THYROID HORMONAL AND ESTROGENIC ACTIVITY OF OH-PCB AND OH-PBDE IN CELL CULTURE

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Abstract
The thyroid hormone-disrupting activity of hydroxylated PCBs and polybromo-diphenyl ethers (PBDEs) was examined. 4-Hydroxy-2,2',3,4',5,5'-hexachlorobiphenyl (4-OH-2,2',3,4',5,5'-HxCB), 4-hydroxy-3,3',4',5-tetrachlorobiphenyl (4-OH-3,3',4',5-TCB), 4,4'-dihydroxy-3,3',5,5'-tetrachlorobiphenyl (4,4'-diOH-3,3',5,5'-TCB), 4-hydroxy-2,2',3,4',5-pentabromodiphenyl ether and tetrabromobisphenol A (TBBPA) markedly inhibited the binding of triiodothyronine to thyroid hormone receptor (TR). However, 4-hydroxy-2',4',6'-trichlorobiphenyl (4-OH-2',4',6'-TCB), 4-hydroxy-2,2',5,5'-tetrachlorobiphenyl, 4-hydroxy-2,3,3',4'-tetrachlorobiphenyl, 2,3',5,5'-tetrachlorobiphenyl and 2,2',3,4,4',5'-hexabromodiphenyl ether did not show affinity for TR. The thyroid hormonal activity of these chemicals was also examined using rat pituitary cell line GH3 cells, which grow and release growth hormone in a thyroid hormone-dependent manner. 4-OH-2,2',3,4',5,5'-HxCB, 4,4'-diOH-3,3',5,5'-TCB, 4-OH-3,3',4',5-TCB and TBBPA enhanced the proliferation of GH3 cells and stimulated their production of growth hormone. In contrast, 4-OH-2',4',6'-TCB and 4'-hydroxy-2,2',4',5-tetrabromodiphenyl ether exhibited a significant estrogenic activity using estrogen-responsive reporter assay in MCF-7 cells. However, the 3,5-dichloro substitution of 4-hydroxylated PCBs markedly decreased the estrogenic activity. These results suggest that a 4-hydroxyl group in PCBs and PBDEs is essential for thyroid hormonal and estrogenic activities, and that 3,5-dichloro or dibromo substitution favors thyroid hormonal activity, but not estrogenic activity.

Introduction

It has been reported that hydroxylated PCBs and PBDEs can disrupt thyroid hormone status. Hydroxylated PCBs showed high binding affinity for the serum thyroid hormone binding protein transthyretin, thereby displacing the natural ligand, T4.[1,2] In vivo toxicity of hydroxylated PCBs on thyroid hormone homeostasis was also suggested on the basis of their high binding affinity to transthyretin.[3,4] However, the exact mechanisms of interference with thyroid hormonal action are not fully understood.

In the present presentation, we deal with the thyroid hormonal and anti-thyroid hormonal activities of hydroxylated PCBs and PBDEs as examined by means of binding assay with TR, as well as thyroid hormone-dependent growth assay and assay of production of growth hormone (GH) in pituitary cell line GH3 cells. The results were compared with the estrogenic activity observed in ERE-luciferase reporter assay.

Materials and Methods

Hydroxy-PCBs were synthesized by the previously reported method.[5] Hydroxy-PBDEs were obtained from Accu Standard (New Haven, CT, USA).

Competitive binding assay to thyroid hormone receptor - Nuclear extracts of MtT/E were used for the assay, since this cell line expresses high amounts of thyroid hormone receptor. Test chemicals and ^{125}I-T3 were incubated with the nuclear suspension. After incubation, radioactivity of the pellets was counted with a gamma counter.

GH production assay and thyroid hormone-dependent growth assay in GH3 cells - These assay were performed as reported previously.[6]

Assay of estrogenic activity of hydroxylated PCBs - ERE-luciferase reporter assay using MCF-7 cells was performed according to the previously reported method.[7]

Results and Discussion

Competitive binding assay for thyroid hormone-like compounds

The inhibitory effects of PCBs and PBDEs on binding of T3 to TR were examined. T3 competitively inhibited the binding of ^{125}I-T3 ($1x10^{-10}$ M) to TR in the range of $1x10^{-9}$ – $1x10^{-8}$ M. 4-OH-2,2',3,4',5,5'-HxCB, 4-OH-3,3',4',5-TCB, 4,4'-diOH-3,3',5,5'-TCB, 4-OH-2',3,5,5'-TCB, 4-OH-3,3',4',5,5'-PCB, 4-OH-2,2',3,4',5,5',6-HpCB, 4-OH-3,4',5-TCB, 4-OH-2',3,4',5,6'-PCB, 4-OH-2',3,4',5-TCB, 4-hydroxy-2,2',3,4',5-pentabromo-diphenyl ether and TBBPA also markedly inhibited the binding of ^{125}I-T3 to the receptor in the concentration range of $1x10^{-5}$ – $1x10^{-4}$ M. Among these compounds, 4-OH-2,2',3,4',5,5',6-HpCB exhibited the greatest activity, followed by 4-OH-2,3,4',5,6'-PCB, 4-OH-3,3',4,5,5'-PCB, 4-OH-3,3',4',5-TCB. 2,3',5,5'-TCB, 2,3',4',5,5'-PCB, 4-OH-2',4',6'-TCB, 2-OH-3,3',4,4'-TCB, 3-OH-2,2',5,5'-TCB, 4-OH-2,3,3',4'-TCB, 4-OH-2,2',5,5'-TCB, 2,2',3,4,4',5'-hexabromodiphenyl ether and decabromodiphenyl ether showed little or no affinity. Elevated binding affinity was observed for 4-hydroxylated PCBs and PBDEs with halogen substitution at both the 3- and 5-positions of the phenyl group. 4-Hydroxy-PCBs chlorinated at the 3- or 5-position, but not both, showed little activity. PCBs with a hydroxyl group at the 3- or 2-position of the phenyl ring, and 4-methoxy-3,3',4',5-TCB also showed little affinity.

Thyroid hormonal activity evaluated by GH production and growth by GH3 cells

The thyroid hormonal activity of hydroxylated PCBs and TBBPA were examined by measuring the ability of these compounds to induce the production of GH by GH3 cells and by assay of thyroid hormone-dependent growth of GH3 cells. GH release activity was observed with T3 in the range of $1x10^{-10}$ - $1x10^{-8}$ M. When GH release from GH3 cells was measured after the addition of 4-OH-2,2',3,4',5,5'-HxCB and 4-OH-3,3',4',5-TCB, an increase was observed in the concentration range of $1x10^{-6}$ to $1x10^{-5}$ M. Positive result was also observed in 4,4'-diOH-3,3',5,5'-TCB and TBBPA at $1x10^{-5}$ M. However, 2,3',5,5'-TCB, 3-OH-2,2',5,5'-TCB and 4-OH-2,2',5,5'-TCB showed no significant effect. The inhibitory effects of PCBs on the hormonal activity of T3 on GH3 cells were examined. These compounds at $1x10^{-5}$ and $1x10^{-4}$ M did not show antagonistic action towards GH production induced by $1x10^{-10}$ and $1x10^{-9}$ M T3. These results suggest that some hydroxylated PCBs tested in this study and TBBPA act as thyroid hormone agonists, but not antagonists.

The growth-inducing effect of T3 on the cells was observed over the concentration range of $1x10^{-10}$ - $1x10^{-8}$ M. When 4-OH-2,2',3,4',5,5'-HxCB, 4-OH-3,3',4',5-TCB, 4,4'-diOH-3,3',5,5'-TCB and TBBPA were added to the cells, growth was also stimulated at $1x10^{-6}$ - $1x10^{-5}$ M. 4-OH-2,2',3,4',5,5'-HxCB showed the highest activity, followed by 4-OH-3,3',4',5-TCB and 4,4'-diOH-3,3',5,5'-TCB. These compounds at concentrations of $1x10^{-5}$ and $1x10^{-4}$ M did not inhibit the induction of GH3 cell growth by $1x10^{-10}$ and $1x10^{-9}$ M T3. These results confirmed that these hydroxylated PCBs and TBBPA act as thyroid hormone-disruptors by exhibiting agonistic activity.

Estrogenic activity of hydroxylated PCBs

4-OH-2',4',6'-TCB and 4'-hydroxy-2,2',4',5-tetrabromodiphenyl ether exhibited a significant estrogenic activity using estrogen-responsive reporter assay in MCF-7 cells in the concentration range of $1x10^{-7}$ - $1x10^{-5}$ M. 4-OH-2,3,3',4'-TCB, 2-OH-3,3',4,4'-TCB, 4-OH-2,2',5,5'-TCB, 4-OH-3,3',4',5-TCB, 3-OH-2,2',5,5'-TCB and TBBPA exhibited low activity in the concentration of $1x10^{-5}$ M. However, no estrogenic activity of 4-OH-2,2',3,4',5,5'-HxCB, 4,4'-diOH-3,3',5,5'-TCB, 4-OH-2,2',3,4',5,5'-HxCB, 4-OH-3,3',4',5-TCB, 3-OH-2,2',5,5'-TCB, 4-OH-2,2',5,5'-TCB, 2,3',5,5'-TCB, 4-hydroxy-2,2',3,4',5-pentabromodiphenyl ether or 2,3',4',5,5'-PCB was observed. These experiments indicate that a hydroxyl group of PCBs and PBDEs is essential for estrogenic activity, but 4-hydroxyl PCBs with an ortho-chlorine substituent show decreased estrogenic activity, though thyroid hormonal activity is unaffected.

Here, we present the evidence that some hydroxylated PCBs and PBDEs exhibit thyroid hormonal activity through interaction with TR. Recently, thyroid hormone-disrupting action of some hydroxy-PCBs has been discussed. These hydroxy-PCBs were reported to have binding capability to TR, as well as the serum transport protein transthyretin.[3,8,9] Iwasaki et al.[10] reported that 4-hydroxy-2',3,3',4',5'-pentachlorobiphenyl acts as an antagonist by suppression of the interaction of TR and a coactivator. In the current study using GH3 cells, we found thyroid hormone agonistic activities of hydroxylated PCBs and TBBPA. A rat pituitary cell line, GH3, isolated from a rat pituitary tumor has been widely used as a standard pituitary cell model. Cell proliferation, as

well as growth hormone secretion, has been shown to depend on thyroid hormones, but only slightly on estrogen.[11] The reason for the difference between the agonistic and antagonistic actions of hydroxy-PCBs and TBBPA may be due to the different responses of the cells used.

A 4-hydroxyl group and adjacent 3,5-halogen substituents on the phenyl group seem to be essential structural factors for binding to TR. Another chlorinated phenyl ring substituted at the 1-position of the phenyl ring bearing the 4-hydroxyl group also seems to be necessary for thyroid hormonal activity, because 4-hydroxy-3,5-dichlorobenzene lacks the activity. In contrast, we found that 4-OH-2',4',6'-TCB, a 4-hydroxy-PCB without 3,5-chlorine atoms, is estrogenic by means of an estrogen-responsive reporter test in a human breast cancer cell line MCF-7.[12] However, 4-hydroxy-3,5-dichlorinated PCBs exhibited little estrogenic activity. These results are consistent with the estrogenic activity of hydroxylated PCBs reported elsewhere.[13,14] It is interesting that when 4-hydroxy-PCBs have 3,5-dichloro substitution, their estrogenic activity is markedly decreased, whereas their thyroid hormonal activity is not. The results suggest that hydroxylated PCBs exhibit endocrine-disrupting action via effects on at least two different hormonal activities *in vivo*.

In conclusion, the structural requirements of hydroxylated PCBs and PBDEs for thyroid hormonal activity are a 4-hydroxyl group and 3,5-dihalogen atoms substituted adjacent to the hydroxyl group. The requirement for estrogenic activity is a 4-hydroxyl group, but 3,5-dihalogen substitution of 4-hydroxy-PCB and 4-hydroxy-PBDE reduces the estrogenic activity.

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