Cytochrome P450 Induction in Rats after Pre- and Postnatal Exposure to PCB#126, PCB#118, PCB#153 or 2,3,4,7,8-PnCDF

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1. Introduction

Developmental and reproductive effects after pre- and postnatal exposure to TCDD or commercial PCB mixtures have been described for various species [1)]. The mechanism of action, however, is presently unresolved. Observations included ambiguous results on decreased steroid hormone levels. During the late gestational and early neonatal phase the hypothalamic-pituitary GH secretion pattern in rats is imprinted by neonatal androgen. A pulsatile GH secretion will constitute a male phenotype cytochrome P450 expression in the liver, continuous secreted GH a female expression pattern [2)]. This includes for instance CYP1A2 (female-predominant), CYP2B1 and 2B2 (male-predominant) [3)]. Beside metabolism of exogenous compounds a range of hepatic P450s is involved in deactivation of steroid hormones: CYP1A1 and 1A2 mainly hydroxylate 17β-estradiol [4)], CYP2B1 and 2B2 testosterone [2)]. However, recently it was suggested that metabolites of steroid hormones might be of importance in reproductive functioning [5)]. Thus, we hypothesized that: 1) pre- and postnatal exposure to PCDDs, PCDFs and PCBs during the imprinting phase might result in a permanently altered P450 expression in the liver; 2) altered P450 expression might change in altered steroid hormone levels and possibly play a role in reproduction failures. To study this hypothesis dose-response studies in rats were performed with 3,3',4,4',5-PnCB (PCB#126), 2,3',4,4',5-PnCB (PCB#118), 2,2',4,4',5,5'-HxCB (PCB#153) and 2,3,4,7,8-PnCDF. In this paper effects of pre- and postnatal exposure on hepatic CYP1A1, 1A2 and 2B1 activities, determined as EROD, MROD and PROD activities at postnatal day (PND) 21 (weaning) and 112 (sexual maturation) are described as well as gross reproduction effects.

2. Experimental design

Virgin female Wistar rats (SPF) were placed overnight with male rats for mating. As soon as a sperm positive vaginal smear was detected the females were housed individually (day 0). On day 1 of gestation a single dose of 6, 16, 51, 147, 375 nmol/kg bodyweight (2-121 μg/kg) PCB#126; 2, 7, 22, 75, 251 μmol/kg bw (0.8-81 mg/kg) PCB#118; 53, 147, 200, 402, 768 μmol/kg bw (19-275 mg/kg) PCB#153 or 1, 4, 12, 43, 131 nmol/kg bw

(0.4-44 µg/kg) 2,3,4,7,8-PnCDF was administered by gavage. Doses ranged from 0.0001 to 0.1 µmol/kg bw TEQ [6,7]. During the experiments tap water and a cereal-based rodent diet (meal mash) were provided *ad libitum*. On PND 4, litters were normalized to four males and four females per litter. On PND 21 and PND 112 six males and six females from different litters per dose group were sacrificed for determination of hepatic PROD (CYP2B1), EROD (CYP1A1), MROD activity (CYP1A2) and protein content [8-10]. In addition, from two high dose groups of each compound (PCB#126: 51, 147 nmol/kg; PCB#118: 75, 251 µmol/kg; PCB#153: 200, 768 µmol/kg; PnCDF: 43, 131 nmol/kg) 12 males and 12 females were selected, raised to adulthood and tested for reproduction capacity. Exposed rats were mated with untreated females or males and allowed to litter. On PND 4 all experiments were terminated. Observations and parameters recorded during postnatal development included body weight, food consumption, pregnancy, duration of gestation, litter size, litter sex ratio, pup mortality, pup malformation, preputial separation (PND 28-35), vaginal examination (PND 90-100), precoital time and sperm analyses. Statistical analyses of data were performed by using Analysis of Variance and subsequent t-test.

3. Results

Cytochrome P450 At PND 21 pups pre- and postnatally exposed to either PCB#126, PCB#118, PCB#153 or PnCDF showed dose-dependent induction of hepatic P450-related activities when compared to control pups. After exposure to PCB#126, PCB#118 or PnCDF EROD (CYP1A1) and MROD (CYP1A2) activities were induced (table 1-3).

Table 1: Hepatic EROD and MROD activities in 3 week- and 16 week-old rats after a single oral dose of PCB#126 to the dams on day 1 of gestation

Dose	EROD	pmol/min/mg	MROD	pmol/min/mg
nmol/kg	male	female	male	female
PND 21				
0	18 ± 6[a]	20 ± 9[a]	12 ± 4	13 ± 5
6	68 (54,81)[f]***	89 ± 17[e]***	47 (34,60)***	54 ± 11***
16	335 ± 82[e]***	379 ± 147[e]***	208 ± 98***	214 ± 94***
51	833 ± 121[d]***	855 ± 136[e]***	609 ± 189***	545 ± 200***
147	1180 ± 187[d]***	1131 ± 139[d]***	777 ± 220***	679 ± 140***
PND 112				
0	63 ± 13[a]	91 ± 18[a]	45 ± 10	57 ± 13
6	58 ± 8[b]	80 ± 12[d]	51 ± 6	51 ± 9
16	58 (51,65)[f]	81 ± 18[d]	48 (46,49)	55 ± 11
51	58 ± 11[c]	96 ± 17[b]	53 ± 6	57 ± 11
147	89 ± 17[c]***	96 ± 36[c]	68 ± 13***	96 ± 36

[a] n>7; [b] n=7; [c] n=6; [d] n=5; [e] n=4; [f] n=2; *** P<0.001

Induction of PROD (CYP2B1) activity was observed in pups exposed to PCB#118 or PCB#153 (table 3,4). Even in the lowest dose groups of PCB#126, PnCDF and PCB#153 P450-related activities were significantly induced; 4-, 2- and 5-fold respectively. In contrast, only doses of >22 µmol/kg bw PCB#118 resulted in induction of EROD (4-fold), MROD (4-fold) and PROD (2-fold) activities in the pups. The highest EROD and MROD activities were observed in PCB#126-exposed pups; 52-66 fold induction after a

dose of 147 nmol/kg. (No pups of the highest dose group had survived.) EROD and MROD induction in pups of the highest dose groups of PnCDF and PCB#118 were 22-fold and 35-53 fold respectively. PROD activity was induced 7-fold in the highest dose group of PCB#118 and approximately 40-fold in PCB#153-exposed pups. At PND 21 no sex differences were observed in EROD, MROD or PROD activities in the pups.

Table 2: Hepatic EROD and MROD activities in 3 week- and 16 week-old rats after a single oral dose of 2,3,4,7,8-PnCDF to the dams on day 1 of gestation

Dose	EROD	pmol/min/mg	MROD	pmol/min/mg
nmol/kg	male	female	male	female
PND 21				
0	18 ± 6[a]	20 ± 9[a]	12 ± 4	13 ± 5
1	41 ± 20[c]***	37 ± 8[d]**	26 ± 12***	22 ± 2***
4	77 ± 15[c]***	65 ± 13[d]***	51 ± 12***	50 ± 15***
12	198 ± 72[e]***	179 ± 84[d]***	72 ± 19***	83 ± 24***
43	275 ± 36[d]***	273 ± 38[d]***	164 ± 39***	145 ± 22***
131	400 ± 68[d]***	433 ± 42[c]***	266 ± 58***	271 ± 19***
PND 112				
0	63 ± 13[a]	91 ± 18[a]	45 ± 10	57 ± 13
1	64 ± 14[c]	95 ± 11[d]	45 ± 6	61 ± 8
4	59 ± 14[c]	86 ± 23[c]	47 ± 6	64 ± 11
12	71 ± 15[d]	117 ± 10[d]**	49 ± 12	73 ± 10*
43	64 ± 10[b]	100 ± 13[b]	53 ± 9*	64 ± 11
131	77 ± 10[c]*	141 ± 24[c]***	57 ± 6**	70 ± 12*

[a] n>7; [b] n=7; [c] n=6; [d] n=5; [e] n=4; * P<0.05; ** P<0.01; *** P<0.001

Table 4: Hepatic PROD activity in 3 week- and 16 week-old rats after a single oral dose of PCB#153 to the dams on day 1 of gestation

Dose	PROD	pmol/min/mg
µmol/kg	male	female
PND 21		
0	3.1 ± 0.6[a]	3.0 ± 1.1[a]
53	15.1 ± 7.3[c]***	13.4 ± 5.3[c]***
147	35.9 ± 24.0[e]***	35.1 ± 7.4[e]***
200	67.4 ± 19.8[c]***	78.1 ± 17.9[d]***
402	100.8 ± 29.1[c]***	104.2 ± 34.0[c]***
768	123.3 ± 7.8[c]***	128.6 ± 28.2[d]***
PND 112		
0	10.3 ± 2.4[a]	8.7 ± 1.2[a]
53	11.2 ± 2.2[c]	7.4 ± 0.7[c]
147	10.7 ± 2.5[e]	10.0 ± 3.8[c]
200	13.8 ± 1.4[c]*	8.3 ± 1.1[d]
402	16.8 ± 4.4[c]**	10.5 ± 0.9[c]*
768	38.0 ± 15.3[e]***	16.8 ± 3.9[d]***

[a] n>7; [b] n=7; [c] n=6; [d] n=5; [e] n=4; * P<0.05; ** P<0.01; *** P<0.001

Table 3: Hepatic EROD, MROD and PROD activities in 3 week-old rats after a single oral dose of PCB#118 to the dams on day 1 of gestation

Dose	EROD	pmol/min/mg	MROD	pmol/min/mg	PROD	pmol/min/mg
µmol/kg	male	female	male	female	male	female
PND 21						
0	18 ± 6[a]	20 ± 9[a]	12 ± 4	13 ± 5	3.1 ± 0.6	3.0 ± 1.1
2	23 ± 8[e]	19 ± 5[e]	14 ± 4	12 ± 2	3.8 ± 1.0	3.0 ± 0.6
7	27 ± 10[f]	23 ± 5[f]	17 ± 4	15 ± 4	3.6 ± 1.2	3.8 ± 0.9
22	76 ± 38[c]***	74 ± 20[d]***	46 ± 29***	42 ± 10***	4.9 ± 1.5**	6.4 ± 3.9***
75	312 ± 110[c]***	314 ± 68[d]***	161 ± 61***	166 ± 96***	9.5 ± 2.9***	9.1 ± 1.4***
251	946 ± 337[b]***	799 ± 163[d]***	593 ± 309***	455 ± 144***	22.8 ± 7.1***	19.6 ± 4.7***

[a] n>7; [b] n=7; [c] n=6; [d] n=5; [e] n=4; [f] n=3; ** P<0.01; *** P<0.001

At PND 112 only slight inductions in EROD, MROD and PROD activities were observed in male and female offspring. In male offspring of the highest PCB#126 dose group both EROD and MROD activity were significantly induced, 1.4 and 1.5-fold respectively (table 1). Also male rats exposed to the highest PnCDF dose still showed significant induction of EROD and MROD at PND 112 (table 2). In contrast to PCB#126-exposed females, EROD and MROD activities of female offspring exposed to 12 and 131 nmol/kg bw PnCDF were also significantly induced. Pre- and postnatal exposure to PCB#118 did not result in induction of either EROD, MROD or PROD in offspring at PND 112 (data not shown). Finally, the highest induction of PROD (maximally 3.7-fold) was found in male rats exposed to >200 µmol/kg bw PCB#153. Also in female offspring exposed to >402 µmol/kg bw HxCB PROD activity was still significantly induced (1.9-fold) at PND 112 (table 4). At PND 112 sex-dependent differences were found in EROD, MROD and PROD activities.

Reproduction A slight dose-related delay in preputial separation was observed in offspring exposed to PCB#126, PCB#118 and PnCDF; sperm counts of these animals were slightly affected, sperm morphology was normal. At the time of mating, vaginal malformations were observed in females of the PCB#126, PCB#118 and PnCDF dose groups. In spite of it, copulation by untreated males was successful. However, the pregnancy rate was low. Exposed male rats were also less successful in achieving pregnancies in untreated females. Potency of the tested compounds decreased as follows: PCB#126 > PnCDF > PCB#118. No effects were observed in PCB#153-exposed female and male offspring.

4. Discussion and conclusions

At PND 112 only minimal induction of CYP1A1, 1A2 and 2B1-related activities was observed in male and female rats after pre- and postnatal exposure to PCB#126, PnCDF or PCB#153. In offspring of PCB#118-exposed dams no induction was found at PND 112. Most likely minor concentrations of the congeners still present in the livers of the offspring can be held responsible. Hepatic concentrations have not yet been determined, but will be in the near future. From these results disturbance of neonatal imprinting of hepatic CYP1A1, 1A2 and 2B1 by these congeners is questionable. However, the high CYP1A1 and 1A2 induction levels at PND 21 might have played a role in the observed reproductive failures after sexual maturation of the PCB#126-, PnCDF- and PCB#118-exposed offspring. At the moment sex-dependent CYP2A1 (female), 2C11 (male), 2C12 (female) and 3A1/2 activities are measured in the livers of the offspring as well as steroid hormone levels. Of the congeners tested PCB#126 was the most potent, both in induction of CYP1A1 and 1A2 activities at PND 21 and reproductive failures in male and female offspring after sexual maturation.

5. References

1) Peterson R.E., H.M. Theobald, and G.L. Kimmel (1993): Developmental and reproductive toxicity of dioxins and related compounds: cross-species comparisons. (review) CRC Crit. Rev. Toxicol. 23, 283-335.

2) Waxman D.J. (1988): Interactions of hepatic cytochromes P-450 with steroid hormones. Biochem. Pharmacol. 37, 71-84.

3) Kato R., and Y. Yamazoe (1993): Hormonal regulation of cytochrome P450 in rat liver. In Cytochrome P450. Handbook of Experimental Pharmacology 105 (J.B. Schenkman, and H. Greim, Eds.) Springer-Verlag, Berlin, Heidelberg, 447-491.

4) Ball S.E., L.M. Forrester, C.R. Wolf, and D.J. Back (1990): Differences in the cytochrome P-450 isoenzymes involved in the 2-hydroxylation of oestradiol and 17α-ethinyloestradiol. Biochem. J. 267, 221-226.

5) Gustafsson J.Å. (1994): The hypothalamic-pituitary-liver axis. Growth hormone controls liver sex. In The liver: biology and pathobiology, 3th edition (I.M. Arias, J.L. Bowyer, N. Fausto, W.B. Jakoby, D.A. Schachter, and D.A. Schafritz, Eds.) Raven Press, Ltd., New York, 1209-1215.

6) Ahlborg U.G., G.C. Becking, L.S. Birnbaum, A. Brouwer, H.J.G.M. Derks, M. Feeley, G. Golor, A. Hanberg, J.C. Larsen, A.K.D. Liem, S.H. Safe, C. Schlatter, F. Waern, M. Younes, and E. Yrjänheikki (1993): Toxic equivalency factors for dioxin-like PCBs. Chemosphere 28, 1049-1067.

7) Safe, S. (1990): Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechnistic considerations which support the development of toxic equivalency factors (TEFs). CRC Crit. Rev. Toxicol. 21, 51-88.

8) Burke M.D., and R.T. Mayer (1983): Differential effects of phenobarbitone and 3-methylcholanthrene induction on the hepatic microsomal metabolism and cytochrome P-450-binding of phenoxazone and a homologous series of its n-alkyl ethers (alkoxyresorufins). Chem.-Biol. Interactions 45, 243-258.

9) Lubet R.A., C.R. Jones, D.L. Stockus, S.D. Fox, and R.W. Nims (1991): Induction of cytochrome P450 and other drug metabolizing enzymes in rat liver following dietary exposure to Arochlor 1254. Toxicol. Appl. Pharmacol. 108, 355-365.

10) Bradford M.M. (1976): A rapid and sensitive method for the quantitaion of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

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