Interaction of polybrominated diphenyl ether metabolites (PBDE-OH) with human transthyretin *in vitro*

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Introduction

Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame retardants in polymers used in computers, TV sets, building materials and textiles[1]. The PBDEs are ubiquitous environmental contaminants and present in sediment[2], biota [3] and also in humans [4,5]. Limited amount of data are available about the toxicity of PBDEs. Since PBDEs and in particular their hydroxylated metabolites have a profound structural resemblance with the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3), it seems likely that these metabolites may interfere with thyroid hormone metabolism and transport, e.g. by competition with T_4 on transthyretin.

In this study we report on the potency of 17 different pure PBDE-congeners and their possible metabolites to compete with T_4 for binding to the plasma thyroid hormone transport protein, human transthyretin (TTR).

Material and Methods

Pure PBDE-congeners were synthesised as described before[6,7].

In order to obtain hydroxylated metabolites of PBDEs a method was developed to couple *in vitro* metabolic activation to competitive binding studies.

Preparation of hepatic microsomes

Wistar WU rats (300 g) were treated with β-naphtoflavone (β-NF, 3 daily ip injections of 30 mg/kg body weight dissolved in corn oil), phenobarbital (PB, 0.1% w/v in the drinking water for 7 days) or clofibrate (CLOF, 4 daily oral administrations of 200 mg/kg bw). One day after the last treatment, the rats were sacrificed under ether anesthesia and the livers removed. Livers of rats per treatment group were pooled, hepatic microsomes were prepared[8] and stored at -80°C.

Metabolism of PBDE *in vitro*

Metabolism of PBDE congeners was achieved by incubations of 10 µM PBDE with hepatic microsomes (1 mg/ml) in glass tubes at 37°C. NADPH (1 mM) was used as a cofactor. Metabolites formed after 30 minutes were extracted with diisopropylether, dried under nitrogen and stored at 4°C until further analysis (but not longer than 1 week). Blanks were carried out by performing identical incubations without the addition of NADPH. For T_4-TTR competition binding studies the dried extracts were dissolved in 50 µl methanol.

In vitro T_4-TTR competition binding studies

The T_4-TTR competition binding studies were performed as described before[9]. Since no reference PBDE-metabolites are presently available it was only possible to determine the T_4-TTR competition by dilution technique. Competition binding curves were made by plotting relative ^{125}I-T_4-protein binding (% of control, with control incubations of microsomes set to 100%) against the dilution factor.

Results and Discussion

The numbering of the PBDEs is the same as the PCBs, so BDE-77 has the same substituents (3,3',4,4') as PCB-77.

In figure 1 it is shown that after microsomal incubation (PB-pretreated) of 2,2',4,6'-tetraBDE (BDE-51) hydroxylated PBDE-metabolites are formed that competitively displace T_4 from TTR with a fairly high potency. Incubation of BDE-51 with NF-pretreated microsomes gives no T_4-TTR inhibition. It is possible that the metabolites formed are not capable of inhibiting T_4-TTR binding, but it could also be that NF-pretreated microsomes are not able to metabolize this BDE-congener.

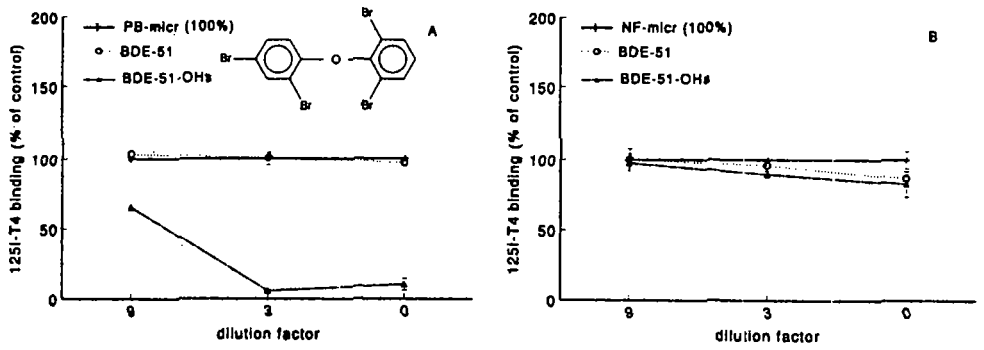

Figure 1. T_4-TTR competition binding of 2,2',4,6'-tetraBDE (BDE-51) prior to (dotted line) and of PBDE-metabolites after microsomal transformation with phenobarbital- (A), or β-naphtoflavone-(B) induced microsomes. Data present mean ± standard deviation (n=2).

In table I the T_4-TTR binding inhibition potency of all 17 tested PBDEs incubated with the three different microsome types are presented.

BDE tested	PB-microsomes	NF-microsomes	CLOF-microsomes
15	++	++	-
28	++	+	+
30	++	++	++
32	-	+	+
47	++	-	-
51	++	-	+
71	+	+	+
75	++	+	+
77	++	+	+
85	+	-	-
99	+	-	-
100	++	-	-
119	++	-	-
138	-	-	-
153	-	-	-
166	+	-	-
190	-	-	-

Table I. Inhibition of T_4-TTR binding by PBDE-metabolites obtained after incubation with PB-, NF- or CLOF-induced microsomes. Inhibition potencies are given from the undiluted extract. ++ = 60% inhibition, + = 20% inhibition, - = 0-20% inhibition.

The potency of a PBDE to compete with T_4-TTR binding appears to be both congener and cytochrome P450 specific. No competition was observed for any of the parent compounds (not shown). This is in agreement with previous studies which showed that the structural requirements of PCBs and other polyhalogenated aromatic hydrocarbons for TTR interactions are hydroxy-substitution on the *para-* or *meta-* positions of one or both the phenyl rings, with adjacent chlorine substitutions[9].

In table I it can also be seen that no T_4-TTR inhibition occurs with the higher brominated diphenyl ethers (e.g. BDE-138, 153, 166 and 190) after microsomal incubations. It is hypothesized that these PBDEs are not metabolized during the microsomal incubations, explaining the fact that no inhibition potency is found especially for these higher brominated diphenyl ethers. Further studies will be necessary to confirm this. There are little data on the

metabolism of PBDEs. DecaBDE has shown to be metabolized by the rat[10] and a recent study showed that 2,2',4,4'-tetraBDE (BDE-47), the major PBDE congener present in wildlife and man, is metabolized by the rat and mouse[11].

The data presented here clearly indicate that hydroxylated metabolites of PBDEs that may be formed *in vitro*, are potent competitors for T_4-binding to TTR. This suggests a potential thyroid hormone modulating effect of these PBDE metabolites if present in wildlife species and humans. The identity of the metabolites formed is unknown at the moment but will be unravelled by a combined effort of large scale metabolic conversion, coupled to clean-up and GC-MS analysis. To study the impact of the competitive displacement of T_4 from TTR in the whole animal we are going to perform an *in vivo* experiment in the near future.

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