaviors in mice. In *the 4th Annual Meeting of Japan Society of Endocrine Disrupters Research Abstracts*, Ibaraki, Japan, p. 402.
17) Kester, M. H., Bulduk, S., Tibboel, D., Meinl, W., Glatt, H., Falany, C. N., Coughtrie, M. W., Bergman, A., Safe, S. H., Kuiper, G. G., Schuur, A. G., Brouwer, A. and Visser, T. J. (2000) Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology*., **141**, 1897–1900.
18) Korach, K. S., Sarver, P., Chae, K., McLachlan, J. A. and McKinney, J. D. (1988) Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes. *Mol. Pharmacol*., **33**, 120–126.