Inhibition of thyroid hormone sulfation by hydroxylated metabolites of polychlorinated biphenyls *in vitro*

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1. Introduction

Hydroxylated polychlorinated biphenyls (PCBs) are known for their competitive inhibition of thyroid hormone binding to proteins such as transthyretin and type I deiodinase [1,2,3]. Various halogenated phenols including pentachlorophenol were already found to be strong inhibitors of sulfotransferase (ST) activities towards thyroid hormone. It is possible, therefore, that hydroxylated PCB metabolites also inhibit thyroid hormone sulfation. Since sulfation of thyroid hormone is an important step in regulation of free hormone levels in the fetal compartment, an interference with ST activity by xenobiotics may compromise the developmental processes [4].

In this study, some hydroxylated PCB metabolites including one that has been shown to accumulate in the fetus (4-OH-2,3,3',4',5-pentachlorobiphenyl; metabolite D) [5], have been tested for their inhibitory effects on thyroid hormone sulfation.

2. Materials and methods

ST activity was determined with 1 μM 3,3'-diiodothyronine (T_2) and circa 80,000 cpm [125I]T_2, 50 μM 3'-phosphosphoadenosine-5'-phosphosulfate (PAPS) and 25 μg protein/ml male rat liver cytosol in 200 μl 75 mM Tris-HCl, 1.5 mM EDTA (pH 7.2). T_3 was also used as a substrate at a final concentration of 1 μM and 100 μg protein/ml rat liver cytosol. Incubations were carried out at 37 °C for 30 min. The reaction was stopped on ice by adding 750 μl 0.1 M HCl, followed by Sephadex LH-20 chromatography. Hydroxylated PCBs (PCB-OHs) tested in this assay are shown in Figure 1. PCB-OHs, dissolved in DMSO, were used in a final concentration series from 10 nM to 5 μM.

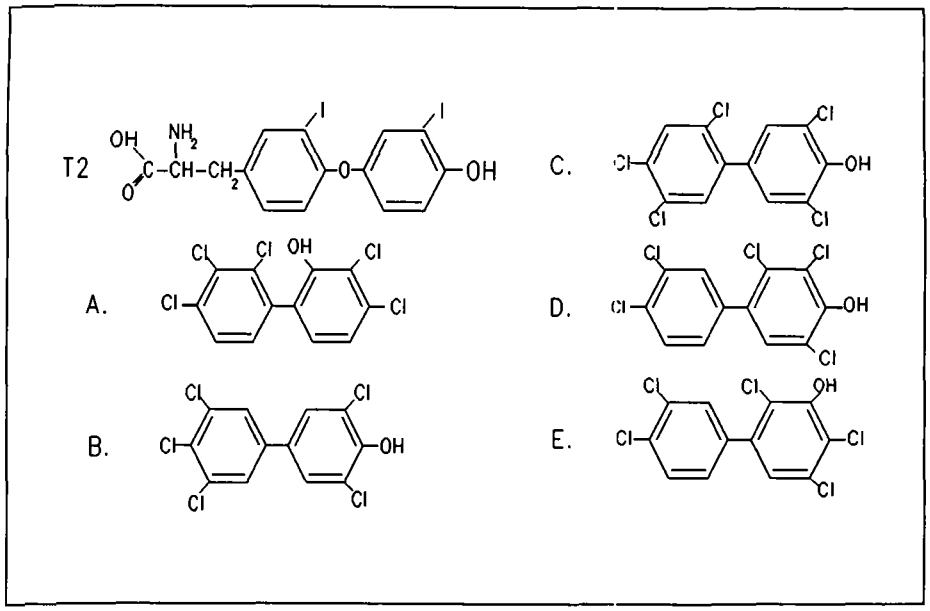

Figure 1. Structures of hydroxylated PCBs as compared to 3,3'-diiodothyronine (T_2). A. 2-OH-2',3,3',4,4'-pentachlorobiphenyl, B. 4-OH-3,3',4',5,5'-pentachlorobiphenyl, C. 4-OH-2',3,4',5,5'-pentachlorobiphenyl, D. 4-OH-2,3,3',4',5-pentachlorobiphenyl, E. 3-OH-2,3',4,4',5-pentachlorobiphenyl

3. Results

As shown in Figure 2, hydroxylated PCB metabolites are inhibitors of the T_2 sulfotransferase activity, with a different inhibition potency depending on their structure. Metabolite A eg, with the hydroxyl group on the ortho-position is not an inhibitor at all. In addition, it is demonstrated that metabolite B and C differ in inhibition potency, although their substitutions on the hydroxylated phenyl ring are identical.

Inhibitor concentration values at 50% inhibition (IC_{50}) were calculated for both T_2 and T_3 as substrates (Table 1). It is demonstrated that there is only a slight difference in IC_{50} values comparing T_2 with T_3 as a substrate for the hydroxylated PCBs tested.

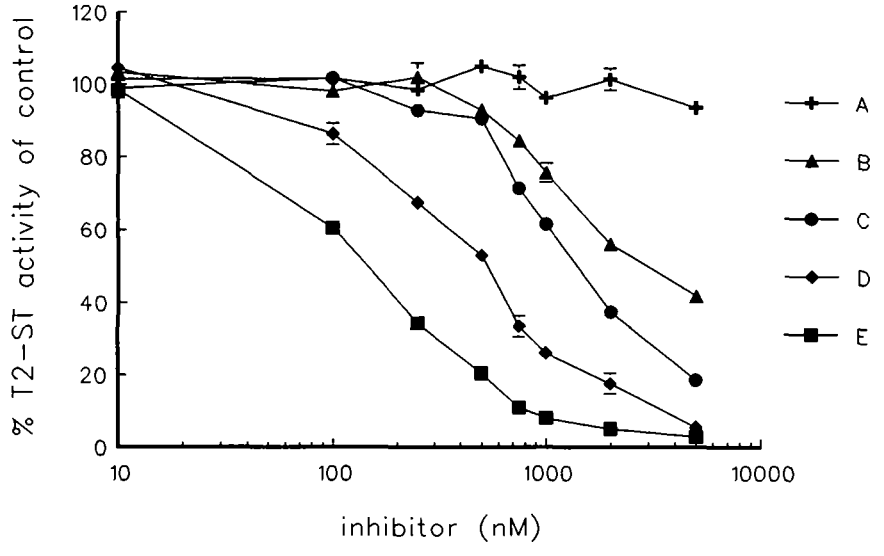

Figure 2. Inhibition of sulfotransferase activity towards T_2 by hydroxylated PCBs. T_2-ST activity is given in percentage of control. Data represent mean (n=2) ± standard deviation.

Table 1. IC_{50} concentrations in μM of hydroxylated PCB metabolites on ST activity using 1 μM T_2 or T_3 as a substrate.
Final male rat liver cytosol concentrations are 25 μg/ml for T_2 and 100 μg/ml for T_3. IC_{50} concentrations are given as mean (n=2) ± standard deviation. (n.d. is not done)

metabolite	IC_{50} (μM) on T_2-ST	IC_{50} (μM) on T_3-ST
A.	> 5.0	> 5.0
B.	3.00 ± 0.40	n.d.
C.	1.25 ± 0.30	3.58 ± 0.53
D.	0.58 ± 0.08	n.d.
E.	0.17 ± 0.02	0.30 ± 0.11

4. Discussion and conclusions

The data presented here indicate that the hydroxylated PCBs are *in vitro* potent inhibitors of sulfotransferase activity towards thyroid hormones. A hydroxyl group on the para or meta position of the hydroxy-PCBs is one structural requirement for this inhibition. Future research aims at elucidating further structural determinants for inhibition of T_2 sulfotransferase activity by hydroxylated PCBs and structurally related compounds.

IC_{50} concentrations of PCB metabolites were observed in the same range as concentrations of metabolites that were found to accumulate in fetuses from pregnant rats exposed to Aroclor 1254 5). Besides, it was demonstrated that the inhibition of sulfotransferase activity towards T_2 is comparable to that towards T_3 in this *in vitro* assay.

Since T_3 is the active hormone, playing a very important role in somatic and brain development and the fact that hydroxylated PCBs can accumulate in fetuses 5,6), inhibition of sulfation could be a possible mechanism for the developmental neurotoxicity of PCBs.

5. Acknowledgements

The investigations were supported by the Life Sciences Foundation (SLW) which is subsidized by The Netherlands Organization for Scientific Research (NWO) and by the Commission of the European Communities (contract BMH1-ST92-0097, Biomedical and Health Research Program).

6. References

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