Polychlorinated Biphenyls Suppress Thyroid Hormone Receptor-mediated Transcription through a Novel Mechanism*

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Polychlorinated biphenyls (PCBs) are environmental compounds that disrupt the endocrine system, and exposure to low doses causes abnormalities, particularly in the developing central nervous system. Because they are structurally similar to thyroid hormone (TH), PCBs might affect systems involving this hormone. We previously found, using reporter assays, that hydroxylated-PCB at doses as low as $10^{-10} \text{M}$ suppress TH-induced transcriptional activation of TH receptor (TR). To understand the molecular mechanisms of this process, we examined whether PCBS alter coactivator or corepressor interaction with TR. Polychlorinated biphenyls suppressed steroid receptor coactivator-1 enhanced TR-mediated transcription, but did not reduce TR/steroid receptor coactivator-1 interaction in mammalian two-hybrid and glutathione S-transferase pull-down studies. Thus, the suppression was probably not caused by coactivator dissociation. Nuclear receptor co-repressor was not recruited to TR by PCBs either in vivo or in vitro, indicating that TR corepressor binding did not induce the suppression. We then examined whether PCB influences TR binding to the thyroid hormone-response element (TRE). Electrophoretic mobility shift assays revealed that the TR/retinoid X receptor heterodimer complex was partially dissociated from TRE in the presence of PCB. These results indicate that partial dissociation of TR/retinoid X receptor heterodimer complex from the TRE is involved in the suppression of transcription induced by PCB.

Polychlorinated biphenyls (PCBs)† were produced for industrial use from the early 1930s until the beginning of the 1970s (1–4). These compounds contain 209 congeners, each of which is chlorinated to various degrees (1–3). These compounds are still detectable, even at the edge of the Arctic (5, 6) and in the depths of the oceans (7). After absorption, PCBs undergo little catalysis. They 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, 8). Thus, PCB contamination is inheritable.

At low doses, PCB might affect embryonic and neonatal development (9, 11–13). Brain development seems to be particularly affected by PCB. Cognitive development among children exposed to PCBs in utero and during lactation is poor (10, 14), and the results of studies using rodents have supported this finding (9–13). Because perinatal hypothyroidism also induces abnormal brain development similar to that seen in PCB-affected animal models and in humans, PCB might disrupt the thyroid hormone (TH) system (15). Thyroid hormones (thyroxine (T$_4$) and triiodothyronine (T$_3$)) play important roles in the growth and development of many organs in embryos and neonates (16–18). In particular, TH is crucial to the development of the brain in human fetuses and newborns, because maternal hypothyroidism during the perinatal period causes cretinism with severe cognitive and/or mental disorders in offspring (19). We and others have generated experimental evidence that TH affects the brain only for a short period during its development (19–21). Hypothyroidism during this critical period causes diminished axonal growth and dendritic arborization in the cerebral cortex, visual and auditory cortex, hippocampus, and cerebellum (22, 23).

Thyroid hormone receptors (TRs) are ligand-regulated transcription factors that are expressed in many organs, including the developing brain (16, 17). In the absence of a ligand, TR binds to a specific nucleotide sequence, known as the thyroid hormone response element (TRE), located at the promoter region of its target gene by forming a complex with a coactivator, such as nuclear receptor corepressor (N-CoR) and its related protein, histone deacetylase, and induces the repression of transcription. Once a ligand binds to TR, the complex dissociates, and other complexes, including coactivators such as steroid receptor coactivator-1 (SRC-1), are recruited to induce transcriptional activation (16, 17, 24).

Several postulated mechanisms might account for PCB action on the TR system. Laboratory animals perinatally exposed

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† The abbreviations used are: PCB, polychlorinated biphenyl; TH, thyroid hormone; T$_4$, thyroxine; T$_3$, triiodothyronine; TR, thyroid hormone receptor; TRE, thyroid hormone response element; N-CoR, nuclear receptor co-repressor; SRC-1, steroid receptor coactivator-1; CB, chlorobiphenyl; RXR, retinoid X receptor; GR, glucocorticoid receptor; LUC, luciferase; TK, thymidine kinase; UAS, upstream activating sequence; DBD, DNA-binding domain; NBD, nuclear receptor-binding domain; LBD, ligand-binding domain; GST, glutathione S-transferase; EMSA, electrophoretic mobility shift assay.

ANOVA, analysis of variance; EMSA, electrophoretic mobility shift assay.
to PCBs have abnormal thyroid function and neurological impairment. Many investigations have shown that exposure to PCBs results in thyroid enlargement and reduced serum $T_4$ levels with normal $T_3$, which is the active compound of TH (25–27). Thus, PCB seems to affect the TH system mainly by inhibiting TH secretion in the thyroid gland, which could cause abnormal brain development. However, the general growth of animals exposed to PCBs was not significantly altered, and the $T_4$ levels in each organ remained within the normal range (28). On the other hand, several PCB congeners, particularly hydroxylated forms, can cross the blood-brain barrier and accumulate in the brain (15, 29–31). Thus, abnormal brain development might be induced by the direct action of PCBs. Because the molecular structure of PCBs (especially hydroxylated PCB, an in vitro metabolite of PCB found in the blood) is similar to that of TH, PCB might act through TR (15). However, binding studies have shown that the affinity of TR$\beta_1$ for hydroxylated PCB is less than 1/10,000 that of $T_3$ (15).

We previously examined whether PCB directly acts on TR, and reported that 10$^{-10}$ M hydroxylated PCB (4(OH)-2',3',3'-pentapenta CB) suppressed TR-mediated transcription in the presence of $T_3$. The suppression induced by PCB was cell-specific, because clonal TE671 cells, which are derived from cerebellar granule cells, were particularly susceptible. After transfection with SRC-1, PCB suppressed TR-mediated transcription in the presence of $T_3$. We also found that the suppression of TR-mediated transcription by PCBs was probably not caused by competition between PCB and $T_3$ for TR binding (32). The present study further examines the molecular mechanism of the suppressive action of PCB on TR-mediated transcription. We used the hydroxylated mono-ortho form of PCB because this type is produced from highly toxic parent PCBs, and it could be a key factor in abnormal brain development (33–35).

**EXPERIMENTAL PROCEDURES**

**Polychlorinated Biphenyls—**Aroclor 1254 was purchased from Sigma. 4(OH)-2',3',3'-pentapenta CB, 4(OH)-2',3',3'-A',5,5'-hexa CB, and 2,3,3',4',5'-hexa CB were purchased from Accustandard Chemicals (New Haven, CT). Their chemical structures are shown in Fig. 1A. **Plasmids—**Expression vectors of TR$\beta_1$, retinoid X receptor (RXR)$\beta$ (36), and the glucocorticoid receptor (GR) (37), have been described previously (36). GST-N-CoR (aa 2053–2414) was constructed by inserting PCR-generated fragments of N-CoR (a gift from Dr. M. G. Rosenfeld, University of California-San Diego, La Jolla, CA) into the insert of the VP16 activation domain in AASV-VP16. The Gal4-blank plasmid was used as an internal control. Sixteen to 24 h after transfection, cells were refilled with fresh medium containing the indicated concentration of specific ligand and/or PCBs. After 24 h, cells were harvested to measure luciferase activities as described previously (32). Total amounts of DNA for each well were balanced by adding pcDNA3 plasmids (Invitrogen). The luciferase activities were normalized to $\beta$-galactosidase activity and then calculated as relative luciferase activity. All transfection studies were repeated at least twice in triplicate. Data shown represent mean ± S.E. of experiments performed in triplicate. The data were analyzed by ANOVA.

**Trypan Blue Exclusion—**Trypan blue exclusion was described previously (42). Briefly, CV-1 cells were plated in 100-mm diameter plates 2 days before adding PCB. Each cell was incubated in the presence or absence of 10$^{-6}$-10$^{-8}$ M 4(OH)-2',3',3'-4',5'-penta CB. Twenty-four hours after changing medium, cells were incubated for 2–5 min in a solution of 0.2% trypan blue in phosphate-buffered saline. In each experiment, nonviable cells (colorless) and viable cells (stained blue) were counted. The data were analyzed by ANOVA.

**In Vitro-Translated Proteins and GST Pull-down Assay—**The GST fusion proteins were produced in *Escherichia coli* BL21 (DE3) and purified by glutathione-Sepharose resin (Amersham Biosciences). In vitro binding assays were performed by incubating GST resin (20 $\mu$l, 2 $\mu$g) and [35S]methionine-labeled, in vitro-translated proteins ($\mu$l) that were produced by rabbit reticulocyte lysate (Promega, Madison, WI) according to the manufacturer's instructions. Similar amounts of binding fusion proteins bound to the beads were used, as determined by Coomassie Blue staining/SDS-polyacrylamide gel electrophoresis analysis. Proteins were incubated at 4°C for 16 h in the binding buffer (20 mM HEPES, pH 7.4, 50 mM NaCl, 75 mM KCl, 1 mM EDTA, 0.05% Triton X-100, 10% glycerol, 1 mM dithiothreitol) in the presence or absence of $T_3$ (10$^{-6}$ M) and/or 4(OH)-2',3',3'-4',5'-penta CB (10$^{-6}$ Mg). Bound proteins were washed three times with binding buffer in the presence or absence of TR and subjected to SDS-PAGE and autoradiography.

**Electrophoretic Mobility Shift Assay (EMSA)—**Methods for EMSA were described previously (43). In brief, double-stranded oligonucleotides containing F2 (sense, 5'-agtattgaggcagctggagctag-3'; antisense, 5'-ctgaattgacgctgacaggtgct-3'); F3 (sense, 5'-ccgaatgtgagctgagcgctgag-3'; antisense, 5'-gtctgactgctgctgctgctg-3'); and/or GST-fused SRC-1, N-CoR, and 1-LBD was produced by *Escherichia coli* that were produced by ethanol precipitation. In vitro-transcribed and -translated TR$\beta_1$, RXR$\beta$, and/or GST-fused SRC-1, N-CoR, and 1$\times$ 10$^{4}$ cpm of labeled templates were incubated in binding buffer (25 mM HEPES, pH 7.6, 5 mM MgCl$_2$, 4 mM EDTA, 110 mM NaCl, 5 $\mu$g/ml bovine serum albumin, 1 $\mu$g/ml poly(dI-dC), 20% glycerol, and 2 mM dithiothreitol) and in the presence or absence of 10$^{-6}$ M $T_3$ and/or 10$^{-6}$ M 4(OH)-2',3',3'-4',5'-penta CB for 30 min on ice. Different amounts of control reticulocyte lysate were added to some samples so that the total volume of the reticulocyte lysate was consistent. After incubation, samples were subjected to electrophoresis and analyzed with the use of autoradiography.

**RESULTS**

**The Suppression of TR-mediated Transcription Is Congener-dependent—**We initially tested a series of PCB congeners including Aroclor 1254, a mixture of non-hydroxylated and hydroxylated PCBs, because the toxicity of congeners differs (2). All congeners used here suppressed TR-mediated transcription on the F2-TRE at various degrees of magnitude (Fig. 1A). These congeners also suppressed transcription when SRC-1 was co-transfected (Fig. 1B). However, the effect of PCB was not always dose-dependent, suggesting that competitive binding between PCB and $T_3$ probably does not cause the suppression. Trypan blue exclusion confirmed that the suppression by PCBs ($10^{-6}-10^{-8}$ M) did not result from cell death (data not shown). We obtained similar results of PCB action on DR4-TRE and 2',5'-pal-TRE (Fig. 2A). On the other hand, the PCBs did not inhibit RXR-mediated transcription in the presence of 10$^{-7}$ M 9-cis-retinoic acid (Fig. 2A) or GR-mediated transcription in the presence of 10$^{-7}$ M dexamethasone with or without SRC-1 co-transfection (Fig. 2B and Ref. 32), suggesting that this suppression is likely to be TR-specific.

**Basal Transcription in CV-1 Cells Was Not Affected by PCB—**We examined whether PCB affects basal transcription. In the absence of $T_3$, we cotransfected F2-TRE-LUC containing the TK promoter with TR$\beta_1$ or vector alone. Transcription in the presence or absence of TR was not affected by PCB (Fig.
A). Together with the results shown in Figs. 1 and 2, these findings suggest that PCB did not affect basal transcription under our experimental conditions.
PCB Mediates TR Dissociation from TRE

PCB Did Not Affect TR-mediated Transcription in the Absence of T3—Because PCB might bind to TR as a result of structural similarity, we further examined whether PCB suppresses TR-mediated transcription in the absence of T3. TR reduced transcription in the absence of T3. Transcription was not affected by 10−10, 10−8 M PCB in the absence of T3 (Fig. 3A). Cotransfection with N-CoR further repressed transcription in the absence of T3 (Fig. 3B). Adding PCB did not affect transcription, suggesting that PCB did not affect N-CoR-mediated suppression (Fig. 3B).

SRC-1 Was Not Dissociated from TR by PCB—We investigated the effect of PCB on binding between TR and SRC-1. Using a mammalian two-hybrid assay in CV-1 cells, we tested interactions between SRC-1-NBD-1 and TRβ1-LBD in the presence of T3 and PCB. The NBD-1 of SRC-1 was fused to the Gal4-DNA binding domain, and the LBD of TRβ1 was fused to the transactivation domain of VP16 (Fig. 4A). Transactivation mediated by Gal4-SRC-1-NBD-1 and VP16-TRβ1-LBD proceeded in the presence of T3, but not in the absence of the VP16 construct and T3 (data not shown). Activated transcription induced by SRC-1-NBD-1 and VP16-TRβ1-LBD binding with T3 was not significantly reduced by 10−8 M hydroxylated PCB (p < 0.01) (Fig. 4B). Neither VP16 alone nor PCB significantly increased transcription. These results suggest that PCB did not affect the binding of SRC-1 and TRβ1-LBD in the presence of T3. We further confirmed this observation using GST pull-down studies (Fig. 5). GST-fused SRC-1-NBD-1 did not dissociate from TRβ1 in the presence of 10−8 M T3 and 10−8 M PCB. These results suggest that the suppressive effect of PCB on TR-mediated transcription is not caused by changes in TR and SRC-1 interaction.

PCB Did Not Recruit N-CoR to TR—Moriyama et al. (40) have shown that bisphenol A, another endocrine-disrupting chemical, inhibits TR-mediated transcription by acting as an antagonist and recruits complexes containing N-CoR to TR in the presence of T3. We performed mammalian two-hybrid assays to examine whether PCB recruits N-CoR to TR. We cotransfected Gal4-fused N-CoR with VP16-TRβ1 and 5× UAS-TK-LUC into CV-1 cells. Transcription was not activated by increasing the concentration of PCBs (Fig. 6), suggesting that PCB did not recruit N-CoR to TRβ1 in the presence of T3 in vivo. We confirmed interaction between N-CoR and TRβ1 using GST-pull down studies. GST-fused N-CoR bound to TR in the absence of T3, whereas N-CoR dissociated from TR and PCB did not recruit N-CoR to TR in the presence of T3 (Fig. 7). These results indicated that PCB did not recruit the corepressor complex containing N-CoR to TR.

Fig. 3. PCB did not affect the F2-TRE-mediated transcription without TR in CV-1 cells. A, expression plasmids encoding TRβ1 (10 ng) or empty vectors were cotransfected with F2-TK-LUC (100 ng) into CV-1 cells. Cells were incubated with indicated amounts of 4(OH)-2, 3, 3′, 4, 5′-penta CB without T3. Total amounts of DNA for each well were balanced by adding vector pcDNA3. No statistical significance was determined by ANOVA, except where indicated (*, p < 0.05).

Fig. 4. PCB did not inhibit SRC-1 binding to TRβ1 in vivo. A, schematic diagram of Gal4-SRC-1-NBD-1 and VP16-TRβ1-LBD. Functionally active LXXLL motifs are located in central (residues 633–637, 690–694, and 749–753) and C-terminal (residues 1434–1438) regions, termed NBD-1 and NBD-2, respectively. Gal4-SRC-1-NBD-1 contains amino acid residues 595–790 of SRC-1. B, expression plasmids encoding Gal4-DBD-fused SRC-1-NBD-1 (10 ng) were cotransfected with VP16-construcst (50 ng) and 5× UAS-TK-LUC-reporter plasmids (170 ng) into CV-1 cells. Cells were incubated with or without T3 (10−7 M) and 10−8 M 4(OH)-2, 3, 3′, 4, 5′-penta CB. Total amounts of DNA for each well were balanced by adding vector pcDNA3. Data represent mean ± S.E. of experiments performed in triplicate. No statistical significance was uncovered by ANOVA (p < 0.01).
Partial Dissociation of TR from TRE in the Presence of PCB—To examine whether PCB induced suppression of TR-mediated transcription is associated with TR-LBD, we performed mammalian one-hybrid assays in CV-1 cells. In contrast to co-transfection with TRE-LUC, increasing amounts of PCB tended to activate transcription in the presence of T₃ (Fig. 8), suggesting that the effect of PCB on TRβ1 probably does not occur through LBD. Therefore, we postulated that PCB affects TR-mediated transcription by interfering with the binding of TR to TRE. Finally, we investigated the effect of PCB on the binding of TRβ1 to TRE using EMSAs. In the presence of 10⁻⁶ M T₃, TRβ1/RXR/β complex was partially dissociated (48.5 ± 1.7%, n = 3) from the inverted palindrome TRE, F₂-TRE, by 10⁻⁸ M PCB (Fig. 9A, lane 6), suggesting that TR-mediated suppression of transcription induced by PCB is at least partly caused by dissociation of TR/RXR complexes from the TRE. In the absence of T₃, TR/RXR complexes were also partially dissociated (46.6 ± 4.2%, n = 3, Fig. 9A, lane 4). The results were similar using DR₄-TRE (data not shown). In case of TRβ1 homodimer, about half of the TRβ1 homodimer was dissociated (47.8 ± 3.2%, n = 3) from the TRE by PCB in the absence of T₃ (Fig. 9A, lane 8). In the presence of T₃, TRβ1 homodimer was dissociated from TRE (Fig. 9A, lane 9), and the addition of PCB did not affect the binding (Fig. 9A, lane 10). We further examined the TRβ1-TRE dissociation when SRC-1 was added to TRβ1/RXR/β hetero- or TRβ1 homodimer complex using EMSA (Fig. 10, A and B). In the presence of RXR, SRC-1-NBD-1 bound to the TR/RXR complex (Fig. 10A, lane 6) in the presence of 10⁻⁶ M T₃. Addition of 10⁻⁷ M PCB caused the partial dissociation of the TR/RXR/SRC-1 complex (50.5 ± 2.1%, n = 3) from the TRE (Fig. 10A, lane 7). TRβ1 homodimer bound to TRE in the absence of T₃ (Fig. 10B, lane 2). In the presence of 10⁻⁶ M T₃, the TRβ1 homodimer dissociated from TRE (Fig. 10B, lane 5) as described previously. The addition of SRC-1 resulted in TR/TR/SRC-1 complex formation (Fig. 10B, lane 6). The addition of PCB caused partial dissociation of the TR/TR/SRC-1 complex to a lesser extent than in the TR/RXR/SRC-1 complex (20.8 ± 1.0%, n = 3, Fig. 10B, lane 7).

We also examined TR-TRE binding with N-CoR (Fig. 11). In the absence of T₃, N-CoR bound to the TR homodimer (Fig. 11, lane 3). Adding PCB did not affect the binding (Fig. 11, lane 4), which was compatible with the functional data. In the presence of T₃, N-CoR did not bind to the TR homodimer (Fig. 11, lane 5). The addition of 10⁻⁷ M PCB did not recruit the N-CoR (Fig. 11, lane 7), which is compatible with the results of the mammalian two-hybrid assay (Fig. 6). When the TR homodimer was replaced with the TR/RXR heterodimer, N-CoR did not bind to the heterodimer even without T₃ (data not shown). Cohen et al. (44) reported similar results with DR₄-TRE. In addition, PCB did not recruit N-CoR to TR/RXR complex in the presence of T₃.
DISCUSSION

We showed that the suppression of TR-mediated transcription by PCB is probably induced through the partial dissociation of TR from TRE in the presence of T₃. This result was compatible with the functional data showing that about 50% of transcription in CV-1 cells is suppressed by PCB. We also showed that the magnitude of suppression varies according to congeners.

Polychlorinated biphenyl compounds might bind to TR because of structural similarity to TH. Thus, we previously examined the dose dependence of the effect of mono-ortho hydroxylated PCB (4(OH)-2',3',3',4',5'-penta CB) on TR-mediated transcription. However, the magnitude of transcriptional suppression between 10⁻¹⁰ and 10⁻⁸ M PCB was not statistically significant (32). The present study further confirmed, using several different PCB congeners, that PCB suppresses transcription. If the PCB effect is induced by the competitive inhibition of T₃ binding TR, then PCB action should not be dose-independent. Furthermore, the affinity of several different PCB congeners is less than 1/10,000 that of T₃ (15). Thus, the effect of PCBs on TR-mediated transcription is probably not caused by competition with TH for TR binding. The suppressive effect of PCB was not induced by cell death, because Trypan blue exclusion showed that PCB did not affect cell viability (data not shown).

We initially postulated that the effect of PCB could be induced by dissociation of the coactivator complex from TR or by recruitment of a corepressor complex, because bisphenol A, which suppresses TR action, recruits corepressor complexes containing N-CoR (40). However, PCB did not dissociate SRC-1 from TR either in vivo or in vitro. PCB also did not recruit complexes containing N-CoR to TR. Thus, the mechanism of PCB action on TR-mediated transcription differs from that of bisphenol A. On the other hand, PCB did not affect transcription in the absence of T₃ in either the presence or the absence of TR, suggesting that PCB does not affect basal transcription of the TK promoter in CV-1 cells. Involvement of the aryl hydrocarbon receptor, to which some PCB congeners bind (45, 46), might also be excluded, because aryl hydrocarbon receptor is not expressed in CV-1 cells (47).

By using EMSA, we finally found that PCB partially dissociated TR from TRE in the presence of T₃. The magnitude of dissociation was ~50%, which is compatible with the findings of the transcriptional analysis. TR/RXR/SRC-1 complex was also partially dissociated (50%) from TRE by PCB. On the other hand, the effect of PCB on TR/TR/SRC-1 complex was much weaker (20.6%). The cause of such difference is obscure. Because PCB was not effective on RXR-mediated transcription, the site of PCB action is likely to be within TR. The conformational difference of TR in homo- and heterodimer complex may have altered PCB sensitivity. On the other hand, in EMSA,
albeit PCB also partially dissociated TR homo- or heterodimer from TRE without T$_3$, it did not dissociate TR/TR/N-CoR complex from TRE. This result is compatible with transcriptional analysis, because PCB did not alter TR-mediated transcription without T$_3$, when corepressor may bind to TR. These results indicate that conformational change of TR in TR/corepressor complex may alter the PCB sensitivity.

In mammalian one-hybrid study using Gal4-DBD-TR-LBD and full-length SRC-1, adding PCB slightly increased transcription (Fig. 8). Because the main cause for TR-mediated transcriptional suppression by PCB in the present study may be partial dissociation of TR-TRE binding, such increase in TR-SRC-1 binding might not alter the effect of PCB on TR-mediated transcription. However, this result indicates that PCB might act upon several regions of TR such as DBD and LBD.

Studies in vivo have led to the notion that the site of PCB action on the thyroid hormone system is the thyroid gland. Maternal PCB crosses the placenta and is secreted into milk, which might cause the fetal or newborn thyroid gland to reduce thyroid hormone secretion. Consequently, TR-mediated brain development might be disrupted. However, although the plasma T$_4$ concentration is decreased by perinatal PCB exposure, such animals generally grow normally, which is usually retarded by perinatal hypothyroidism. Furthermore, despite reduced plasma T$_4$, the T$_3$ concentration in peripheral organs is not altered by PCB exposure. Therefore, the peripheral organs might not become severely hypothyroid after PCB exposure. The present study showed that PCB acts directly on the TR to suppress transcription. Thus, we believe that the main site of action of PCB on the TH system is TR and not the thyroid gland. If so, why is the effect of PCB greater in the brain than in other organs? Additional studies are required to address this issue. Many tissue-specific factors might alter PCB action on TR-mediated transcription.

A key quandary of PCB is that at low doses, which do not cause maternal problems, PCB compounds and their hydroxylated forms are transferred to the embryo through the placenta and are secreted into milk, and they might disrupt brain development or cause other abnormalities (10, 14). Synaptogenesis mainly proceeds in the developing human brain during the perinatal to the infant period (22, 23) when the blood-brain barrier is not yet well developed. Thus, toxic chemicals in the blood can easily enter the fetal brain during the critical period when functional neuronal connections are being established (33). Recently, Kuroda and colleagues (33, 48) established primary cell cultures of the rat cerebellar cortex, in which neurons form vast numbers of synapses in the presence of TH. A similar low dose of hydroxylated PCB also inhibited the TH-dependent extension of dendrites of Purkinje neurons in this system (33). Together with the present study, these two independent studies show a low-dose effect of PCBs on TR-associated biological events.

In summary, our results suggest that PCBs suppress TR-mediated transcription directly through partial dissociation of TR from the TRE. The site of PCB action might not be thyroid gland, but rather TR, which is expressed in many organs, including the central nervous system. We hope that this study will significantly contribute to establishing appropriate restrictions on PCB intake to avoid the specter of complications arising in the future generations.

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