ALTERATIONS IN GENE EXPRESSION BY EXPOSURE TO HYDROXYLATED POLYCHLORINATED BIPHENYLS IN DEVELOPING RAT BRAIN

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Abstract

Some congeners of hydroxylated polychlorinated biphenyl (OH-PCB) metabolized from parent PCB in the body have endocrine disrupting potencies. Thus, there is an increasing concern about adverse effect(s) of OH-PCB exposure on brain development, because developing brain is most sensitive to endogenous hormones throughout its life. In the present study, we evaluated gene expression altered by hydroxylated PCBs exposure in the developing rat brain (cerebral cortex, hippocampus and striatum).

Prenatal exposure to 4-hydroxy-2,2',3',4',5'-pentachlorobiphenyl (OH-PCB106) which has thyroid hormone-disrupting activities at the dose of 1 mg/kg/day from gestational day 7 to postnatal day 1 significantly altered mRNA expression levels of glutamate receptors and exocytosis-related genes, showing significant region-specificities.

Neonatal exposure to 4-hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl (OH-PCB187), a major congener in human blood, at the dose of 1 mg/kg at postnatal day 2 altered mRNA expression levels of genes involved in transcriptional/translational activation at 5 hours after injection, which was indicated by comprehensive gene expression analyses, although apparent pathway could not be identified by quantitative validations in this study. However, mRNAs of two thyroid hormone-responsive genes, neurogranin and myelin basic protein, were decreased by OH-PCB187 exposure, suggesting ant-thyroid hormone like activity of this congener in the developing brain.

Introduction

Polychlorinated biphenyls (PCBs), a major environmental contaminant, raise a public concern about adverse effects on human health. In human, PCBs were hydroxylated at phase I metabolism, which was followed by glucuronidation and excretion. Recent studies revealed that some congeners of hydroxylated PCBs have physiological activities such as thyroid hormone-disrupting activities in *in vitro* conditions[1].

Brain development has a critical period of exposure to physiologically active chemicals around birth, because it highly depends on endogenous hormones such as thyroid hormone. And there is a possibility that perturbation during the critical period leads to irreversible alteration of brain function[2].

In the present study, we evaluated gene expression altered by hydroxylated PCBs exposure in the developing rat brain and two congeners of hydroxylated PCBs were used. One was 4-hydroxy-2,2',3',4',5'-pentachlorobiphenyl (OH-PCB106) which has thyroid hormone-disrupting activities[3,4]. The other was 4-hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl (OH-PCB187), a major congener in human blood[5], although physiological potencies has not been clear to date.

Materials and Methods

Animals

Experiment 1 (OH-PCB106)

Pregnant F344/N rats were exposed to OH-PCB106 at the dose of 1 mg/kg/day via subcutaneously implanted osmotic pump from gestational day 7. At postnatal day (PND) 1, neonatal brains were dissected and separated anatomically into three brain regions (cerebral cortex, hippocampus and striatum), which were subjected to further experiments.

Experiment 2 (OH-PCB187)

Neonatal rats at PND 2 were exposed to OH-PCB 187 at the dose of 1 mg/kg via single subcutaneous injection. At 5 hour after injection, the cerebral cortex, hippocampus and striatum were sampled and the hippocampus was subjected to comprehensive gene expression analyses.

Total RNA preparation

Total RNA was prepared using TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA). Quality of individual total RNA was checked by BioAnalyzer (Agilent Technologies, Palo Alto, CA).

Real-time quantitative fluorescence-based PCR

Expression levels of specific genes in cDNA samples synthesized from total-RNA using SuperScript II (Invitrogen) were quantified by fluorescence-based real time PCR using Smart Cycler System (Cepheid, CA) with SYBR Premix ExTaq (Takara). Cyclophilin-A (Ppia) was used as a housekeeping gene. PCR primers were designed using Oligo 6.0 primer analysis software (Molecular Biology Insights, CO).

DNA microarray

Experiment 1 (OH-PCB106)

Equal amount of Individual total RNA samples were mixed within each region and group. These mixed samples were considered as average total RNA of each group. The mixed RNA was reverse transcribed with RNA Transcript SureLABEL Core kit (Takara, Shiga, Japan), which was followed by transcription into promoter-tagged second strand cDNA for linear amplification. During linear amplification, 5mM aminoallyl (aa)-UTP (Applied Biosystems, CA) was incorporated into the cRNA, which is subsequently coupled with Cy3 (OH-PCB106) or Cy5 (control). Moreover, these samples were purified and concentrated by RNeasy MinElute cleanup kit (QIAGEN Science, Maryland). Finally, Cy3- (OH-PCB106) and Cy5- (control) labeled cRNA were mixed, fragmented by RNA Fragmentation Reagents (Applied Biosystems), and hybridized to oligonucleotide microarray (Rat V2 Oligo Microarray Kit, Agilent Technologies), according to the operations recommended by the manufacturer.

Experiment 2 (OH-PCB187)

Pooled total RNA from the hippocampus of control neonates were labeled with Cy5 as described above. Similarly, four mixed hipppocampal RNA samples from eight OH-PCB187-exposed individuals were labeled with Cy3. Four competitive hybridizations were performed to increase reliability of results.

Microarray data acquisition and data normalization

After hybridization, microarrays were scanned by DNAscope^{TM}IV (GeneFocus Biomedical Photometrics Inc., Canada). The fluorescence intensity signals of gene-specific spots were quantified after background correction by ImaGene 5.5 (Biodiscovery, CA) and normalized using the global normalization.

Results and Discussion

Experiment 1 (OH-PCB106)

There was no effect of exposure to OH-PCB106 on the body weight of mothers and the number, gender ratio and body weight of male offspring. Expression levels of mRNA of thyroid hormone-related genes, thyroid hormone receptor α (THR α) and β (THR β), neurogranin (RC3/Ngn), myelin basic protein (MBP), hairless (Hr) and reelin (Reln), were evaluated, because this congener has thyroid hormone-disrupting activity. In the cerebral cortex, THR α mRNA was increased by OH-PCB106 exposure, while mRNAs of THR β, MBP and Hr were decreased. In the hippocampus, no thyroid hormone-related gene expression was influenced. In the striatum, increase of THR α mRNA and decrease of MBP mRNA by OH-PCB106 exposure were observed. These results suggest that OH-PCB106 has anti-thyroid hormone-like activity even in *in vivo* developing brain and that there is significant region-specificities in response to OH-PCB106 exposure.

Comprehensive gene expression analyses using DNA microarray indicated alterations in mRNA expression levels of 1659, 504 and 1285 genes in the cerebral cortex, hippocampus and striatum, respectively, and a total of 2918 genes were altered at least in one region and perturbations of mRNA expression levels of genes related to neurotransmission, especially glutamatergic transmission, and secretory pathway (data not shown). Based on these results, we evaluated quantitatively mRNA expression levels of glutamate receptors (Table 1). These significant alterations of mRNA expression levels of glutamate receptor genes suggest aberrant glutamatergic neurotransmission, although there were significant region-specificities. In addition, mRNAs of exocytosis-related genes, VAMP1 and VAMP2, were significantly increased by OH-PCB106 exposure in all three regions (data not shown).

Table 1
Altered gene expression of glutamate receptors by OH-PCB106 exposuer in developing brain.

Genes	Cerebral cortex	Hippocampus	Striatum
NMDA receptors (NR)			
NR1	-	-	-
NR2A	-	decrease	-
NR2B	decrease	-	-
NR2C	-	decrease	increase
NR3A	-	decrease	-
NR3B	-	-	increase
Metabotropic glutamate receptors (mGluR)			
mGluR1	-	decrease	-
mGluR2	-	decrease	-
mGluR3	-	-	-
mGluR4	-	-	-
mGluR5	-	decrease	-
mGluR6	-	decrease	increase
mGluR7	decrease	decrease	-
mGluR8	-	decrease	-

- : no alteration

Experiment 2 (OH-PCB187)

No gross abnormality was observed by exposure to OH-PCB187 at the dose of 1 mg/kg even at 3 days after injection. In the hippocampus at 5 hours after injection, comprehensive analyses using DNA microarray indicated perturbations of "cell-cell contact and cytoskeletal regulation" and "transcriptional/translational activation" as primary response to OH-PCB187 exposure. However, no apparent pathway was identified when validated by quantitative PCR. When we evaluated mRNA expression levels of thyroid hormone-related genes, RC3/Ngn in the cerebral cortex and MBP mRNAs in the cerebral cortex and striatum were decreased by OH-PCB187 exposure (Fig. 1), suggesting anti-thyroid hormone-like activity of this congener in *in vivo* brain with region specificities However mRNA expression levels of glutamate receptors were not influenced by OH-PCB187 exposure (data not shown), which was different from OH-PCB106 exposure.

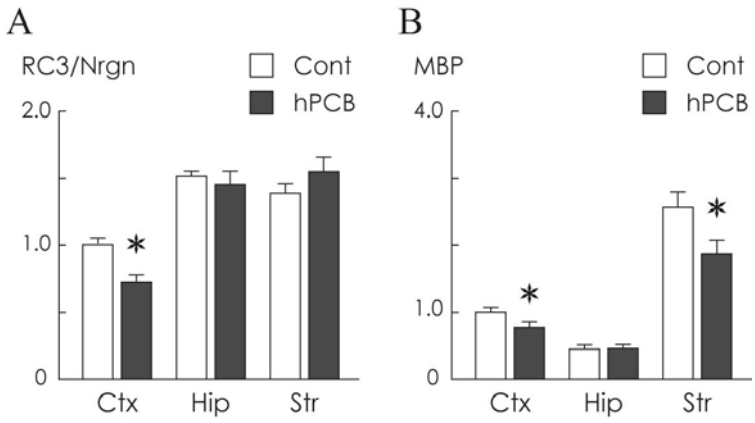

Fig. 1.Effect of exposure to OH-PCB187 (hPCB) on expression levels of mRNA of neurogranin (RC3/Ngn) (A) and myelin basic protein (MBP) (B). Each mRNA level is expressed relative to the value of the cerebral cortex of control neonates (mean±S.E.M n=6/group). *: $p<0.05$

Both congeners used in this study showed anti-thyroid hormone-like effect on mRNA expression levels of thyroid hormone-responsive genes, which suggests that thyroid hormone dependent brain development is a potential and common target of exposure to some of hydroxylated PCBs.

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