Thyroid hormone binding proteins as targets for hydroxylated PCB, PCDD and PCDF metabolites; an overview

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Introduction

Disturbances in thyroid hormone levels and metabolism are among the endocrine effects observed in animals following exposure to PCBs and related compounds. Exposure of rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[1], 3,3',4,4'-tetrachlorobiphenyl (TCB)[2], 3,3',4,4',5,5'-hexabromobiphenyl (HCB)[3], Aroclor 1254[4], Kanechlor 400[5] and several polybrominated biphenyls resulted in increased glucuronidation and biliary clearance of thyroxin (T_4) and decreased plasma T_4 levels. TCDD and TCB are also reported to decrease thyroxine type-1-deiodinase (type-1-D) activity in rat liver *in vivo*[6,7].

In addition to the effects of these parent compounds on thyroid hormone metabolism, a hydroxylated TCB-metabolite (4-OH-3,3',4',5-TCB) was found to interact with transthyretin (TTR), a thyroid hormone binding transport protein in the blood, after exposure of rats to TCB[8]. Other hydroxylated-TCB metabolites could also inhibit TTR-T_4 binding in *in vitro* binding-competition studies with human TTR[9]. These findings prompted us to investigate several thyroxine binding proteins (e.g. human and rat TTR, human thyroxine binding globulin (TBG) and rat type-1-D) as possible targets for different hydroxylated PCB-, PCDD, and PCDF metabolites, *in vitro* and/or *in vivo*.

A. *In vitro* inhibition of T_4 binding to TTR or TBG and inhibition of type-1-deiodinase activity by hydroxylated PCBs, PCDDs and PCDFs

Inhibition of T_4-binding to transthyretin (TTR)

Several hydroxylated polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) were tested in an *in vitro* competitive binding assay, using purified human TTR and ^{125}I-T_4 as a displaceable radioligand[10]. All hydroxylated PCBs, but not the parent compounds tested, competitively displaced ^{125}I-T_4 from TTR with differential potency. The highest competitive binding potency was observed for hydroxylated PCB congeners with the hydroxygroup substituted on *meta* or *para* positions and one or more chlorine atoms substituted adjacent to the hydroxy-group on either or both aromatic rings (IC_{50} range 6.5 - 25 nM; Ka range: 0.78 - 3.95 * 10^8 M^{-1}). The relative potency of all *meta* or *para* hydroxylated PCBs was higher than that of the physiological ligand, T_4 (relative potency range: 3.5 - 13.6 compared to T_4). There were no marked distinctions in TTR-T_4 competitive binding potencies between the *ortho*- and non-*ortho* chlorine substituted hydroxy-PCB congeners tested. Marked differences in

TTR-T_4 binding competition potency were observed between the limited number of hydroxylated PCDDs and PCDFs tested. The hydroxy-PCDD/Fs, with chlorine substitution adjacent to the hydroxy-group i.e., 7-OH-2,3,8-trichlorodibenzo-*p*-dioxin, 2-OH-1,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3-OH-2,6,7,8-tetrachlorodibenzofuran, all showed a similar or higher relative binding potency, i.e. 1, 4.4 and 4.5 times higher respectively, than T_4. No detectable ^{125}I-T_4 displacement was observed with 2-OH-7,8-dichlorodibenzofuran, 8-OH-2,3,4-trichlorodibenzofuran and 8-OH-2,3-dichlorodibenzo-*p*-dioxin, which did not contain chlorine substitution adjacent to the OH-group. These results indicate a profound similarity in structural requirements for TTR binding between hydroxy-PCB, -PCDD and -PCDF metabolites and the physiological ligand, T_4, e.g., halogen substitution adjacent to the *para* hydroxy-group, while planarity does not seem to influence the ligand binding potency.

Inhibition of T_4-binding to thyroxine-binding globulin (TBG)

TTR is the major T_4 transport protein in plasma of rodents. In man, however, thyroxine-binding globulin transports most T_4 in blood. In a next study, hydroxylated PCBs, PCDDs and PCDFs were tested in a comparable *in vitro* competitive binding assay, using purified human thyroxine-binding globulin and ^{125}I-T_4 as the displaceable radioligand[11]. None of the tested hydroxylated PCBs, PCDDs and PCDFs inhibited ^{125}I-T_4 binding to thyroxine-binding globulin. In addition, some T_4 derived compounds; e.g. tyrosine, mono-iodotyrosine, di-iodotyrosine and tri-iodophenol were tested on both transthyretin and thyroxine-binding globulin to investigate possible differences in structural characteristics determining T_4 binding to thyroxine-binding globulin and transthyretin. The T_4 derived compounds also did not inhibit ^{125}I-T_4 binding to thyroxine-binding globulin as tested in the *in vitro* assay. However, tri-iodophenol and to a lesser extent di-iodotyrosine inhibited ^{125}I-T_4-transthyretin binding. So a marked difference in structural requirements for T_4 binding to thyroxine-binding globulin or transthyretin was found. The hydroxylated PCBs, PCDDs and PCDFs can inhibit T_4 binding to transthyretin, but not to thyroxine-binding globulin, and thus may cause different effects in rodents and man.

Inhibition of hepatic type-1-deiodinase activity

The possible inhibition of rat liver type-1-D, another T_4 binding protein involved in the enzymic conversion of T_4 to T_3 and/or reverse T_3, was also assayed for several hydroxylated PCBs, PCDDs and PCDFs. Rat hepatic microsomes were used in an *in vitro* type-1-D activity assay, with ^{125}I-reverse T_3 as the substrate and increasing amounts of hydroxylated PCBs, PCDDs and PCDFs as inhibitors. The formation of free labeled iodine by the conversion of rT_3 to T_2 was used as a measure for outer ring type-1-deiodinase activity. Only the dihydroxylated metabolites (4,4'-$(OH)_2$-3,3',5,5'-tetraCB, 4,4'-$(OH)_2$-2,3,3',5,5'-pentaCB and 4,4'-OH_2-3,3'-diBB) strongly inhibited type-1-D activity. The inhibition constants (Ki) of these compounds ($3.7*10^{-8}$ - $2.7*10^{-7}$ M) were in the same order of magnitude as the Km value for the preferred natural substrate reverse T_3 (rT_3, $6.4*10^{-8}$ M). Monohydroxylated metabolites of all classes of compounds inhibited type-1-D activity at concentrations 10 to 100 times higher than the di-hydroxylated metabolites. These and other data[6,12] indicate that the structural requirements for type-1-D inhibition by metabolites of PCBs, PCDDs and PCDFs are preferentially dihydroxylation and halogen substitution adjacent to (at least) one hydroxy-group. The observed inhibition of type-1-D activity by hydroxylated PCBs, PCDDs and PCDFs in this *in vitro* system suggests that these metabolites may play a role in the decrease in type-1-D activity found in rats after exposure to PCBs and related compounds *in vivo*.

B. ***In vivo* interference of Aroclor 1254 and TCDD and hydroxylated metabolites with thyroid hormone plasma transport and metabolism in rats.**

Two *in vivo* studies were performed to determine the contribution of both mechanisms (i.e. increased T_4 glucuronidation and TTR-T_4 binding inhibition) to the decrease of plasma T_4 levels of rats exposed to 2,3,7,8-TCDD or Aroclor 1254. For this purpose, female Wistar rats (18-20 weeks) were treated with 25 μg 2,3,7,8-TCDD/kg b.w. (TCDD), 50 mg (A50), 500 mg (ARO500) Aroclor 1254/kg b.w. or cornoil (CON). After several days rats pretreated with TCDD or Aroclor 1254 were treated with ^{125}I-T_4 (10-15 μCi), bloodsamples were taken after 3,6 and 24 hours. On the next day these rats and rats not treated with ^{125}I-T_4, were killed and organs/tissues were removed and stored at -80 °C. T_4 levels were measured using a chemoluminescense immunoassay (Amerlite, Amersham). T_4 glucuronidation, cytochrome P4501A1 levels and (EROD)-activity and type-1-D activity in rat liver were measured. ^{125}I-T_4 binding to TTR in plasma was determined by PAGE-gelelectroforese and by counting the radioactivity of the separated ^{125}I-T_4-binding proteins: albumin and TTR. Hydroxylated metabolites of TCDD or Aroclor 1254 (E. Klasson-Wehler, Wallenberg Lab Stockholm, Sweden) were analysed in rat plasma by GC/MS.

All exposed groups, except the ARO50 group, showed a marked decrease in plasma total T_4 levels. EROD activity was significantly induced by TCDD, ARO50 and ARO500 treatment. Simultaneously, T_4-UGT activity was increased for both low ARO50 and high dosed ARO500 groups and significantly induced by TCDD. However, due to large interindividual variation the T_4-UGT activity in Aroclor 1254 rats was not significantly increased. Type-1-deiodinase activity in rat liver was significantly inhibited in TCDD-treated rats, but not in Aroclor 1254 treated rats. Specific binding of ^{125}I-T_4 to TTR or albumin in sera of TCDD or ARO500 exposed rats was decreased. In ARO500 rats a drastic decrease in the ratios of specific ^{125}I-T_4 binding to TTR vs. albumin was found immediately at 3, 6, and 24 hours after ^{125}I-T_4 exposure. This reduction in ^{125}I-T_4 binding ratios could be caused by the selective inhibition of T_4-TTR binding by the high levels of hydroxylated PCB-metabolite 4-OH-2,3,3',4',5-pentachlorobiphenyl (4-OH-PeCB) found in plasma of Aroclor 1254 treated rats. In TCDD exposed rats, the ratio of ^{125}I-T_4 binding to TTR vs. albumin was normal at 3 hours but decreased at 6 and 24 hours after ^{125}I-T_4 exposure. Hydroxy-metabolites of TCDD do have a similar high affinity for T_4 binding site on TTR as the 4-OH-PeCB. However, analysis of rat plasma did not show any presence of OH-metabolites of TCDD. The decrease with time in ^{125}I-T_4 binding to plasma TTR in TCDD treated rats could be caused by the induced T_4-glucuronidation, while ^{125}I-T_4 binding to plasma TTR in Aroclor 1254 treated rats is constantly low due to the abundant presence of the 4-OH-PeCB metabolite.

C. **General Conclusions**

The *in vitro* studies described here show that several different hydroxylated PCB-, PCDF- and PCDD-metabolites but not the parent compounds could displace T_4, the natural ligand, from TTR. This was explained by the striking structural resemblance of these hydroxy PCB metabolites with T_4. Surprisingly, the same hydroxylated metabolites did not interact with TBG, the major thyroid hormone plasma transport protein in man. The third thyroxine-binding protein, type-1-D, was inhibited *in vitro* mainly by di-hydroxy-PCB-metabolites. The potency of the different PCB, PCDD and PCDF-metabolites was considerably lower for inhibition of the type-1-D activity as compared to the inhibition of T_4 binding to TTR. The tested thyroid hormone binding proteins thus seem to have different binding sites, and do not share full similarity in the structural requirements for ligand binding.

From the *in vivo* studies we can conclude that plasma T_4 reduction by TCDD will be mainly caused by the increased T_4 glucuronidation, while both increased T_4 glucuronidation and T_4 displacement from TTR by OH-PCBs may be involved in T_4 reduction by AROCLOR 1254 and other PCB mixtures or metabolisable PCB congeners. In TCDD but not in Aroclor 1254 treated rats changes in type-1-D activities were found, suggesting less interference of hydroxylated metabolites with this thyroid hormone binding protein compared to *in vitro* effects. The changes found in TCDD treated rats may be a physiological response to lowered plasma T_4 levels.

Hydroxylated metabolites of PCBs and related compounds can interact with some thyroid hormone binding proteins (TTR, type-1-deiodinase) due to the structural resemblance of these compounds with T_4. Bergman et al.[13] showed the presence of significant amounts of hydroxylated metabolites of PCBs in plasma of environmentally exposed seal, polar bear and man. The major metabolite present in these plasma samples, 4-OH-2,3,3',4',5-pentachlorobiphenyl (4-OH-PeCB), which was also found in our Aroclor 1254 rat study, can have a high affinity for the TTR protein because it meets the structural requirements as found *in vitro* for T_4-TTR inhibition potency. Furthermore, hydroxylated PCB metabolites can selectively accumulate in fetus to high concentrations in pregnant rats exposed to Aroclor 1254[14], indicating the importance of further research on the presence and toxic action of these hydroxylated metabolites of PCBs and related compounds.

Acknowledgements

Hydroxylated metabolites used in *in vitro* studies have been kindly provided by S.Safe, Department of Veterinary Physiology and Pharmacology, A&M University, College Station, Texas (USA) and E. Klasson-Wehler, Wallenberg Laboratory, University of Stockholm, Sweden.

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