Competitive Inhibition of ^{125}I-Thyroxin (T4) Binding to Choroid Plexus by Hydroxylated PCB Metabolites

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Introduction

Several polyhalogenated organic pollutants, among these polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDE), exert toxico-dynamic as well as kinetic effects on the thyroid and its hormones (1-4). Certain phenolic metabolites of PCBs bind to the thyroxin (T4) carrier transthyretin (TTR) and reduce plasma T4 levels (5), and a high binding affinity to TTR has been observed (6-7). The phenolic PCB metabolites have been detected in blood plasma of several mammals including man (8). Hydroxylated PCBs have been also detected in foetal rat brain (9).

TTR is the only T4-binding plasma protein which is synthesised by choroid plexus (CP) and involved in T4 transport from blood to the brain (10-12). In both rodents and man, TTR is the major protein involved in T4 transport to the brain (11-14). Reduction of T4 levels in brain during the perinatal period may affect brain maturation, which could result in permanent alterations e.g. in memory and behaviour (15).Therefore the aim of this investigation is to examine if the brain CP is a target tissue for binding of PCBs, PBDEs or preformed phenolic PCB metabolites.

Materials and Methods

Chemicals and Animals: 3,4,3′,4′-tetrachlorobiphenyl (TCB), 4-OH-3,5,3′,4′-tetrachlorobiphenyl (4-OH-TCB), 2,3,4,3′,4′-pentachlorobiphenyl (PeCB), 4-hydroxy-3,5,2′,3′,4′- and 4-hydroxy-2,3,5,3′,4′-pentachlorobiphenyl (4-OH-PeCB1 and 4-OH-PeCB2), 4-OH-3,3′,4′-trichlorobiphenyl (4-OH-TriCB) and 2,4,2′,4′-tetrabromodiphenyl ether (TBDE) (chemical purity > 98%) were kindly provided by Dr. Eva Jakobsson and co-workers, Stockholm University. The synthesis of the pure congeners was performed as described (8). Aroclor 1254 was purchased from a commercial source. T4 and L-triiodothyronine (T3) were from Sigma, Sweden and ^{125}T4 (specific activity 150 μCi/μg and radiochemical purity > 95%) from DuPont NEN, Sweden. Male and female Sprague-Dawley rats (200-300 g) were used.

Dissection and CP incubation: CP was taken from the brain ventricles and placed in the ice-cold incubation buffer contained 20 mM tricine, 1.5 mM EDTA, 2.5 mM DTT, 36% v/v of glycerol, pH 8.2 and kept frozen at -70 °C. The pooled tissue (from 6-12 animals) was homogenised by glass/glass homogeniser on ice-bath. The CP homogenate protein content was determined (16). About 0.2 mg CP protein (200 µl) was incubated at 37 C ° in 2 hours with 0.05 µCi of ^{125}I-T4 with or without (control) various concentrations of unlabelled T4 or competitors diluted to the desired concentrations to a volume of 5 ul n-propanol. The total incubation volume was 0.5 ml. The protein-bound and free ^{125}I-T4 was separated by hydroxyl apatite (HAP) as described by (17). The adsorbed protein-bound ^{125}I-T4 in the HAP pellet was determined by gamma counting (Cobra, Packard-Canberra, Meriden, USA).

*Experiment I (*in vitro *exposure):* The ability of T4, T3, TCB, TBDE, 4-OH-TriCB, 4-OH-TCB, 4-OH-PeCB1 and 2 to displace ^{125}I-T4 binding sites in CP from male rats, was tested by *in vitro* incubation of CP homogenate with ^{125}I-T4 and various concentration of the unlabelled compounds. Bound ^{125}I-T4 (% of control) = (bound dpm/µg protein with competitor/bound dpm/µg protein in control) x 100. The relative binding affinity of the tested compounds was calculated from the ratio of competitor and unlabelled T4 concentrations that reduce ^{125}I-T4 binding to CP to 50%.

*Experiment II (*in vivo *exposure):* Female rats (n=6-8/group) were administered orally with Aroclor 1254 (4 mg/kg/day), TBDE (6 or 18 mg/kg/day) or corn oil (control), for a total period of two weeks. The animals were killed 24 hr after the last administration, and the CP dissected. The homogenate was incubated with ^{125}I-T4.

Results and Discussion

*Experiment I (*in vitro *exposure):* The specific binding of ^{125}I-T4 to the CP homogenate was studied by the use of unlabelled T4, which competitive and concentration dependent reduced the association of ^{125}I-T4 to the protein binding sites (Fig. 1A and B). The inhibition potency of the hydroxylated PCBs 4-OH-TriCB, 4-OH-TCB, 4-OH-PeCB1, and 4-OH-PeCB2 was in range of 4-17 times higher than that of T4 (Table 1). No inhibition effect of TCB, TBDE or PeCB on ^{125}I-T4 binding to the sites in CP was observed.

*Experiment II (*in vivo *exposure):* IP administration of 4 mg Aroclor 1254/kg body wt. resulted in only 56% binding of ^{125}I-T4 to CP homogenate comparing with that from injected rats with the vehicle (controls). CP homogenate from dosed animals with 6 and 18 mg of TBDE/kg body wt. showed 80 and 63% ^{125}I-T4 binding of that in controls.

Reduction in binding ^{125}I-T4 to incubated CP after *in vitro* exposure to hydroxylated PCBs is believed a consequence of competitive binding of these substances to TTR, which is synthesised in the CP. Earlier findings from *in vitro* studies have showed the high binding affinity of hydroxylated PCBs to plasma TTR (6). Our observed reduction in ^{125}I-T4 binding to CP from dosed animals with the PCB mixture Aroclor 1254 and with TBDE may be explained by that metabolites to these compounds cross the blood brain barrier and bind to CP protein/s. An earlier observation (9) of accumulated 4-OH- PeCB2 in brain to prenatally exposed foetus to Aroclor 1254 support our data. This finding of T4 displacement in CP by hydroxylated metabolites of PCBs and probably also of PBDEs may affect CP dependent T4 transport to the brain and consequently result in anomalies in CNS development.

Fig. 1. Competition of ^{125}I-T4 binding to CP by T4, T3, TCB, 4-OH-TCB and TBDE (A), and T4, 4-OH-TriCB and PeCB and their metabolites 4-OH-PeCB1 and 4-OH-PeCB2 (B) (expressed as bound ^{125}I-T4 in % of control). Each point represent the mean of two experiments, and the max. and min. values for all points were at most 5.4 and 5.7%, respectively, higher and lower than the mean.

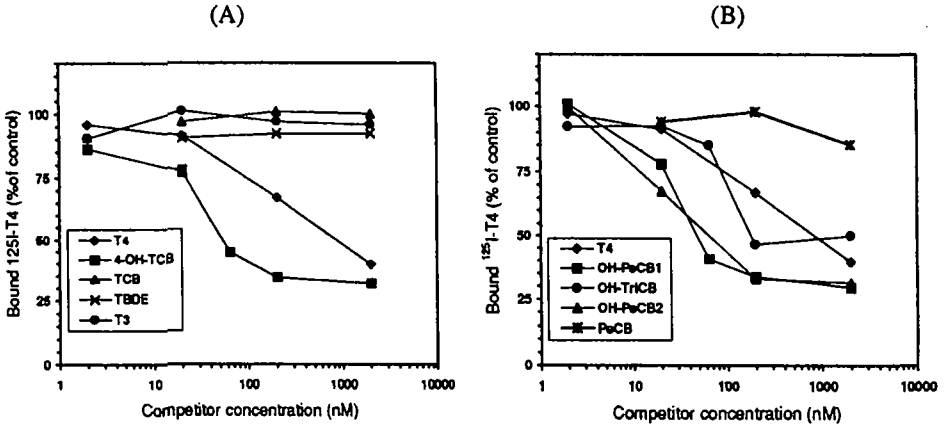

Table 1. Competitors concentration at 50% inhibition of ^{125}I-T4 binding to T4 binding sites in CP.

Compound	Concentration (nM)	Relative inhibitory potency
T4	860	1
T3	>> 2000	<< 1
4-OH-TCB	57	15
TCB	>> 2000	<< 1
TBDE	>> 2000	<< 1
4-OH-PeCB1	51	17
4-OH-PeCB2	66	13
PeCB	>> 2000	<< 1
4-OH-TriCB	200	4

Table 2. The effect of *in vivo* exposure of rat to Aroclor 1254 and TBDE on in vitro binding of ^{125}I-T4 to CP. Each value represent the mean with the min. and max. of two experiments.

	mg/kg body wt.	Bound ^{125}I-T4 (% of dpm in control)
Aroclor 1254	4	55.7 (53.3, 58.0)
TBDE	6	79.5 (74.9, 84.1)
- " -	18	62.7 (62.0, 63.3)

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