Assessing The Role of *Ortho*-substitution on Polychlorinated Biphenyl (PCB) Binding to Transthyretin (TTR), a Thyroxine Transport Protein

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Introduction

The molecular events leading to PCB perturbations in thyroid hormone (TH) homeostasis are not fully understood. However, PCBs bear a close structural relationship to TH and can compete for specific sites on receptor (nuclear TH receptor) or non-receptor type (transthyretin, deiodinase) proteins in biological systems. A general model for TH binding to proteins has been suggested in which the hormone completes the hydrophobic core of the binding domain in active protein conformers (1). Transthyretin (also known as prealbumin) binding by certain PCBs is consistent with such a model.

Studies in our laboratory (2,3) as well as others (4,5) have previously demonstrated that some PCBs and their hydroxylated derivatives (potential metabolites) bind strongly to transthyretin and can potentially compete with T_4 in biological systems. Our strusture-activity relationship (SAR) studies indicated that lateral chlorine substitution on PCBs is important for the binding. The best binders were 4-8 times better than T_4 and *ortho* substitution in the presence of lateral substitution does not appreciably lower binding. Furthermore, it was observed that a single lateral chlorine along with *ortho* substitutions (2,4,6-pattern) could lead to significant binding activity (2,3). However, these earlier studies did not fully assess the effects of *ortho* substitution, especially in the absence of lateral substitution.

The present study was undertaken for the systematic evaluation of competitive binding of *ortho* only PCBs to transthyretin, a T_4 transport protein, and compared with lateral only and *ortho*-lateral PCB congeners. Since individual research groups observed different binding affinities for certain lipophilic PCBs (2, 4, 5), priority was given to optimize the conditions of the binding assay, which include the choice of buffer (imadazole versus tris) and temperature (4°C

versus room temperature).

Material and Methods

Chemicals: PCB congeners (purity > 99%) were purchased from either Ultra Scientific (North Kingstown, RI) or AccuStandard (New Haven, Conn.). 2,3,3',4',5,5'- (IUPAC # 162) and 2,3,3',4,4',5 - (IUPAC # 156) Hexachlorobiphenyls were synthesized and fully characterized (6). Stock solutions of PCB congeners were prepared by dissolving them in dimethyl sulfoxide (DMSO). A 1 µl aliquot of stock solution (different concentrations) was added to the buffer to yield the desired final concentration. L-Thyroxine (T_4); 3,5,3-triiodothyronine (T_3), and Sephadex G-25, particle size 50-150 µm, were purchased from Sigma Chemical Co. (St. Louis, MO). Sephadex was deaerated by boiling for at least 1 hr in buffer. For gel filtration, minicolumns (2 ml bed volume) were used.

[^{125}I]-T_4(L) with a specific activity of 1250 µCi/µg and radiochemical purity of >99% was purchased from New England Nuclear Corp. (NEN). Human prealbumin (TTR; 95%) was purchased from Calbiochem Corp. (La Jolla, CA).

Competitive [^{125}I]-T_4 Binding Assay: A binding assay using the gel filtration procedure described by Somack et al. (6) was used to measure the ability of various halogenated biphenyls to displace [^{125}I] L-T_4 from the high affinity TTR binding site. Conditions for binding assay were optimized with imidazole-acetate (IA) or Tris buffer (figure 1a, 1b), room temperature (RT; 25°C; 1 hr) or 4°C (24 hr) (figure 2a, 2b).

The assay mixture contained Tris-HCl buffer (pH 8.0), 10 nM TTR, 20 nM L-T_4 (including 0.66 nM of [125I] L-T_4, 150,000 cpm), and competitors (cold T_4 or PCBs) with increasing concentrations (1 nM to 1000 nM). The final volume of the assay mixture was 0.5 ml. After incubation at 25°C for 1 hr, the mixtures were quickly cooled to 4°C, and a 0.4 ml portion was filtered at 4°C on Sephadex G-25 minicolumns. With an additional volume of 1.2 ml, the protein-bound [^{125}I] L-T_4 and competitor compound respectively were removed from the column (total 1.6-ml fraction). Slight nitrogen pressure was applied to achieve an elution time of this fraction of 40-60 s, minimizing the dissociation of the complex. Radioactivity was counted by Packard Cobra-auto gamma counter (Packard Instruments, Meriden, Conn.). Non-specific binding was determined in the presence of 1000 nM cold L-T_4 and subtracted from the total binding to get the specific binding. Competitive binding curves were made by plotting specific TTR bound [^{125}I] L-T_4 (% of control) against added nM competitor concentration.

Analyses of binding data: The binding data (mean of 3 experiments done in duplicates ± standard deviation) were analyzed by a two-way ANOVA with PCB as one factor and concentration as the other. Post-hoc comparisons were made using Dunnetts t-test. IC_{50} (concentration that inhibits the control activity by 50%) values were estimated from the binding curves. The binding potencies of each analogue relative to that of thyroxine was calculated by the ratio of unlabeled T_4 concentration at 50% of total binding (IC_{50}- L-T_4) vs. competitor concentration at 50 % of total binding (IC_{50}-competitor) [IC_{50} (L-T_4)/ IC_{50} (competitor)].

Results and Discussion

There is controversy in the literature over the effects of parent PCB congeners on [^{125}I] L-T_4-

binding to TTR. Rickenbacher et al. (2) reported a significant effect of parent PCBs on [^{125}I] L-T_4 binding to TTR while Lans et al. (5) reported no significant [^{125}I] L-T_4 binding competition with TTR. This discrepancy could be due to the choice of buffer and/or incubation temperature. Our preliminary experiments were focused on resolving this issue. The results in Figure 1a and b summarize the competitive binding of selected PCBs in two buffer systems [Imidazole acetate (IA) and Tris-HCl buffer], where no significant difference was observed in binding potencies. However, a significant difference was observed among assay incubation temperature conditions (RT vs. 4^{0}C). The results summarized in Figure 2a and 2b show marked effect of selected PCB congeners on [^{125}I] L-T_4 binding at RT when compared to that of at 4^{0}C.

All the other competitive binding experiments were conducted using Tris-buffer and the assay was conducted at RT. The IC_{50} value for thyroxine on the TTR binding is 46 nM, which is in agreement with our previous reports (2,3). Solvents (DMSO, methanol or isopropanol), at the concentrations used (1 µl/0.5 ml assay mixture), did not alter [^{125}I] L-T_4 binding to TTR.

The results from competitive binding studies with *ortho* only series along with lateral only and *ortho*-lateral PCBs are presented in figure 3 and 4, and Table 1. These results provided important information demonstrating some *ortho* PCBs indeed bind to TTR. Specific binding increased with added *ortho* chlorine substitutions, but was completely abolished upon full *ortho* substitution as seen for 2,2',6,6'-tetrachloro biphenyl. The binding pattern seen with these *ortho* only PCBs is agreement with the activity of these PCBs in neuronal cells and brain homogenate preparations (8). The results from lateral only PCB series showed greater binding affinity when compared to *ortho* only PCBs. Also, lateral substitution (*meta* as well as *para*) on *ortho* PCB increased the binding affinity. The observation seen with *ortho*- only and *ortho*-lateral series parallel the proposed neuroactive properties of these PCBs and strongly suggest involvement of intracellular binding site that may be identical or very similar in size and shape to the binding site on transthyretin.

Implication

Information obtained from the present study could be used to develop a quantitative predictive model that could permit combined consideration of some of the more important classes of chemicals of concern and help set priority for their testing.

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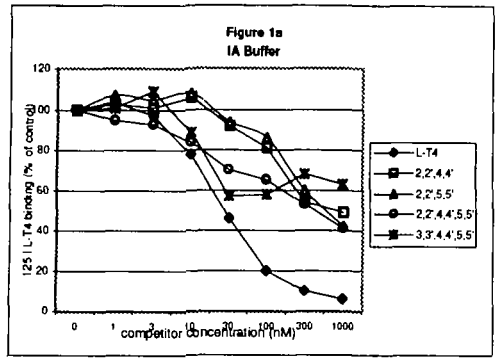

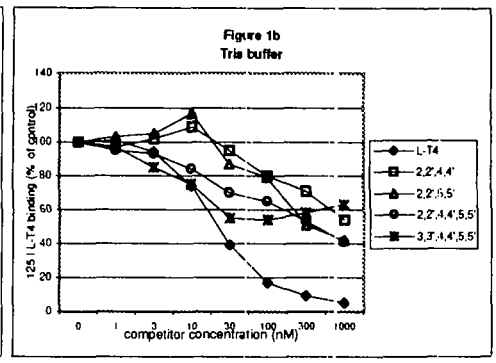

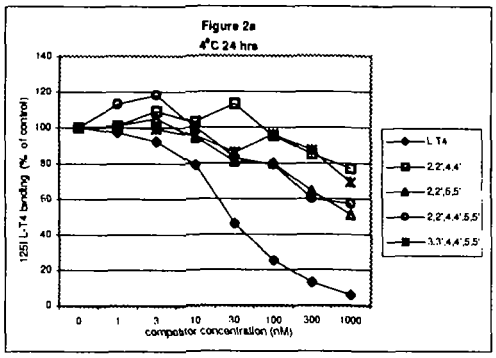

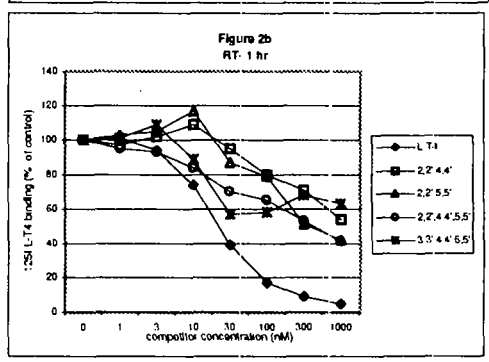

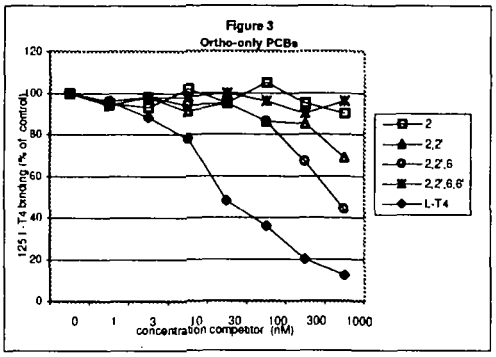

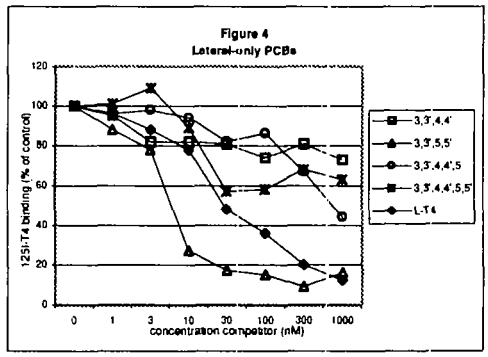

Table 1 Competitive ^{125}I-T_4-TTR binding inhibition concentrations (IC_{50}), and relative potencies of ortho substituted polychlorinated biphenyls (PCBs), lateral substituted PCBs and Ortho-lateral substituted PCBs

IUPAC #	Structure	IC_{50} (nM)	Rel. Pot.
	L-Thyroxine	46	1
1	2-CB	>10,000	<< 1
4	2,2'-DCB	5,500	0.008
19	2,2',6-TrCB	650	0.07
54	2,2',6,6'-TCB	>10,000	<< 1
77	3,3',4,4'-tetra CB	>10,000	<< 1
80	3,3',5,5'-tetra CB	7	6.7

IUPAC #	Structure	IC_{50} (nM)	Rel. Pot.
126	3,3',4,4',5-penta CB	1,250	0.037
169	3,3',4,4',5,5'-hexa CB	92*	0.5*
47	2,2',4,4'-tetra CB	700	0.065
52	2,2',5,5'-tetra CB	325	0.141
153	2,2',4,4',5,5'-hexa CB	300	0.153
162	2,3,3',4',5,5'-hexa CB	28	1.64
156	2,3,3',4,4',5-hexa CB	1,500	0.03

* inconsistency in the results may be due to solubility