Different effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and Aroclor 1254 on thyroxine metabolism and transport

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Abstract
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and AROCLOR 1254 exposure to rats decreased plasma thyroxine (T_4) levels and induced thyroxine glucuronidation (T_4-UGT). In AROCLOR 1254 treated rats both T_4-UGT induction and selective inhibition of plasma T_4 transport by transthyretin (TTR) were observed. This may be due to the presence of hydroxylated metabolites in plasma of especially AROCLOR 1254 but not TCDD-treated rats.

Introduction
PCBs, PCDDs and PCDFs can exhibit a common biological toxic response mediated by Ah-receptor binding, like thymic atrophy, hepatotoxicity, chloracne, induction of liver cytochrome P450 1A1 and EROD activity[1]. Concurrently, thyroid hormone levels and metabolism are changed after exposure to 3,3',4,4'-tetrachlorobiphenyl[2] (CB 77), 3,3',4,4',5,5'-hexachlorobiphenyl[3] (CB 169), AROCLOR 1254[4] and TCDD[5,6]. Induction of thyroxine (T_4) glucuronidation (T_4-UGT) and increased biliary clearance of T_4 has been suggested as the main cause of plasma T_4 depletion by TCDD and related compounds. Another mechanism involved in decreasing T_4 levels was found for easily metabolisable PCBs, such as CB 77[7]. Hydroxylated metabolites of these compounds, like 4-OH-3,3',4',5-tetrachlorobiphenyl, a metabolite of CB 77, showed specific inhibition of T_4 binding to transthyretin (TTR)[7], the major T_4 transport protein in rodents, causing T4 levels to drop.

The aim of the present studies was 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.

Materials and methods

Experiment 1

8 Female Wistar rats (18-20 weeks) were treated with 25 μg 2,3,7,8-TCDD/kg b.w. (TCDD) or cornoil (CON). After 4 days four CON or TCDD rats were treated with ^{125}I-T_4 (10-15 μCi), bloodsamples were taken after 3,6 and 24 hours. On day 5 these rats and the 4 CON or TCDD rats not treated with ^{125}I-T_4, were killed and organs/tissues were removed and stored at -80°C.

Experiment 2

14 Female Wistar rats (18-20 weeks) were treated with 50 mg AROCLOR 1254/kg b.w.(ARO50), 500 mg AROCLOR 1254/kg b.w. (ARO500) or cornoil (CON). After 2 and 7 days 3 animals of each group were treated with ^{125}I-T_4 (10-15 μCi), bloodsamples were taken after 3,6 and 24 hours. On day 3 and 8 these animals and 4 ARO50, ARO500 and CON rats not treated with ^{125}I-T_4 were killed and organs/tissues were removed and stored at -80°C.

Biochemical parameters

EROD-activity was measured according to Lubet et al.[9]. T_4 levels were measured using a chemoluminescense immunoassay (Amerlite, Amersham). T_4 glucuronidation was measured by the method as described by Beetstra et al.[10] (1991). ^{125}I-T_4 binding to TTR in plasma was determined by PAGE-gelelectroforese and counting radioactivity of the separated ^{125}I-T_4-binding proteins: albumin and TTR.

Results

The plasma T_4 levels and binding and hepatic EROD and T_4-UGT results are listed in Table 1. All exposed groups, except the ARO50 group, showed a marked decrease in plasma total T_4 levels. EROD activity was significantly induced by TCDD and ARO50 and ARO500 treatment (resp. 170, 11 and 58 times induced). Simultaneously, T_4-UGT activity was increased for both low ARO50 and high dosed ARO500 groups after 3 or 8 days and significantly induced by TCDD after 4 days. However, due to large interindividual variation the T_4-UGT activity in AROCLOR rats was not significantly increased. 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, while in TCDD exposed rats, the ratio of ^{125}I-T_4 binding to TTR vs. albumin was normal at 3 hours but decreasing at 6 and 24 hours after ^{125}I-T_4 exposure.

Discussion

Exposure of rats to TCDD or low or high doses of AROCLOR 1254, led to deceases in plasma total T_4 levels, as earlier described by Bastomsky[4,6], Brouwer[7] and Lans[5]. Concommitantly, T_4-UGT activity was induced in tandem with EROD-activity in both TCDD and low and high AROCLOR 1254 treated groups. Ratios of ^{125}I-T_4 binding to TTR vs. albumin, used as indicators of selective displacement of T_4 from TTR[7], were reduced especially in high dosed AROCLOR 1254 rats and less in TCDD treated animals. This reduction in ^{125}I-T_4 binding ratios indicate the presence of hydroxylated metabolites in AROCLOR 1254 treated rats. Klasson-Wehler[11] showed the presence of

Table 1 Effects of 2,3,7,8-TCDD and low and high dose AROCLOR 1254 exposure on plasma T_4 levels, EROD activity, T_4-glucuronidation and specific binding of ^{125}I-T_4 to TTR and albumin. Ratios of ^{125}I-T_4 binding to TTR over albumin are given at T=3,6 and 24 hours after ^{125}I-T_4 exposure. Results shown are means ± S.D. of tri- or quadruplicate measurements. Significant differences (Student's t-Test): $^*P < 0.05$

Group	Plasma TT_4 (nM)	EROD-act nmol/min/ mg	T_4-UGT pmol/min/ mg	T_4-binding TTR/alb (3h)	T_4-binding TTR/alb (6h)	T_4-binding TTR/alb (24 h)
Control Exp.1	34.9 ± 8.2	0.020 ± 0.003	1.73 ± 0.13	4.2	3.9	4.6
TCDD	18.7 ± 1.1*	3.390 ± 0.731*	6.78 ± 1.17*	4.4	3.1	2.5
Control Exp.2	29.3 ± 3.7	0.039 ± 0.007	2.39 ± 0.85	4.1	3.7	8.0
ARO50 day 3	32.9 ± 6.0	0.469 ± 0.461	3.34 ± 1.62	4.4	3.8	3.8
ARO500 day 3	12.9 ± 11.9*	2.336 ± 1.026*	6.84 ± 2.34	2.7	1.7	2.3
ARO50 day 8	31.2 ± 7.9	0.345 ± 0.344	2.55 ± 1.34	4.1	3.6	4.2
ARO500 day 8	10.1 ± 5.2*	2.288 ± 0.799*	11.80 ± 8.23	1.5	1.2	1.6

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), has high affinity for the TTR protein[7]. In addition this metabolite is found in plasma of rats exposed to AROCLOR 1254[11]. Some hydroxylated PCB-metabolites are capable of inhibiting TTR-T_4 binding as was found in vitro[8], requiring a meta- or para hydroxylation with adjacent halogen substitution(s).

TCDD treated animals also showed some reduction in ^{125}I-T_4 binding ratios. 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[12].

In conclusion, plasma T_4 reduction by TCDD is mainly caused by T_4-UGT induction, while both T_4-UGT induction and T_4 displacement from TTR by OH-PCBs may be involved in T_4 reduction by AROCLOR 1254 and other PCB mixtures.

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