HEPATIC THYROID HORMONE 5'-DEIODINASE, ANOTHER TARGET-PROTEIN FOR MONOHYDROXY METABOLITES OF 3,3',4,4'-TETRACHLOROBIPHENYL

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ABSTRACT

Exposure of Wistar rats to 3,3',4,4'-tetrachlorobiphenyl (TCB) resulted in a drastic reduction of hepatic thyroid hormone 5'-deiodinase (5'-D) activity. In vitro studies revealed competitive inhibition of hepatic 5'-D activity by monohydroxy metabolites of TCB, but not by the parent compound TCB.

INTRODUCTION

Several target proteins have been reported to be involved in the onset of distinct pathobiochemical changes induced by toxic congeners of polyhalogenated aromatic compounds, such as polychlorinated biphenyls (PCBs). The most familiar and most thouroughly investigated target protein, directly involved in microsomal enzyme induction, is the cytosolic Ah-receptor [1]. The Ah-receptor protein, upon binding of toxic parent compounds, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and TCB, mediates the onset of "de novo" synthesis of cytochrome P450IA$_1$ and accompanying ethoxyresorufin-o-deethylase (EROD) and arylhydrocarbon hydroxylase (AHH) activities, in particular [2,3].

Another, more recently discovered target protein for toxic PCBs and related compounds is transthyretin (TTR), a protein involved in the plasma transport of thyroid hormone and vitamin A [4,5]. Binding of, primarily, monohydroxy metabolites of PCBs to this target protein was observed both in vitro and in vivo, resulting in a

disturbance of binding of thyroxine (T4) and retinol-bound retinol binding protein (RBP) to TTR [4-6]. These results prompted us to also investigate possible interaction of TCB and/or its monohydroxy metabolites with other proteins involved in thyroid hormone metabolism. In this paper the interaction of TCB and its monohydroxy metabolites with hepatic microsomal type I 5'-deiodinase (5'-D), an enzyme critically involved in the activation/elimination of T4, is presented.

MATERIALS AND METHODS

Animal treatment

Male Wistar rats (4 per group) weighing 200-250 g were exposed to a single ip dose of 50 mg/kg body weight of TCB (99% pure, dibenzo-p-dioxin and dibenzofuran free; Chrompack, Middelburg, The Netherlands) dissolved in corn oil, or to corn oil alone (5 ml/kg). After 4 days the livers were isolated and microsomes were prepared immediately, according to previously published methods [7]. Protein contents were measured with the Bio-Rad assay, using bovine serum albumin (Sigma Chemical Company, St. Louis, USA) as a standard. The hepatic microsomal fractions were stored at - 80 °C until further analysis.

Microsomal 5'-D activity measurements

Hepatic type I 5'-D activity was determined in microsomal preparations by the formation of $^{125}I^-$ from 3,[3',5'-^{125}I]-triiodothyronine ([^{125}I]rT_3) [8]. Briefly, 20 -200 µg microsomal protein/ml was incubated for 20 min at 37 °C with 0.2 µM ^{125}I-rT_3 in a 200 mM phosphate buffer, pH 7.2, containing 4 mM EDTA and 3mM dithiotreitol at 37 °C. The reaction was stopped by addition of 750 µl of 1 N HCl. The $^{125}I^-$ produced was separated from the reaction mixture by Sephadex LH-20 chromatography. Inhibition studies were performed by addition of 0 to 40 µM of TCB or the various monohydroxy-TCBs to the reaction mixtures.

Synthesis and purification of monohydroxy-TCBs

The hydroxylated TCB metabolites, 5-OH-3,3',4,4'-TCB, 4-OH-3,3',4',5-TCB, 2-OH-3,3',4-,4'-TCB and 6-OH-3,3',4,4'-TCB were prepared from the corresponding methoxy-chlorobiphenyls (synthesis described elsewhere [9 ,10]) by demethylation with boron tribromide [11]. The demethylation was quantitative and no further clean-up was needed.

RESULTS

Exposure of male Wistar rats to a single ip dose of 50 mg/kg of TCB resulted in a 43 % reduction of the hepatic microsomal type I 5'-D activity, tested 4 days after TCB administration (Table 1).

Table 1

Effect of 3,3',4,4'-tetrachlorobiphenyl on the microsomal type I 5'-deiodinase activity in the rat liver

Treatment	number of animals	hepatic 5'-D activity (pmol/min/mg protein)
corn oil (5 ml/kg)	4	804 ± 73
TCB (50 mg/kg)	4	*457 ± 75

Note: Data are presented as means ± SD; * $P < 0.05$ (Student's t test)

In vitro incubation of hepatic microsomal preparations from untreated Wistar rats with 0.1 μM [^{125}I]rT_3 in the presence of 0 to 10 μM of the various OH-TCB metabolites resulted in a significant reduction in the 5'-D activity (Fig. 1). No reduction in 5'-D activity was observed following addition of 0.01 to 10 μM of the parent compound TCB.

The inhibition of microsomal 5'-D activity by OH-TCBs appeared to be of a competitive nature, with an apparent inhibition constant (K_i) of 1 μM for 5-OH-3,3',4,4'-tetrachlorobiphenyl, i.e., the most potent 5'-D inhibitor of the OH-TCBs tested

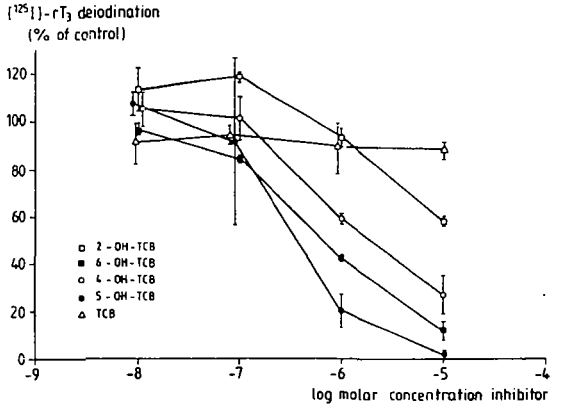

Figure 1: Inhibition of hepatic microsomal type I 5'-deiodinase by monohydroxy-TCB metabolites. Data points represent means ± SD (N = 2).

DISCUSSION

The hepatic microsomal enzyme, type I 5'-deiodinase involved in the metabolism of thyroid hormones, was found to be inhibited by monohydroxy-TCB metabolites, but not by the parent compound TCB. The inhibition appeared to be of competitive nature, indicating a direct interaction of the OH-TCBs with the substrate binding site of 5'-D. The apparent Ki for the most potent OH-TCB inhibitor, e.g., 5-OH-TCB was 1 μM, which is about two times lower than the Km for T4 (1.9-2.3 μM) [7], a physiological substrate for 5'-D.

In vivo exposure of Wistar rats to TCB also resulted in a significantly reduced hepatic microsomal 5'-D activity. This suggests that the in vivo hepatic production of the biologically active thyroid hormone, 3,3',5-triiodothyronine (T3), may be seriously hampered by TCB treatment.

This study demonstrates that hepatic 5'-D is the third protein involved in thyroid hormone metabolism that is affected by toxic PCBs. TTR, the plasma carrier of T4 in rats, was found to be a binding protein for especially monohydroxy-metabolites of PCBs [4,5]. In addition, the nuclear thyroid hormone receptor was reported to bind toxic PCBs and adipamide-derivatives of 2,3,7,8-TCDD and 2,3,7,8-TCDF [12]. These findings suggest that multiple proteins involved in thyroid hormone metabolism are target entities for toxic PCBs and related chemicals.

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