Alteration of Brain Neurotransmitters in Female Rat Offspring Induced by Prenatal Administration of 16 and 64 mg/kg of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153)

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Abstract: PCB153 (2,2',4,4',5,5'-hexachlorobiphenyl), a non-coplanar PCB and the congener most widely distributed in the environment, was orally administered to pregnant Sprague-Dawley (Crj: CD (SD) IGS) rats from gestation day 10 through 16 at doses of 0 (control), 16 and 64 mg/kg body weight. Female pups were sacrificed at 1, 3, 6, and 9 wk, and at 1 yr of age to evaluate the differences in brain neurotransmitters and their metabolites between PCB153-exposed and control groups. Brain levels of norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5HT), 5-hydroxyindoleacetic acid (5HIAA), acetylcholine (ACh), and choline (Ch) in discrete brain regions or in whole brain were measured. At 1 to 3 wk after birth, brain levels of DA, DOPAC, HVA, 5HT and 5HIAA in PCB-exposed groups were higher than those of the control group. At 9 wk after birth, DA turnover was reduced in half of the four brain areas examined (forebrain and hindbrain), and 5HIAA levels were increased in all brain areas in the PCB-treated group compared to those of the control group. At 1 yr after birth, the levels of DA, DOPAC, and HVA in the hippocampus, hypothalamus, and medulla oblongata were lower in the PCB-exposed groups than in the control group. Prenatal exposure to PCB153 stimulated the turnover of 5HT neurons in the brain of female offspring at early stages (1 to 9 wk) of development. On the other hand, the turnover of DA neurons in the PCB-exposed groups was reduced in late stages (9 wk to 1 yr) of development compared with that of the control group. The brain neurotransmitters of dams treated with PCB were assayed at 3 wk after delivery (15 wk old), and decreases in DA, DOPAC, and HVA were observed. PCB153 reduced the activity of DA neurons in the brain of dams. These results are discussed in relation to health effects observed in humans exposed to PCBs.

Key words: PCB153, Prenatal exposure, IGS rat, Offspring, Brain, Neurotransmitters, Norepinephrine, Dopamine, Serotonin, Acetylcholine

Introduction

In 1968, many Japanese people were intoxicated with polychlorinated biphenyls (PCBs). This was the first inci-

dence in which the neurotoxicity of PCBs (Yusho) was recognized. Similar intoxication cases occurred later in Taiwan (YuCheng). PCBs are suspected of possessing endocrine disrupting activity^{1–3}, and when administered to pregnant rats, may cause the retardation of development in offspring through the disruption of thyroid hor-

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mone^{4,5}). According to Wang *et al.*⁶), a decrease in thyroid hormone in humans was related to the accumulation of PCBs. In addition, epidemiological studies have indicated relationships between prenatal exposure to PCBs and deficits in cognitive function in children^{7,8}). These reports have suggested that PCB administration to pregnant females may cause disruption of nerve function in newborns, children, or even adults. In order to investigate this possibility, we examined changes in brain substances of offspring after PCB administration to pregnant rats.

There are 209 congeners of PCBs that are different in structure and the position of chlorination. Among these, twelve co-planar PCBs resemble dioxins in structure, and are considered to be more toxic than the other congeners, such as non-co-planar PCBs. 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB153) is a non-co-planar PCB, and is the congener most widely distributed in the environment. According to Chou *et al.*⁹), PCB153 is the most abundant congener in all samples from the Taiwan coast. PCB153 consistently comprised between 20% to 30% of 19 PCB congeners examined (SigmaPCBs). There are studies regarding the toxicity of PCB153¹⁰), however the data of prenatal administration effects are insufficient. In the study of Oskam *et al.*¹⁰), female goats were administered PCB153 at 98 µg/kg body weight per day, from gestational day 60 until delivery. Offspring received PCB via lactation until postnatal day (PND) 40. PCB153-exposed male offspring showed a smaller diameter testis compared with that of the control group. Plasma luteinizing hormone (LH) and testosterone levels of PCB153-exposed offspring differed from controls. Damage to sperm DNA was greater in the PCB153-exposed group than controls, and it was concluded that PCB153 induced alterations in the reproductive system of offspring.

In the present study, we investigated the effects of PCB administration to pregnant rats on the cerebral neurotransmitters and related substances of offspring. Some brain functions are closely related to the activity of specific neurons. For example, dopamine (DA) and serotonin (5HT) neurons are involved in the control of cognitive function and mood, respectively^{11,12}), and it is of interest to investigate the effects of prenatal administration of PCB153 to pregnant rats on the cerebral neurotransmitters and related substances of offspring. Accordingly, we investigated changes in norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG, a NE metabolite), DA, 3,4-dihydroxyphenylacetic acid (DOPAC, a DA metabolite), homovanillic acid (HVA, a DA metabolite), 5HT, 5-hydroxyindoleacetic acid (5HIAA, a 5HT metabolite), acetylcholine (ACh), and choline (Ch, an ACh precursor and metabolite) in discrete brain regions of female offspring. Measurements were

performed at 1, 3, 6, and 9 wk, and 1 yr after birth. Analyses of PCB effects were performed in both of male and female offspring, however, the data from female offspring only are reported in the present paper because the quantity of data on the nervous system is very large. This paper deals with a portion of a comprehensive study of PCB153 effects including neurochemistry, laboratory animal ethology, and endocrinology. Similar experiments were performed previously using bisphenol A in our laboratory, and those experiments have been published in part^{13,14}).

Materials and Methods

Animals and chemicals

All experiments were conducted under the "Institutional guidelines for animal care and use" of the National Institute of Occupational Safety and Health (Japan). Thirty pregnant 9-wk-old rats of the Crj: CD (SD) IGS strain were used. These rats were purchased from Charles River Japan Inc. on gestation day (GD) 3; GD 0 was confirmed by the presence of a copulatory plug. They were individually housed under a 12/12 h light/dark cycle with lights on at 0800 h, and with free access to sterilized feed (CE-2, Japan Clea, Inc.) and sterilized water. Room temperature and humidity were maintained at 23 ± 1°C and 55 ± 5%, respectively. Three groups of 10 pregnant rats were given standard PCB153 (> 99.6% pure; AccuStandard, Inc., USA), dissolved in corn oil (4 ml/kg) at 0 (control), 16, or 64 mg/kg body weight (BW).

Administration of PCB153 to pregnant rats

PCB153 was administered to rats by oral gavage between 0830 and 0930 h from GD 10 through 16 after an acclimatization period of 7 d. Control rats were administered corn oil without the addition of PCB153 in a similar manner. The day of birth by 1000 h was considered to be PND 0. The litter size was standardized to 10 pups (5:5 for male: female ratio, when possible) per dam on PND 7. Pups were sacrificed at 1, 3, 6, and 9 wk, and 1 yr of age between 1300 and 1600 h. Five to eight female pups were sacrificed from each PCB153 dose group at 3, 6, and 9 wk and 1 yr of age. Pups chosen for sacrifice in each of the PCB153 dose groups were culled from different dams. Because of the imbalance of male and female numbers and different pup numbers among dams, some pups remained after the standardization of litter size on PND 7. These pups were used for the analysis at 1 wk of age, and the numbers of female pups sacrificed in each dose group at 1 wk of age ranged from 9 to 14.

Offspring were weaned on PND 21, and males and females were separately housed. The highest dose of

PCB153, 64 mg/kg body weight, was selected on the basis of the study by Ness *et al.*⁵⁾, who observed a decline of serum thyroxine (T4) in offspring after maternal administration of PCB153 at doses of 16 and 64 mg/kg/day from GD10 through 16.

Extraction and measurement of brain substances

In order to measure the brain neurotransmitters, pups were sacrificed and brains were treated as described in our previous paper¹³⁾. Dams were sacrificed on the day of the weaning of pups (3 wk after delivery; 15 wk of age). The 1-wk-old pups were sacrificed by decapitation under ice-cold conditions. Dams and their 3- and 9-wk-old pups were sacrificed by exsanguation from an abdominal vein under ether anesthesia and decapitation. At 6 wk and 1 yr after birth, the pups were sacrificed by microwave exposure (5.0 KW, 0.9 sec) focused on the head (Microwave applicator, Muromachi Kikai Co., Tokyo)^{15, 16)}. The extracted brains were dissected into small brain regions. In 1-wk-old pups, brain size was too small to divide exactly into individual brain regions, therefore, whole brain was used for homogenization. The brains of 3- and 9-wk-old pups were dissected into 4 brain areas (forebrain, hindbrain, medulla oblongata, and cerebellum), as described previously¹³⁾. The brains of dams and 6-wk- and 1-yr-old pups were dissected into 8 brain regions (frontal cortex, occipital cortex, hippocampus, midbrain, striatum, hypothalamus, medulla oblongata, and cerebellum), as described by Glowinski and Iversen^{17, 18)}. The ACh and Ch contents could not be measured in offspring at 1, 3, 9 wk after birth and dams because the use of microwaves must be avoided in sacrifices performed to obtain organs other than the brain. All brain samples were stored at -80°C . The procedure of extraction of brain substances was described previously¹³⁾.

High-performance liquid chromatography (HPLC) analysis

Monoamines and metabolites were extracted from the brain homogenates by centrifugation. Supernatants were divided into two portions (Portions A and B). Portion A was used in HPLC to quantitate ACh and Ch according to the method previously described^{16, 19, 20)}. Portion B was injected to HPLC equipped with a CoulArray[®] detector (ESA Inc., USA) after passing through a filter of 0.45- μm pore size, and NE, MHPG, DA, DOPAC, HVA, 5HT, and 5HIAA were analyzed. These monoamines and metabolites were separated by reverse phase ion pair chromatography (MCM Column, 4.6 mm I.D. $\times$ 150 mm, MC Medical, INC., Japan) using a mobile phase comprising 0.1 M phosphate-0.05 M citrate buffer, pH 3.0. This buffer contained sodium 1-heptanesulfonate, EDTA $\cdot$ Na₂ $\cdot$ H₂O, acetonitrile and methanol. The column temperature was maintained at 23°C in an oven, and

the flow rate was 1.1 ml/min. The voltages for CoulArray[®] detection were 100, 200, 300 and 400 mV.

Statistics

Means $\pm$ SEM of each group were calculated for each amine or metabolite analyzed (nmoles/g tissue). Metabolite to monoamine ratios (MHPG/NE, DOPAC/DA, HVA/DA, 5HIAA/5HT) and the ACh/Ch ratio were calculated for each rat and the mean values of these ratios were obtained for each group. These ratios are used as markers of turnover or metabolism of biogenic amines in cerebral neurons. The statistical significance of differences between the control and PCB-exposed groups was examined by Dunnett's multiple *t*-test using statistics software (SPSS Japan Inc.). Differences between groups at $p < 0.05$ were considered significant.

Results

Effects of PCB153 on the offspring at 1 wk of age

Figure 1 shows how the maternal administration of PCB153 affected the brain content of neurotransmitters and metabolites in female pups at 1 wk after birth. In the offspring prenatally exposed to PCB153 at 16 mg/kg, levels of DA, DOPAC, and HVA were significantly higher than control values, however, no significant differences were found for these substances between the 64 mg/kg group and control. 5HT and 5HIAA levels increased in the PCB153-exposed groups in a dose dependent manner, and the differences between the dosed group and control for these substances were significant in the 64 mg/kg group. The 5HIAA/5HT ratio for the 64 mg/kg group was higher than for controls, but without significant differences.

Fig. 1. Effects of prenatal administration of PCB153 on the monoamine contents of the whole brain in 1-wk-old female rat offspring.

Control: $\square$ 16 mg/kg: $\square$ 64 mg/kg: $\blacksquare$ Results are shown as means $\pm$ SEM (%). Means of brain levels of neurotransmitters, metabolites, and metabolite/neurotransmitter ratios were normalized to those of control group which were set to 100%. N=9-14. *: $p < 0.05$ by Dunnett's multiple *t*-test.

Fig. 2. Effects of prenatal administration of PCB153 on the monoamine contents of the dissected brain regions in 3-wk-old female rat offspring.

See legends for Fig. 1. N=8.

Effects of PCB153 on the offspring at 3 wk of age

Figure 2 shows the levels of neurotransmitters and metabolites, and the ratios of metabolite/monoamines in the brains of 3-wk-old pups. No significant effects of PCB153 were observed on the levels and ratios in the forebrain (data not shown). MHPG, DA, DOPAC, and HVA showed a tendency to increase in the hindbrain of the group exposed to PCB at 16 mg/kg. DA of the 64 mg/kg group and the MHPG/NE and HVA/DA ratios of the 16 mg/kg group had a tendency to increase in the medulla oblongata; however, these changes were not statistically significant. Although the mean levels of NE, DA, DOPAC, 5HT, and 5HIAA in the cerebellum of the 16 mg/kg group were higher than those of controls, the overall tissue concentrations of neurotransmitters in the cerebellum were low, the data varied greatly, and no change in the levels of these substances showed statistical significance (data not shown).

Effects of PCB153 on the offspring at 6 wk of age

Figure 3 shows the results of neurotransmitter analysis of offspring at 6 wk after birth. There were no significant changes in monoamine and metabolite contents or in metabolite/monoamine ratios, except the HVA/DA ratio, in all brain regions of the PCB153-exposed groups. The HVA/DA ratio in the striatum of the 64 mg/kg group was significantly lower than that of controls, however, the decrease was less than 20% of the controls. In the hippocampus, the DOPAC/DA and HVA/DA ratios were elevated in the 64 mg/kg group. In the medulla oblongata, the levels of MHPG, DA, DOPAC, and HVA, and the

Fig. 3. Effects of prenatal administration of PCB153 on the monoamine contents of the dissected brain regions in 6-wk-old female rat offspring.

See legends for Fig. 1. N=5–6. *: $p<0.05$.

MHPG/NE ratio in the 64 mg/kg group were higher than those of controls. In the cerebellum, the MHPG levels and the MHPG/NE ratio in the 64 mg/kg group were higher than those of controls. The effects of PCB153 on monoamine and metabolites contents and metabolite/monoamine ratios in the frontal & occipital cortices, midbrain, and hypothalamus were subtle, therefore, the data for these four brain regions are not shown.

The effects of PCB153 on the cholinergic neurons of rat offspring at 6 wk after birth were estimated by measuring ACh and Ch levels, and calculating the ACh/Ch ratios for each rat. There were no significant differences between PCB153-exposed and control groups in ACh, Ch, and ACh/Ch values in any brain region examined (data not shown).

Fig. 4. Effects of prenatal administration of PCB153 on the monoamine contents of the dissected brain regions in 9-wk-old female rat offspring.

See legends for Fig. 1. N=8. *: $p<0.05$; **: $p<0.01$.

Effects of PCB153 on the offspring at 9 wk of age

Figure 4 shows levels of neurotransmitters, metabolites, and metabolite/monoamine ratios in the brains of offspring at 9 wk after birth. In the forebrain, the HVA level and the DOPAC/DA and HVA/DA ratios decreased significantly in the 64 mg/kg group compared with those of controls; the 5HIAA level and the 5HIAA/5HT ratio in the 16 mg/kg group increased with statistical significance. In the hindbrain, the DOPAC/DA and HVA/DA ratios decreased significantly in the 64 mg/kg group, and 5HIAA levels in the 16 mg/kg group increased significantly. In the medulla oblongata, MHPG decreased significantly and 5HIAA increased significantly in the 64 mg/kg group. All of the above changes in NE, DA, and DA neurons were approximately 20% of the control values or less. In the cerebellum, HVA of the 16 mg/kg group increased by about 50% with statistical significance; however, the increase in HVA in the 64 mg/kg group was neither large nor significant. The 5HIAA level

Fig. 5. Effects of prenatal administration of PCB153 on the monoamine contents of the dissected brain regions in 1-yr-old female rat offspring.

See legends for Fig. 1. N=7–8. *: $p<0.05$; **: $p<0.01$.

of the 64 mg/kg group increased significantly by about 20%.

Effects of PCB153 on the offspring at 1 yr of age

Figure 5 shows the levels of neurotransmitters, metabolites, and metabolite/monoamine ratios in the brains of offspring at 1 yr after birth. In the frontal and occipital cortices, striatum, midbrain, and cerebellum, there were either no significant changes in these levels or the changes in these brain regions were negligible (data not shown). In the hippocampus, the HVA/DA ratio in the 16 mg/kg group was significantly higher than that of controls, while the ratio in the 64 mg/kg group was similar to that of controls. The increase in the HVA level of the 16 mg/kg group was slight but HVA of the 64 mg/kg group was markedly less than in controls. In the hypothalamus, the levels of DOPAC and HVA in the PCB-exposed group were lower than those of controls, though without statistical significance. In the medulla oblongata, the MHPG level and MHPG/NE ratio of the 64 mg/kg group were markedly lower with statistical significance.

Effects of PCB153 on ACh, Ch, and ACh/Ch of offspring at 1 yr after birth are shown in Fig. 6. In the cere-

Fig. 6. Effects of prenatal administration of PCB153 on the levels of acetylcholine and choline, and the acetylcholine/choline ratio in 1-yr-old female rat offspring. See legends for Fig. 1. N=7–8. *: $p < 0.05$.

bellum, ACh levels were too low to perform analysis, therefore, the data from the cerebellum are not presented. Very slight, dose-dependent decreases in ACh levels were observed in the frontal and occipital cortices, midbrain, and medulla oblongata, though a significant decrease was observed in the striatum but only in the 64 mg/kg group. Ch increased in the frontal and occipital cortices, hippocampus, striatum, midbrain, and hypothalamus in the 64 mg/kg group. These increases ranged from 40 to 140% of the control values, however, they were not statistically significant because of large variation in the data.

Effects of PCB153 on the dams

The effects of PCB administration on the levels of neurotransmitters and metabolites of dams are presented in Figs. 7-1 and 7-2. In the frontal cortex, subtle increases in MHPG and the MHPG/NE ratios were found in PCB153-treated groups, although these changes were not significant (Fig. 7-1). In the occipital cortex, the MHPG levels and MHPG/NE ratios of the 16 and 64 mg/kg groups were markedly lowered, and these decreases were about 50% of the control value. However, statistical significance was not shown because of large variations in values. The DA, DOPAC, and HVA levels of the PCB153-treated groups were reduced significantly in both the 16 and 64 mg/kg groups. In the hippocampus, DA, DOPAC, and HVA levels decreased by about 30 to 40%

in the 16 and 64 mg/kg groups with statistical significance. In the striatum, the MHPG levels and MHPG/NE ratios decreased in the 16 and 64 mg/kg groups, although not significantly; HVA decreased dose-dependently and the decrease in the 64 mg/kg group was significant. In the midbrain, increases in MHPG and MHPG/NE and decreases in DA, DOPAC, and HVA were observed, though without statistical significance (Fig. 7-2). In the hypothalamus, decreases in MHPG and MHPG/NE were observed, however these changes were not significant. HVA levels and HVA/DA ratios decreased in PCB153-treated groups, and were significant in the 64 mg/kg group. 5HT increased slightly, and dose-dependently, and the increase in the 64 mg/kg group was significant. In the medulla oblongata, the DA, DOPAC, and HVA levels and HVA/DA ratio decreased in a dose-dependent manner in the PCB153-treated groups with the DA level in the 64 mg/kg group showing statistical significance. In the cerebellum, the DOPAC levels and DOPAC/DA ratios of the PCB153-treated groups decreased, however without statistical significance.

Discussion

The effects of PCB on brain neurotransmitters were reported in Japan for the first time in 1977²¹⁾. Single oral doses of Aroclor1254 and 1260 were administered to rats

Fig. 7-1. Effects of maternal administration of PCB153 on the neurotransmitter contents of the frontal cortex, occipital cortex, hippocampus, and midbrain in dams.

See legends for Fig. 1. N=9–10. *: $p < 0.05$; **: $p < 0.01$.

Fig. 7-2. Effects of maternal administration of PCB153 on the neurotransmitter contents of the striatum, hypothalamus, medulla oblongata, and cerebellum in dams.

See legends for Fig. 1. N=9–10. *: $p < 0.05$.

at 500 and 1,000 mg/kg body weight, and levels of DA, DOPAC, and HVA/DA in the caudate nucleus were reduced²²). Following Aroclor1254 dosing for 30 d, DA and its metabolite were found to be reduced in the caudate nucleus²³). Choksi *et al.* suggested PCBs inhibited tyrosine hydroxylase²⁴). Female rats were intraperitoneally administered 2,2',3,5',6-PCB (PCB95) or 2,2',4,5,5'-PCB (PCB101) at 16 or 32 mg/kg, and hypothalamic DA and serum T_4 decreased in the treated groups²⁵). PCB153 was administered to rats for 13 wk, and DA and 5HT decreased in the frontal cortex in female rats, but not in male rats²⁶). Many studies have reported a decrease in DA and/or DA turnover in response to PCB administration. However, some of those studies used a mixture of PCB congeners, and few studies have used

only a single PCB congener.

In the present study, the effects of PCB on the brain of dams and offspring after maternal exposure were investigated using a single congener, PCB153. Changes in brain neurotransmitters and metabolites observed in maternal rats are summarized in Table 1. As shown in Table 1, MHPG levels and the MHPG/NE ratio were low in some brain regions of the PCB-treated groups. On the other hand, these parameters were increased by PCB treatment in other brain regions. PCB153 caused an increase or decrease in NE turnover rate, which depended on the brain region. DA, DOPAC, and HVA decreased by about 20 to 40% in the occipital cortex and hippocampus of the PCB-treated groups. There were no differences in the degree of the decreases in DA and metabolites between

Table 1. Summary of changes in neurotransmitters and metabolites in the brain of female rats which received PCB153 in pregnancy

	NE neurons	DA neurons	5HT neurons
16 mg/kg	MHPG ↗ MHPG/NE ↗ F.Cortex MHPG ↘ MHPG/NE ↘ O.Cortex MHPG ↘ MHPG/NE ↘ Striatum MHPG ↗ MHPG/NE ↗ Midbrain MHPG/NE ↘ Hypothalamus	DA ↓ HVA ↓ O.Cortex DA ↓ DOPAC ↓ HVA ↓ Hippocampus DA ↘ DOPAC ↘ Midbrain DOPAC ↘ DOPAC/DA ↘ Cerebellum	
64 mg/kg	MHPG ↗ MHPG/NE ↗ F.Cortex MHPG ↘ MHPG/NE ↘ O.Cortex MHPG ↘ MHPG/NE ↘ Striatum MHPG ↗ MHPG/NE ↗ Midbrain MHPG/NE ↘ Hypothalamus	DA ↓ DOPAC ↓ HVA ↓ O.Cortex DA ↓ DOPAC ↓ HVA ↓ Hippocampus HVA ↓ Striatum DA ↘ DOPAC ↘ HVA ↘ Midbrain HVA ↓ HVA/DA ↓ Hypothalamus DA ↓ HVA ↘ M.Oblongata DOPAC ↘ DOPAC/DA ↘ Cerebellum	5HT ↗ Hippocampus 5HT ↑ Hypothalamus

↑ and ↓: Increase or decrease in brain substances from control levels with statistical significance.

↗ and ↘: Increase or decrease in brain substances from control levels by 20% or more without statistical significance.

the 16 and 64 mg/kg groups. The suppression of DA and its metabolites by PCB153 seems to have reached a maximum in the 16 mg/kg group in these brain regions. Decreases in DA, its metabolites, and metabolite/monoamine ratios were observed in the striatum, midbrain, hypothalamus, medulla oblongata, and cerebellum, although the degree of the decrease was not as great as in the occipital cortex and hippocampus. 5HT increased in the hippocampus and hypothalamus of the PCB-treated groups, but the degree of change was small in the hypothalamus, and it was not statistically significant in the hippocampus. PCB affected 5HT levels in dams in a few brain regions.

The dominant effects of PCB153 on the brain neurotransmitters of dam rats were the declines of DA, DA metabolites, and DA turnover in many brain regions. These effects were similar to the results reported in previous studies^{23–26}.

Various changes have been reported in brain neurotransmitters of offspring following prenatal administration of PCB. Aroclor 1254 lowered 5HIAA levels and the 5HIAA/5HT ratio in the brain of male and female offspring, although the levels of NE, DA, DOPAC, and HVA were unaffected²⁷. The administration of 2,2',4,4'-PCB (2-TCB) decreased brain DA of offspring²⁸. Prenatal injection of 3, 3',4, 4'-PCB (3-TCB) increased DA in the frontal cortex. Aroclor 1016 induced few effects on DA and NE and their turnover in the brain of offspring²⁹. Following the injection of Aroclor 1254, DA was unchanged, but NE was reduced in the brain of offspring. Thus, the reported effects of prenatal administration of PCB on the neurotransmitters of offspring vary greatly.

In the present study, the effects of prenatal exposure to PCB153 on female offspring were investigated at several stages of development, and the results are summarized

in Table 2. At 1 wk after birth, the increases in DA, DOPAC, and HVA were significant, but not dose-dependent; the increases in 5HT and 5HIAA were significant and dose-dependent. Prenatal PCB exposure resulted in increases in DA, DA metabolites, 5HT and 5HIAA in several brain regions in the early weeks after birth (1 to 3 wk). Prenatal exposure to PCB153 had only slight effects on brain neurotransmitters of 6-wk-old offspring. In 9-wk-old offspring, PCB exposure reduced HVA levels and the DOPAC/DA and HVA/DA ratios significantly in the forebrain and hindbrain; 5HIAA levels were elevated in all four brain regions. Although the degrees of change were not great, DA turnover was reduced and 5HT turnover was increased in PCB-exposed 9-wk-old female offspring. In 1-yr-old offspring, a slight, but clear tendency was observed for decreases in DA, DOPAC, and HVA levels in the hippocampus; a similar tendency was observed in the hypothalamus.

Our results suggest that the effects of prenatal exposure to PCB153 on brain neurons are dependent on the development stages of female offspring. PCB153 exposure induced similar effects on 5HT neurons at 1 and 9 wk after birth, with increases in 5HT or 5HT metabolites in several brain regions. DA and its metabolites were lowered at 9 wk to 1 yr after birth by PCB153 exposure. The mechanism responsible for these changes in offspring was not clarified in this study.

In the present study, PCB153 doses of 16 and 64 mg/kg body weight were chosen. Because this is the first PCB study in our laboratory, we tried to elucidate the effects of high doses of PCB. Schantz *et al.* assessed spatial learning and memory in rats following gestational exposure to PCB153 at 16 or 64 mg/kg/day, and these doses were also used in the studies by Ness *et al.*^{5,30}. PCB153 at these doses exerted no effects on the body weight of

Table 2. Summary of changes in neurotransmitters and metabolites in the brain of female offspring rats which received prenatal exposure to PCB153

	NE neurons	DA neurons	5HT neurons	ACh neurons
1 wk 16 mg/kg		DA ↑ DOPAC ↑ HVA ↑ Whole brain		ND
64 mg/kg			5HT ↑ 5HIAA ↑ Whole brain	ND
3 wk 16 mg/kg MHPG/NE ↗ M.Oblongata		DA ↘ HVA/DA ↗ M.Oblongata		ND
64 mg/kg		DA ↗ M.Oblongata		ND
6 wk 16 mg/kg MHPG ↗ MHPG/NE ↗ Cerebellum		HVA ↘ HVA/DA ↘ Cerebellum		
64 mg/kg MHPG ↗ Hippocampus		DOPAC ↗ DOPAC/DA ↗ HVA/DA ↗ Hippocampus		
MHPG ↗ MHPG/NE ↗ M.Oblongata		HVA ↘ HVA/DA ↓ Striatum		
MHPG ↗ MHPG/NE ↗ Cerebellum		DOPAC ↗ HVA ↗ M.Oblongata		
		HVA ↘ HVA/DA ↘ Cerebellum		
9 wk 16 mg/kg		DA ↗ HVA ↑ DOPAC/DA ↘ HVA/DA ↗ Cerebellum	5HIAA ↑ 5HIAA/5HT ↑ Forebrain 5HIAA ↑ Hindbrain	ND
64 mg/kg MHPG ↓ MHPG/NE ↘ M.Oblongata		HVA ↓ DOPAC/DA ↓ HVA/DA ↓ Forebrain DOPAC/DA ↓ HVA/DA ↓ Hindbrain HVA ↗ DOPAC/DA ↘ Cerebellum	5HIAA ↑ M.Oblongata 5HIAA ↑ Cerebellum	ND
1 yr 16 mg/kg MHPG/NE ↗ Hypothalamus		HVA/DA ↑ Hippocampus HVA ↘ Hypothalamus DOPAC ↘ HVA ↘ M.Oblongata		Ch ↘ M.Oblongata
64 mg/kg MHPG ↓ MHPG/NE ↓ M.Oblongata		DA ↗ DOPAC ↘ HVA ↘ Hippocampus HVA ↘ HVA/DA ↘ Hypothalamus	5HIAA/5HT ↘ Hippocampus 5HT ↗ 5HIAA/5HT ↘ Hypothalamus	ACh ↓ Striatum Ch ↗ All brain regions except M.Oblongata ACh/Ch ↗ M.Oblongata

See legends for Table 1. ND: No Data.

dams or offspring in our study (data not shown). The results obtained in our present study in which high PCB doses were given to pregnant rats may correlate to exposure to PCBs of pregnant or non-pregnant humans and to PCB exposure of workers. However, there are not many papers dealing with the effects of PCBs on females or workers. A retrospective mortality study of PCB-exposed workers was performed³¹⁾, and in that study, an excess of Parkinson's disease and dementia was found among women exposed to high levels of PCBs. This result suggests that exposure to PCBs increases the incidence of neurodegenerative diseases in women, and it is well known that brain DA and its metabolites are reduced and DA neurons are degenerated in the brains of Parkinson's disease patients. In our present study, the administration of high doses of PCB153 decreased DA and its metabolites in many regions of the brains of dam rats. The effects of PCB153 observed in our study may explain why women exposed to PCBs develop Parkinson's disease.

The results of some epidemiological studies have suggested that deficits in cognitive function such as low IQ and disorders in learning in some children are related to prenatal exposure to PCB^{6, 7)}. According to recent studies concerning cognitive function, low activity of brain DA neurons is involved in deficits of cognitive function in humans^{32, 33)}. In our present study, a persistent decrease in brain DA and DA metabolites from 9 wk to 1 yr after birth was observed in offspring that received

prenatal exposure to PCB153. These decreases reflect the low activity of DA neurons in the PCB-exposed rats, and this change in DA neurons may mimic changes in the human brain prenatally exposed to PCBs. The results obtained in our study suggest that the deficits in cognitive function in children born to mothers exposed to PCBs are due to the lowered activity of DA neurons.

Only a few studies have examined the effects of prenatal exposure to PCBs on brain 5HT neurons in offspring. In our present study, prenatal exposure to PCB153 produced increases in 5HT turnover in the brains of rat offspring from 1 to 9 wk after birth. Morse *et al.* reported that the prenatal administration of Aroclor 1254 to pregnant rats at doses up to 25 mg/kg resulted in increases in the 5HIAA and 5HIAA/5HT ratios in some brain regions of offspring at 90 d after birth³⁴⁾. These results suggest that 5HT-related brain functions such as emotion and sleep would be altered in human children born to PCB-exposed mothers.

In the present study, the effects of PCB exposure on neurotransmitters and metabolites in the brain were dependent on the stage of development of the rat pups. The results of previous reports suggest that the disorder of brain function produced by prenatal exposure to PCBs is due to retardation of brain development caused by lowered thyroid function⁴⁻⁶⁾. If the brain development of PCB-exposed pups is retarded, the levels of neurotransmitters and metabolites should be different from control

pups. The low levels of DA and its metabolites observed in 9-wk- to 1-yr-old pups may be an example of this difference. Some studies have reported that mental retardation is observed in infancy or in the early school years of children exposed prenatally to PCBs, however, the differences disappear after their juvenile period⁸⁾. Some recovery in brain function may occur during development. Apparent increases in 5HT and 5HIAA in PCB-exposed pups may reflect such a recovery process in the brain.

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