Prenatal Exposure to 3,3',4,4',5-Pentachlorobiphenyl (PCB126) Promotes Anxiogenic Behavior in Rats

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ORITO, K., GOTANDA, N., MURAKAMI, M., IKEDA, T., EGASHIRA, N., MISHIMA, K. and FUJIWARA, M. *Prenatal Exposure to 3,3',4,4',5-Pentachlorobiphenyl (PCB126) Promotes Anxiogenic Behavior in Rats*. Tohoku J. Exp. Med., 2007, **212** (2), 151-157 — Polychlorinated biphenyls (PCBs) are environmental contaminants that have adverse effects on the endocrine and nervous systems. As they are still detected in breast milk and adipose tissue in humans, the accumulated PCBs may transfer from mothers to children and damage central nervous system. It is revealed from epidemiological studies that cognitive and motor functions were damaged in children born to mothers who ingested PCBs-contaminated foods. However, it remains unclear whether prenatal exposure to PCBs affects emotionality. In the present study, we therefore examined the effect of prenatal exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB126) on emotionality in rats by focusing on anxiogenic behavior and response of the hypothalamus-pituitary-adrenal axis to stress. Pregnant rats were treated orally with PCB126 at a dose of 30 μ g/kg or corn oil, its vehicle, on gestational day 15, and their male offspring were subjected to the following experiments at 4-5 weeks old. In an open field test, rats with prenatal exposure to PCB126 showed anxiogenic behavioral responses, including decrease in time spent in the center of an open field and the number of rearings and extension of grooming duration. Interactive behavior, which is an index of anxiety level, was shortened in the social interaction test. The increase in the serum corticosterone level induced by forced swim stress was facilitated by prenatal exposure to PCB126. This evidence suggests that PCB126 may exert anxiogenicity on the offspring of exposed dams, and dysfunction of the hypothalamus-pituitary-adrenal axis may at least in part contribute to this abnormality. ——— 3,3',4,4',5-pentachlorobiphenyl (PCB126); prenatal exposure; anxiogenic; corticosterone

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Polychlorinated biphenyls (PCBs) are widespread persistent environmental contaminants. Because of their stability and lipophilic proper-

ties, PCBs are accumulated by biological magnification; indeed, residues of PCBs have been detected in humans, fish, and wildlife (Kalantzi et

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al. 2004; Carlson and Hites 2005; Cok et al. 2007). Epidemiological studies revealed that perinatal exposure to PCBs exerted a developmental neurotoxicologic effect on humans (Nakai and Satoh 2002; Schantz et al. 2003). In Taiwan, pregnant mothers were accidentally exposed to PCBs through the ingestion of contaminated rice oil and neurodevelopmental abnormalities were observed in their offspring (Chen et al. 1992). Children born to mothers who ingested PCB-contaminated fish also exhibited neurological impairment (Jacobson and Jacobson 1996). This evidence confirmed the long-term impact of PCBs on cognitive deficits and intellectual dysfunction. In experimental studies, the effects of PCBs on learning and memory, and the mechanisms of dysfunctions have been reported (Gilbert and Crofton 1999; Faroon et al. 2001); however, the effect of prenatal exposure to PCB with special reference to the impact on emotionality has not been explored in-depth to date.

3,3',4,4',5-pentachlorobiphenyl (PCB126), benzene rings of non-*ortho*-substituted PCB, may assume a planar configuration and have been suggested to be dioxin-like, the most toxic PCB congener (Safe 1990). PCB126 increased adrenocorticotrophic hormone in pituitary cells that stimulate the secretion of corticosterone from the adrenal cortex (Bestervelt et al. 1998). PCB126 raised cortisol levels in human adrenocortical cells (Li and Wang 2005). Serum corticosterone was increased by exposure to Aroclor 1254, a PCB mixture which contains PCB126 (Kodavanti et al. 2001), in mice and rats (Sanders et al. 1974; Miller et al. 1993). Thus, PCB126 elevates serum corticosterone/cortisol levels in both direct and indirect manners. Recently, Zagron and Weinstock (2006) revealed that high-level maternal corticosterone was responsible for the anxiogenic properties of offspring. From this evidence, we hypothesized that maternal exposure to PCB126 would cause emotional disturbance in offspring.

In the present study, rats were exposed to PCB126 during pregnancy, and the emotionality of their offspring was evaluated in terms of anxiogenicity. Moreover, the effect of prenatal exposure

to PCB126 on the HPA axis was examined by measuring serum corticosterone levels before and after forced swim stress. Prenatal exposure to PCBs possibly exerts motor dysfunction (Chen et al. 1985; Nguon et al. 2005); thus, the effect of prenatal exposure to PCB126 on motor coordination was also examined.

MATERIALS AND METHODS

Subjects

Twenty 10-week-old female and ten 12-week-old male Sprague-Dawley rats were purchased from SLC, Japan and acclimated for 1 week prior to the start of the experiment. Rats were housed in a cage at $23 \pm 2^\circ\text{C}$, $60 \pm 10\%$ humidity, and with lights on daily from 7:00 a.m. to 7:00 p.m. in a controlled room, and received laboratory chow and water *ad libitum*. Two female rats were cohabited overnight with a male rat. Females with sperm in their vaginal smears the next morning were regarded as pregnant and the day was designated as gestation day 0. Our preliminary study revealed that prenatal oral exposure to $100 \mu\text{g/kg}$ of PCB126 on gestational day 15 reduced neonatal body weight at birth. On the other hand, $30 \mu\text{g/kg}$ of PCB126 had no effect ($6.9 \pm 0.1 \text{ g}$ [$n = 6$] vs $7.2 \pm 0.3 \text{ g}$ [$n = 6$], normal vs $30 \mu\text{g/kg}$ of PCB126, $p = 0.3778$). Thus, ten pregnant rats were treated orally with PCB126 at a dose of $30 \mu\text{g/kg}$ and the other ten pregnant rats with corn oil, its vehicle, on gestational day 15. All newborns were weighed on postnatal day 1 and each litter was reduced randomly to four males and four females. Only three females were born to one dam that exposed to corn oil although number of male offspring of the dam was nine. In this case, extra one male nursling of the litter was added so that the total number was eight. All female offspring were discarded at weaning. Male offspring were subjected to the present study at 4-5 weeks old. One animal from each litter was used for each behavioral test. Each animal experienced only one behavioral test. Thirty-six male offspring of rats with prenatal exposure to PCB126 and 36 male offspring with prenatal exposure to corn oil were used in the present study. The care and handling of the animals were in accordance with the "Azabu University Animal Experiment Guidelines; April 2000".

Rotating rod test

It was examined whether motor coordination of PCB126 rats was impaired using the rotating rod test. Rats were placed on a rotating rod (10 cm diameter,

Natsume, Tokyo) which was rotated at 3 rev./min for 10 min to habituate them to the apparatus. The next day, the rats were again placed on the rotating rod and the speed was increased in the order of 3, 5, 8, 10, and 15 rev./min every 1 min. The number of rats that successfully walked at each speed was recorded.

Locomotor activity and anxiety-related behavior in an open field

Locomotor activity was measured in a black wooden box (45 × 45 × 40 cm depth). The apparatus was located under concealed lighting which gave low luminance (55 lx). A rat was placed in the center of the box and left for 15 min. Its behavior was recorded by a digital video camera set above. The number of rearings and duration of grooming behavior, which are indices of anxiety (De Souza Spinosa et al. 1999; Moreira et al. 2000; Sonavane et al. 2002; Kalueff and Tuohimaa 2005), were measured by an observer blinded to the rats' history. The distance moved horizontally and the percent time spent in the 25 cm square in the center were measured using a computer-aided behavior analysis system (SMART, Bio Research Center, Aichi). The apparatus was cleaned using ethanol prior to each experiment.

Social interaction

Anxiogenic responses were measured utilizing a modified social interaction test (Sajdyk et al. 2002). A rat with prenatal exposure to either corn oil or PCB126 was put into an arena for the social interaction test (45 × 45 × 40 cm depth) with a novel partner rat of the same sex and of similar weight. Their behavior was video-recorded for 5 min with a digital video camera above. The time spent in social interaction (sniffing, following, grooming the partner, and wrestling) of the test animal provided a measure of anxiety. These measurements were performed by an observer blind to the rats' history.

Serum corticosterone level under non-stressful conditions and after forced swim stress

A Plexiglas cylinder (20 × 50 cm, diameter × height) filled with water (25°C) 30 cm in depth was used as a swim stress device. Each rat was put into the cylinder for 15 min and trunk blood was collected in a glass tube by decapitation immediately after forced swim stress. Rats were quickly removed from their home cage and trunk blood was collected as a non-stressful control. All blood collections were performed between 14:00–15:00. Serum corticosterone levels of trunk blood were measured using an RIA kit (Amersham, UK).

Statistical analyses

Results are expressed as the means ± S.E.M. or means + S.E.M. The effect of prenatal exposure to PCB126 was analyzed with unpaired *t*-test for body weight, locomotor activity and anxiety-related behavior in an open field, social interaction, and serum corticosterone level, and Fisher's exact probability test for the incidence of the rota-rod test. A *p* value < 0.05 was considered significant.

RESULTS

Body weight

The body weights of male rats with prenatal exposure to corn oil and PCB126 on postnatal day 1 were 7.0 ± 0.1 g and 7.0 ± 0.1 g, respectively, with no statistical significance between the two groups.

Rotating rod test

The effect of prenatal exposure to PCB126 on motor coordination was examined using a rotating rod. At 3, 5, and 8 rpm, all rats accomplished the walking task (Table 1). At 10 and 15 rpm, 2–4 rats failed to accomplish the walking

TABLE 1. Effect of prenatal exposure to PCB126 (30 µg/kg, PO) or Corn oil on motor coordination in rotating rod test.

	Revolution (rpm)				
	3	5	8	10	15
Corn oil (<i>n</i> = 8)	8/8	8/8	8/8	6/8	4/8
PCB126 (<i>n</i> = 8)	8/8	8/8	8/8	8/8	5/8

Number of rats walking on the rotating rod for 1 min/Total number of rats is shown in the table.

task (Table 1); however, there was no difference between the two groups.

Locomotor activity and anxiety-related behavior in an open field

Total horizontal distance moved during 15

Fig. 1. Effect of prenatal exposure to PCB126 (30 $\mu\text{g/kg}$, PO) on behavioral parameters. Rats with prenatal exposure to corn oil or PCB126 were put into an open field for 15 min and locomotor activity (A), % time spent in the central area (B), number of rearings (C), and grooming duration (D) were measured. Data are presented as the mean + S.E.M. of 10 animals.

N.S., not significant. * $p < 0.05$ vs corn oil, unpaired t -test.

min in an open field was not affected by prenatal exposure to PCB126 (Fig. 1A). On the other hand, time spent in the center of the open field and the number of rearings fell (Fig. 1B and 1C), and total grooming duration was extended (Fig. 1D).

Social interaction

Time spent in social interaction during the 5 min test period was 19.9 ± 2.6 s in rats with prenatal exposure to PCB126. This time was significantly shorter than that with prenatal exposure to corn oil, 32.5 ± 2.9 s (Fig. 2)

Fig. 2. Effect of prenatal exposure to PCB126 (30 $\mu\text{g/kg}$, PO) on social interaction. Rats with prenatal exposure to corn oil or PCB126 were put together with a novel partner rat and the time spent in social interaction (sniffing, following, grooming the partner, and wrestling) was measured. Data are presented as the mean + S.E.M. of 9 animals.

* $p < 0.05$ vs corn oil, unpaired t -test.

Fig. 3. Serum corticosterone level under non-stressful conditions (A) and after forced swimming (B) in rats with prenatal exposure to corn oil or PCB126. Data are presented as the mean + S.E.M. of 4 (A) and 5 (B) animals.

N.S., not significant. * $p < 0.05$ vs corn oil, unpaired t -test.

Serum corticosterone level under non-stressful conditions and after forced swim stress

The serum corticosterone levels under non-stressful conditions were not affected by prenatal exposure to PCB126 (Fig. 3A); however, the increase in the serum corticosterone level induced by forced swim stress was significant (Fig. 3B).

DISCUSSION

The present study revealed that open field performance was greatly influenced by prenatal exposure to PCB126. In rodents, grooming is particularly sensitive to stress and exogenous manipulation, and generally facilitated under stressful situations (Moyaho and Valencia 2002). In contrast, rearing, an exploratory behavior, is decreased under anxiogenic conditions as anxiogenic agents exerted a decrease in the number of rearings in an open field (De Souza Spinosa et al. 1999; Sonavane et al. 2002). Together with the evidence of decreased time spent in the center of the open field and decreased social interaction, which is a representative index of anxiety (Sajdyk et al. 2002), it is suggested that rats with prenatal exposure to PCB126 are sensitive to stress and have a propensity to develop anxiety.

Maternal stress exerted emotional disturbance, including anxiogenic behavior, through modification of the feedback mechanism of the offspring HPA (Henry et al. 1994; Patin et al. 2005). As the anxiogenic behavior of prenatally-stressed rats was abolished by maternal adrenalectomy, excess maternal corticosterone may cause this emotional disturbance (Zagron and Weinstock 2006). Literature data show that PCB has a property to elevate serum corticosterone when administered orally (De Krey et al. 1993; Miller et al. 1993), and the data of the present study show that prenatal exposure to PCB causes a disturbance of HPA response. It is conceivable from this evidence that the underlying mechanisms of the emotional disturbance induced by prenatal stress and PCB exposure may be common to both. Thus, dysfunction of the HPA axis may, at least in part, contribute to the emotional dysfunction of rats with prenatal exposure to PCB126. As hippocampal corticoid receptors decreased in prena-

tally-stressed rats (Henry et al. 1994), this mechanism may be involved in the dysfunction of HPA induced by prenatal exposure to PCB126. Further studies are necessary to prove this hypothesis.

To stay on the rotating rod, the rats required complex motor skills, including motor coordination and precise postural control. Nevertheless, prenatal PCB126 did not affect their performance, even at the highest rotation speed. Together with evidence that locomotor activity in an open field was not different in the two groups, prenatal PCB126 at the dose examined may not exert motor dysfunction, at least at the age subjected to the experiment. In previous studies, however, maternal exposure to PCB elicited motor dysfunction in the rotating rod test (Nguon et al. 2005) and swimming test (Pantaleoni et al. 1988). The former and latter studies administered Aroclor 1254 and Fenclor 42, respectively, both of which are commercial PCB mixtures. The dosing period was gestation day 11 through postnatal day 21, and gestation day 6 through 15, respectively. We administered PCB126 once on gestation day 15. The different types of PCB and dosing period may have caused the different effects on motor function.

The concentration of PCB126 in adipose tissue of Japanese was 120–730 pg/g (Kannan et al. 1989). When PCB126 was administered orally to rats at a daily dose of 10 μ g/kg for 13 weeks, the concentration in adipose tissue was 645 ng/g (Van Birgelen et al. 1994). Thus, the amount of PCB126 in the adipose tissue of the present study is estimated to be about 1,000 times higher than that in humans. As the toxic effect is dependent on the dose, it is doubtful that PCB affects the central nervous system in humans. Indeed, there is little evidence available about the anxiogenic effect of prenatal exposure to PCB in humans; however, etiological study revealed that chronic inhalation of low chlorinated PCBs in school buildings was associated with increased emotional complaints in humans (Peper et al. 2005); therefore, we cannot reject the possible effect of prenatal exposure to PCB126 on emotionality. Besides the toxicity of PCBs, additive or synergic neurodevelopmental effects of prenatal exposure

to PCBs and MeHg, which is also an environmental contaminant, have been evidenced in terms of motor dysfunction (Roegge and Schantz 2006) and toxicity to pituitary cells (Johansson et al. 2006). A cohort study of child development from the effects of perinatal methylmercury and environmental pollutants (Nakai et al. 2004) may uncover the intrinsic effect of prenatal exposure to PCB.

In summary, prenatal exposure to PCB126 decreased rearing and time spent in a central area, increased grooming in an open field, and decreased social interaction, although it did not affect total locomotor activity. This evidence suggests that rats with prenatal exposure to PCB126 are vulnerable to stress. As the serum corticosterone level increased in stressed rats with prenatal PCB126, dysfunction of the HPA axis may be one of the causes of anxiogenic properties.

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