PCB affect on DNA binding domain of thyroid hormone receptors

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Abstract

Perinatal exposure of polychlorinated biphenyls (PCBs), dibenzo-p-dioxin (PCDDs) and dibenzofurans (PCDFs) may influence the mammalian brain development. However, the mechanisms of action have not yet been clarified. We recently reported that low-dose hydroxylated-PCB, whose TEF is almost negligible, suppressed thyroid hormone (TH) receptor (TR)-mediated transcription by dissociation of TR from TH response element (TRE). Since TH is important factor for brain development, some brain abnormalities induced by PCB/dioxins may be caused through the disruption of TH system. In this study, we investigated the effects of several PCB/dioxins on TR-mediated transcription.

TCDD and PCDFs did not affect TR-mediated transcription, whereas several PCBs and their hydroxylated compounds altered TR action at various degrees. The degree of suppression of TR action was correlated with that of dissociation of TR from TRE. These results suggest that PCBs may act on DNA binding domain (DBD) of TR. Thus, we performed reporter assay with chimeric receptors made from domains of TR and glucocorticoid receptor. The suppressions by PCBs were observed on chimeric receptors containing TR-DBD.

These results indicate that PCBs affects TR-mediated transcription through altering the conformation of DBD. The toxicity index should be rearranged based on several critical factors such as influence on TR-mediated transcription.

Introduction

TH is crucial to the development of the brain in human fetuses and newborns, because maternal hypothyroidism during the prenatal period causes cretinism with severe cognitive and/or mental disorders in offspring[1]. Jacobson JL et al reported that *in utero* exposure of PCBs exerted intellectual impairment[2], which is also observed in cretinism patient. Previously, we have been shown that as low as 10^{-10} M of hydroxylated PCB (4(OH)-2', 3, 3', 4', 5'-penta CB), whose TEF is almost negligible, suppresses the TR-mediated transcription induced by TH.[3] Subsequently, we have shown that this suppression is due to partial dissociation of TR from TRE by PCB.[4] Therefore, we further investigated the effects of dioxins and coplanar PCBs on TR-mediate transcription to consider the relationship between TEF and the effect of TR-mediated transcription. We also investigated which region in TRβ1 is responsible for the suppressive actions of dioxins and PCBs.

Materials and Methods

To compare TR-mediated transcription with various concentrations of PCB/dioxins, we performed transient cotransfection experiments using fibroblast derived CV-1cells and neuroblastoma derived TE671 cells. The degrees of dissociation of TR-TRE binding by such compounds were studied using electrophoretic mobility shift assay (EMSA).

To investigate which domain of TR is responsible for PCB/dioxins action, we performed reporter gene assays with chimeric receptors containing GR or TR functional domains.

Results and Discussion

2,3,7,8-TCDD and PCDFs did not affect on TR-mediated transcription. Various effects were observed by PCBs, from severe suppression to potentiation (Table). The degree of TR dissociation from TRE is correlated with that of suppression by PCBs. These results suggest that TEF of dioxins and PCBs do not always correctly indicate their toxicity.

chemicals	TEF	CV-1	TE671
2,3,7,8-TCDD	1	→	→
2,3,4,7,8-PCDF	0.5	→	↓
2,8-DCDF	n.d.	→	→
2-MCDF	n.d.	→	→
PCB77	0.0001	↓	↓
PCB114	0.0005	↓	↓
PCB118	0.0001	→	→
PCB126	0.1	↓	↓
PCB153	n.d.	↓	↓
4OH-PCB106	n.d.	↓	↓
4OH-PCB159	n.d.	↓	↓
4OH-PCB165	n.d.	→	→
4OH-PCB187	n.d.	↓	↓

Table. Effects of PCBs/dioxins on TR-mediated transcription

The effects of PCBs/dioxins on TR-mediated transcription in the presence of T3 (10^{-7} M) were shown in this table. These effects are indicated by the down-arrows (suppression) and horizontal arrows (no-change) at the dose of 10^{-8}M PCBs/dioxins.

Furthermore, the suppression of PCBs were observed with chimeric receptors containing TR-DBD. When TR-DBD was replaced by GR-DBD, the effect of PCB was not observed with such chimeric receptors (Figure). These results indicate that PCB action is exerted through DBD of TR, possibly by altering the conformation of this domain.

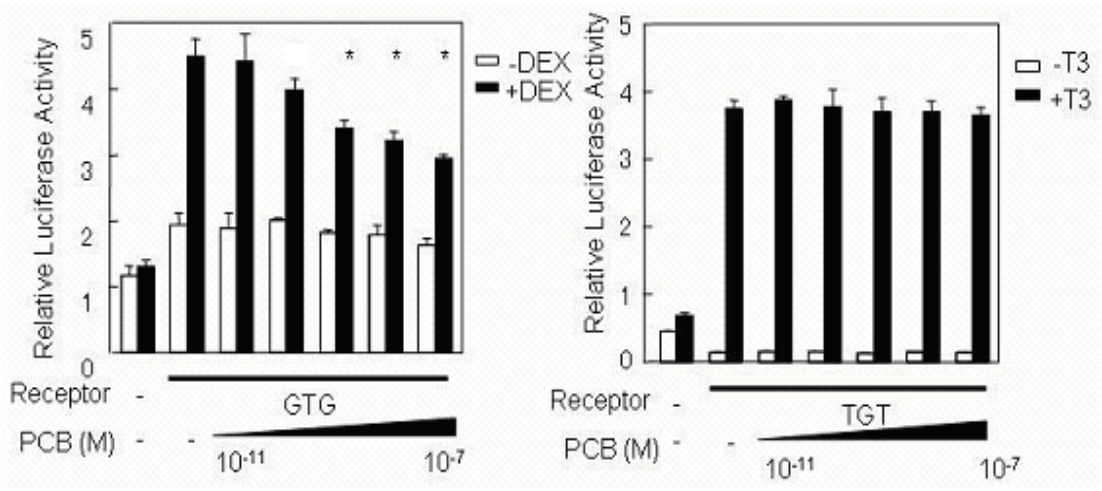

Figure. PCBs affect on TR-mediated transcription through DNA binding domain of TR.

Each chimeric receptors (10 ng) were cotransfected with F2-TK-LUC reporter plasmid (100 ng) or GRE-LUC reporter plasmid (100ng) into CV-1 cells. Cells were incubated with or without T3 (10^{-7} M) or dexamethasone (DEX) (10^{-7} M) and 10^{-8} M of 4OH-PCB106. Total amounts of DNA for each well were balanced by adding vector pcDNA3. Data represents mean in triplicate ± standard error of means (S. E. M.). *: Statistically significant (p<0.01 by ANOVA) vs. TRβ1 (+), T3 (+) and PCB (-).

In the separate experiment, we have also shown that PCB altered intracellular calcium levels, which alter the expression of calcium sensitive genes. Thus, the toxic effects of these compounds on the development of central nervous system might be exerted through several different mechanisms. In conclusion, we propose that the toxicity index should be rearranged based on several critical factors.

Acknowledgement

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