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The Effect of Hydroxylated Polychlorinated Biphenyl (OH-PCB) on Thyroid Hormone Receptor (TR)-mediated Transcription through Native-Thyroid Hormone Response Element (TRE)

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Abstract: Polychlorinated biphenyls (PCBs) are known as environmental contaminants that may cause abnormal effect in various organs. We have previously reported that low dose of hydroxylated PCBs (OH-PCBs) including 4'-OH-2',3,3',4',5'-pentachloro biphenyl (4'-OH-PCB 106), suppressed thyroid hormone (TH) receptor (TR)-mediated transcription on several artificial TH-response elements (TREs) due to partial dissociation of TR from TRE. In the present study, we examined the effect of OH-PCB on TR-mediated transcription on native TRE-containing promoter, using malic enzyme (ME)-TRE. Transcriptional activity was measured by transient transfection based reporter gene assay in CV-1, fibroblast-derived clonal cells. TR-mediated transcription was suppressed by 4'-OH-PCB106 significantly and 4'-OH-PCB187 weakly, but not by 4'-OH-PCB165. To examine TR-TRE bindings under exposure of 4'-OH-PCB106, electrophoretic mobility shift assay (EMSA) was performed. In EMSA, TR was dissociated from ME-TRE by 4'-OH-PCB106. These findings suggest that OH-PCB may disrupt TR-mediated transcription on native promoter.

Key words: TR, Malic enzyme, Hydroxylated-PCB, Transcriptional regulation, Native promoter

Polychlorinated biphenyls (PCBs) had been produced since early 1930 th until the beginning of the 1970 th[1–3]. These chemicals were used widely in industrial and household appliances, such as electric fluid in transformer and capacitor, hydraulic lubricants, paints, copy paper. Although production has been banned in 1972, these are still globally detectable even at the edge of Arctic[4]. PCBs contain 209 congeners, each of which is chlorinated to various degrees[1, 2] (Fig. 1A). All PCB compounds are highly lipophilic, accumulate in the liver and adipose tissue, and easily transfer to the embryo through the placenta and via breast milk[1, 2].

Several reports have shown that PCBs may affect embryonic and neonatal development at low doses[5, 6]. For example, exposure to low dose PCB may cause abnormal development of the central nervous system[6]. Although the molecular mechanisms of PCB action during developing have not yet been fully clarified, a possible involvement of thyroid hormone (TH; thyroxin, T4; triiodothyronine, T3) system has been postulated, because TH and PCB molecules harbor structural similarity, and because TH plays important roles in the growth, development and functional maintenance of many organs[7]. Hypothyroidism during perinatal period causes retardation of general growth as well as abnormal development of many organs including brain, bone, skeletal muscle and liver[7]. Thus, disruption of TH system by PCB may affect development of such organs.

Based on this hypothesis, we previously examined the effect of various PCBs and hydroxylated PCBs (OH-PCBs) on TH receptor (TR)-mediated transcription[8–10]. Several PCBs and OH-PCBs suppressed TH-induced tran-

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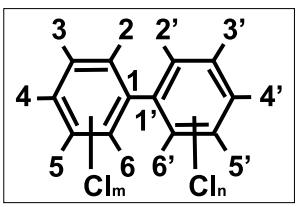
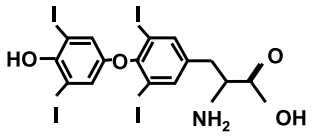
A



Polychlorinated biphenyls (PCB)



L-Thyroxine (T4)

B

	sequence
F2	AAGGGGATCCTTAT**TGACCC**CAGCTG**AGGTCA**AGTTACGGATCTTCCT
DR-4	AAGGGGATCCAGCTTC**AGGTCA**CAGG**AGGTCA**GAGAGCTGATCTTCCT
Pal	AAGGGGATCCAGCTTC**AGGTCATGACCT**GAGAGCTGATCTTCCT
Malic Enzyme (ME)	GATCCATCC**AGGACG**TTG**GGGTTA**GGGG**AGGACA**GTGGA

Fig. 1. Schematic representative of materials used in the present study.

(A) The representative structure of PCB and thyroxine (T4). PCB compounds contain 209 congeners, each of which is chlorinated to various degrees.

(B) Artificial and native TREs used in the present study. Bold letters indicate TRE half sites.

scription at doses as low as 10^{-10} M on artificial TH-response elements (TRE) such as direct repeat (DR)-4, F2, and palindromic (pal) TRE[8, 9] (Fig. 1B). Although the suppression by 10^{-10}–10^{-5} M PCB were significant comparing to the absence of PCB, the magnitude of suppression between 10^{-10} M and 10^{-5} M PCB treatment was not statistically significant, indicating that PCB may not competitively inhibit TH binding to TR[8]. Then we found that PCB may partially dissociate TR from TRE[9] using electrophoretic mobility shift assay (EMSA), by acting to DNA-binding domain of TR[10]. However, we did not examine the effects on native TRE. Thus, we used TRE located at promoter region of malic enzyme (ME) as a representative native TRE in the present study.

We tested the effect of OH-PCBs that are abundantly contained in human organs[11, 12]. We particularly focused on hydroxylated metabolites, because we have previously shown that some OH-PCBs such as 4'-OH-2',3,3',4',5'-pentachloro biphenyl (4'-OH-PCB106) greatly suppressed TR-mediated transcription[8–10].

We carried out the cell culture and transient transfection-based reporter gene assays by same procedure with our previous report[8–10]. Plasmids informations were described previously[8, 9]. A significant suppression of TR-mediated transcription by 10^{-9} M of 4'-OH-PCB106 was observed (Fig. 2A), which is essentially consistent with data on artificial TREs[8–10].

We reported that 4'-OH-PCB165 significantly suppressed the TR-mediated transcription on artificial TREs. However, it did not affect the transcription on ME-TRE (Fig. 2B), although the molecular structure of this congener is similar to that of 4'-OH-PCB106. These results indicate that the effects of OH-PCBs may be different among TREs.

Since hydroxylated-PCB187 (4'-OH-PCB187) is abundantly contained in human serum[11, 12], we investigated the effect of 4'-OH-PCB187. A significant, but weak suppression of TR-mediated transcription was observed on ME-TRE (Fig. 2C). These results indicate that OH-PCB suppresses the TR-mediated transcription not only through artificial TRE but also through native TRE. However, the effect of OH-PCBs on TR-mediated transcription may not be consistent among TREs. While 4'-OH-PCB106 suppressed TR action on ME-TRE as well as on artificial TREs, 4'-OH-PCB165 did not suppressed TR action on this native TRE. As shown in Fig. 1B, the nucleotide sequence of ME-TRE is different from that of typical artificial TRE. It contains 3 nuclear receptor binding motif-like sequences (Fig. 1B). Although 2 downstream motifs are aligned as DR-4, previous studies have shown that another upstream motif is equally important to interact with TR[13]. Such different property of ME-TRE from typical TRE may have caused a differential sensitivity to OH-PCBs.

We previously reported that the mechanism of suppression by PCB is partial dissociation of TR from TRE and the magnitude of suppression may be related to the degree of dissociation[9]. Thus, we investigated the effect of OH-PCB on ME-TRE using EMSA (Fig. 3). Methods for EMSA were described previously[9]. In brief, [α-^{32}P]-labeled TRE was incubated with *in vitro* transcribed and translated TRβ1, retinoid X receptor (RXR) β, a heterodimer partner of TR, in the presence or absence of ligands, followed by electrophoresis and autoradiography to visualize. We observed that TR-RXR bound to ME-TRE (lane 2,3). The TR-RXR heterodimer was dissociated from ME-TRE by OH-PCB (lane 4). The mode of 4'-OH-PCB106 action is the same as that on typical artificial TRE, indicating that the effect of OH-PCBs on TR mediated transcription on ME-TRE is also induced by partial dissociation of TR[8–10].

ME expression is regulated by TH in liver. Previous studies have shown that PCB affects hepatic function such as the expression of cytochrome P450 enzymes[14]. The results of present study have shown that OH-PCB affects ME expression. ME catalyzes the reversible oxidative decarboxylation of malate in a $NADP^+$-dependent manner and links the glycolytic pathway with the citric acid cycle. By reacting with L-malate as a substrate, ME with $NADP^+$ produces pyruvate, CO_2, and NADPH. Thus,

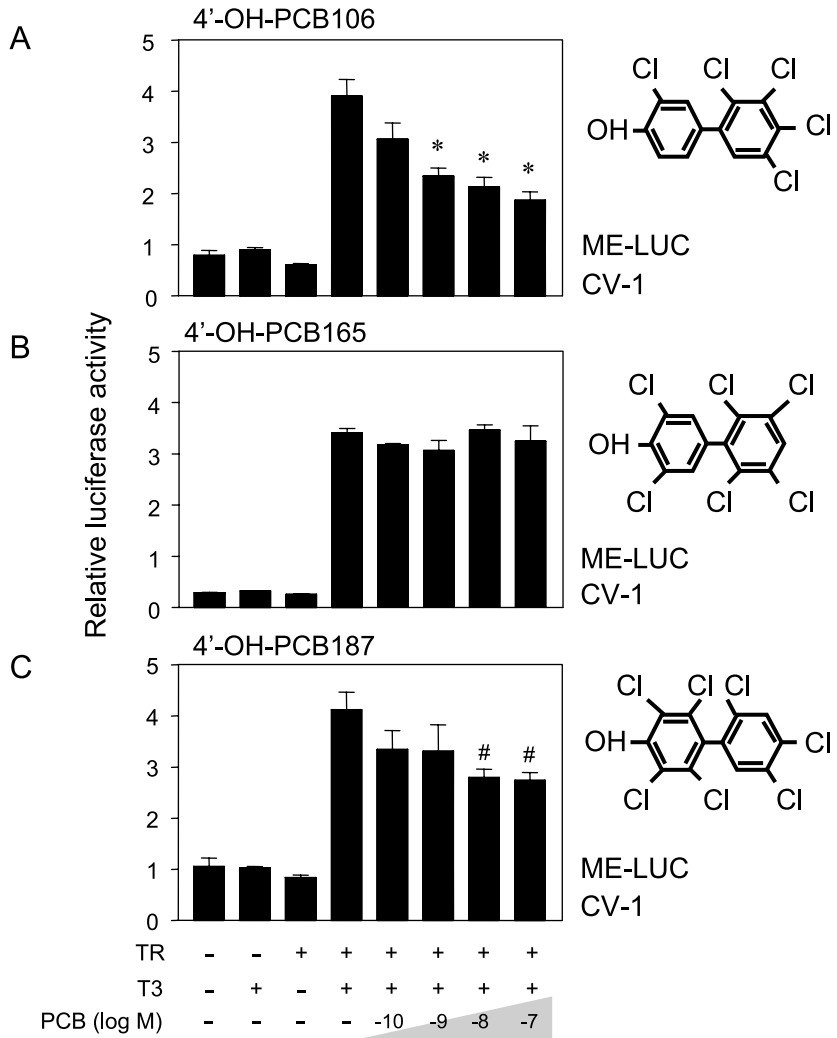


Fig. 2. Effects of PCB congeners on native ME promoter.

Expression plasmids encoding TRβ1 (10 ng) were cotransfected with ME-TRE-TK-LUC reporter plasmid (100 ng) into CV-1 cells. CMV-β-galactosidase plasmid (25 ng) was cotransfected as an internal control. Cells were incubated with or without triiodothyronine (T3) (10^{-7} M) and indicated amounts of 4'-OH-PCB106 (A), 4'-OH-PCB165 (B) or 4'-OH-PCB187 (C). Total amounts of DNA for each well were balanced by adding vector pcDNA3. Data represents mean in triplicate ± S. E. M. of experiments performed in triplicate. All transfection studies were repeated at least twice in triplicate and similar results were obtained. The luciferase activities were normalized to β-galactosidase activity and then calculated as relative luciferase activity. The data were analyzed by ANOVA, followed by *post hoc* comparison with Bonferonni's multiple range test. *: Statistically significant (p<0.01) vs. TRβ1 (+), T3 (+) and PCB (–). #: Statistically significant (p<0.05) vs. TRβ1 (+), T3 (+) and PCB (–).

PCBs may affect hepatic energy and glucose metabolism. In fact, clinical reports from Belgium[15] have shown a close association of diabetes mellitus with several environmental chemicals such as polychlorinated dibenzo-*p*-dioxin, dichloro-diphenyl-trichloroethane (DDT) and PCB. Diabetic patients had significantly increased serum levels of dioxins and coplanar PCBs[15]. Thus, our results in the present study may indicate that these substances may disrupt ME-mediated pathway. Furthermore, not only ME, but also several lipid metabolism-related enzymes are regulated by TH. Thus, PCB may disrupt a broad range of lipid/glucose metabolic pathways by suppressing TR action to induce diabetes and/or hypercholesterolemia. The results of present study may provide an important clue to explain the association between such metabolic diseases and environmental chemicals.

In summary, our results indicate that OH-PCBs suppress TR-mediated transcription through partial dissociation of TR from not only artificial TRE but also native ME-TRE. We hope that this study significantly contributes to increase our understanding on the association of PCB with energy metabolism.

Lane	1	2	3	4	5
Probe (ME)	+	+	+	+	-
Probe (F2)	-	-	-	-	+
TR	-	+	+	+	+
RXR	-	+	+	+	+
T3 (10^{-6} M)	-	-	+	+	+
PCB (10^{-8}M)	-	-	-	+	-

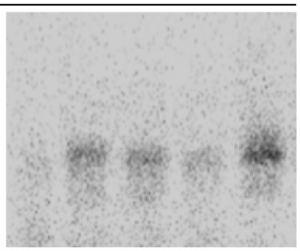


Fig. 3. OH-PCB partially dissociated TRβ1 from native ME-TRE.

Double-stranded oligonucleotides containing F2-and ME-TRE that are shown in Fig. 1B were labeled using Klenow fragment with α-[^{32}P]-dCTP. *In vitro* translated TRβ1 (1.5 μl) and/or RXRβ (3 μl) were incubated with [^{32}P]-labeled ME-TRE or F2-TRE with or without 10^{-6} M T3 and/or 10^{-8} M 4'-OH-PCB106 in binding buffer for 30 min on ice. After incubation, samples were subjected to electrophoresis and analyzed by autoradiography. Shown is a representative autoradiography. Same experiments were performed at least 5 times and similar results were obtained.

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References

1) Tilson HA, Kodavanti PR (1997) Neurochemical effects of polychlorinated biphenyls: an overview and identification of research needs. Neurotoxicology **18**, 727–43.
2) Safe SH (1994) Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit Rev Toxicol **24**, 87–149.
3) McKinney JD, Waller CL (1994) Polychlorinated biphenyls as hormonally active structural analogues. Environ Health Perspect **102**, 290–7.
4) Dewailly E, Ryan JJ, Laliberte C, Bruneau S, Weber JP, Gingras S, Carrier G (1994) Exposure of remote maritime populations to coplanar PCBs. Environ Health Perspect **102** (Suppl 1), 205–9.
5) Jacobson JL, Jacobson SW (1996) Dose-response in perinatal exposure to polychlorinated biphenyls (PCBs): the Michigan and North Carolina cohort studies. Toxicol Ind Health **12**, 435–45.
6) Jacobson JL, Jacobson SW, Humphrey H (1990) Effects of exposure to PCBs and related compounds on growth and activity in children. Neurotoxicol Teratol **12**, 319–26.
7) Koibuchi N, Chin WW (2000) Thyroid hormone action and brain development. Trends Endocrinol Metab **11**, 123–8.
8) Iwasaki T, Miyazaki W, Takeshita A, Kuroda Y, Koibuchi N (2002) Polychlorinated biphenyls suppress thyroid hormone-induced transactivation. Biochem Biophys Res Commun **299**, 384–8.
9) Miyazaki W, Iwasaki T, Takeshiata A, Kuroda Y, Koibuchi N (2004) Polychlorinated biphenyls suppress thyroid hormone receptor-mediated transcription through a novel mechanism. J Biol Chem **279**, 18195–202.
10) Miyazaki W, Iwasaki T, Takeshita A, Tohyama C, Koibuchi N (2008) Identification of functional domain of thyroid hormone receptor responsible for polychlorinated biphenyl-mediated suppression of its action in vitro. Environ Health Perspect **116**, 1231–6.
11) Takasuga T, Senthikumar K, Watanabe K, Takemori H, Shoda T, Kuroda Y (2004) Ultratrace analysis of polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCB) in human serum and cerebrospinal fluid (CSF) samples. Organohalogen Compounds **66**, 2529–34.
12) Park JS, Linderholm L, Charles MJ, Athanasiadou M, Petrik J, Kocan A, Drobna B, Trnovec T, Bergman A, Hertz-Picciotto I (2007) Polychlorinated biphenyls and their hydroxylated metabolites (OH-PCBs) in pregnant women from eastern Slovakia. Environ Health Perspect **115**, 20–7.
13) Miyamoto T, Suzuki S, DeGroot LJ (1993) High affinity and specificity of dimeric binding of thyroid hormone receptors to DNA and their ligand-dependent dissociation. Mol Endocrinol **7**, 224–31.
14) Yang F, Xu Y, Pan H, Wu D (2008) Induction of hepatic cytochrome P4501A1/2B activity and disruption of thyroglobulin synthesis/secretion by mono-ortho polychlorinated biphenyl and its hydroxylated metabolites in rat cell lines. Environ Toxicol Chem **27**, 220–5.
15) Fierens S, Mairesse H, Heilier J, De Burbure C, Focant J, Eppe G, De Pauw E, Bernard A (2003) Dioxin/polychlorinated biphenyl body burden, diabetes and endometriosis: findings in a population-based study in Belgium. Biomarkers **8**, 529–34.