Cytochrome P450 Induction in Weanling and Adult Rats Perinatally Exposed to PCB 126, PCB 118, PCB 153 or 2,3,4,7,8-PnCDF

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1. Introduction

Perinatal exposure to PCBs or TCDD has been found to disturb sexual maturation and reproduction in various species[1)]. In rats the hypothalamic-pituitary secretion of growth hormone (GH) is imprinted by testosterone during late gestation and the early neonatal period. Following, the GH secretion pattern determines the sexual differentiation of cytochrome P450 expression in the liver[2)]. Several of the hepatic P450s induced by PCBs and related compounds are sex-dependent and involved in steroid hormone metabolism: CYP1A1 and 1A2 mainly hydroxylate 17β-estradiol; CYP2B1/2, CYP2A1 and CYP3A1/2 testosterone[2,3)]. In general, steroid hormones are eliminated by metabolism, however, metabolites were recently suggested to play a role in reproductive functioning[2,4)]. Thus, we hypothesized that perinatal exposure to PCBs and related compounds might disturb the imprinting of hepatic P450s resulting in altered P450 expression during adulthood. Next, circulating steroid hormone levels might be altered which possibly could be involved in reproduction failure. To study this hypothesis dose-response studies were performed with rats administered 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 a previous paper effects of perinatal exposure on hepatic EROD (CYP1A1), MROD (CYP1A2) and PROD (CYP2B1) activities at postnatal day (PND) 21 (weaning) and 112 (sexual maturation) from these studies were described as well as major reproduction effects[5)]. The present paper will focus on female-predominant CYP2A1, male-predominant CYP2B1 and CYP3A1/2, and male-specific CYP2C11 activities, in connection with hepatic concentrations of the congeners at PND112.

2. Experimental design

Virgin female Wistar rats (SPF) were mated overnight. 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 bw (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 approximately ranged from 0.5 to 45 nmol TEQ/kg bw[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. On PND 21 and PND 112 six males and six females from different litters per dose group were sacrificed for analyses of P450 activities by hydroxy-testosterone metabolite formation using HPLC: 16α/β-OH-testosterone (CYP2B1), 7α-OHT (CYP2A1), 2α-OHT (CYP2C11), 6β-OHT (CYP3A1/2)[8]. In 16 week-old male offspring plasma testosterone levels were measured using a radio-immuno assay. Hepatic concentrations of the PCBs and PnCDF were determined by GC-ECD after extraction and clean up procedures[9]. 2,3,3',4,4',5-HxCB (PCB 156) served as an internal standard for the PCB congeners, and 1,2,3,7,8-PnCDF served this purpose for 2,3,4,7,8-PnCDF. Statistical analyses of the data were performed by using Analysis of Variance and subsequent Dunnett's t-tests.

3. Results

Cytochrome P450 In the 3 week-old rats perinatally exposed to 147 nmol PCB 126/kg bw female-predominant 7α-OH-testosterone (CYP2A1) activity was induced 1.2-fold, whereas male-predominant 16α-OHT (CYP2B1) and 6β-OHT (CYP3A1/2) activities were reduced 0.5 and 0.8-fold respectively (fig.1). Also exposure to >12 nmol PnCDF/kg bw resulted in reduction of 16α-OHT (0.7-fold) and 6β-OHT activities (0.8-fold) (fig.1B,C). In contrast, perinatal exposure to PCB 153 induced both female- and male-predominant P450 activities dose-dependently (fig.1). The highest induction by PCB 153 was found on CYP2B1 activity, 17-fold; CYP2A1 en CYP3A1/2 were increased 2.2 and 3.2-fold respectively. With PCB 118 only the highest exposed offspring showed significant induction of CYP2A1 (7α-OHT, 1.6-fold) and CYP2B1 (16α-OHT, 2.0-fold) activities; thus, PCB 118 resembled PCB 153 but was less potent (fig.1A,B). At PND 21 no male-specific 2α-OHT activity (CYP2C11) could be measured. No differences in P450 activities were recorded between male and female offspring at PND 21. This was in contrast with the 16 week-old offspring in which the sex specificity of the hepatic P450s was fully expressed.

In the 16 week-old offspring (PND 112) statistically significant induction of CYP2B1 (16β-OHT) and CYP3A1/2-related activities (6β-OHT) was only found in male rats exposed to PCB 126 or PCB 153 (table 1). No effect was observed on female-predominant CYP2A1 (7α-OHT) or male-specific CYP2C11 (2α-OHT). At PND 112 no effect of perinatal exposure to PCB 118 or PnCDF was found on CYP2A1, CYP2B1, CYP2C11 and CYP3A1/2 activities in the offspring. In the 16 week-old female offspring P450 activities did not differ from those of the control group.

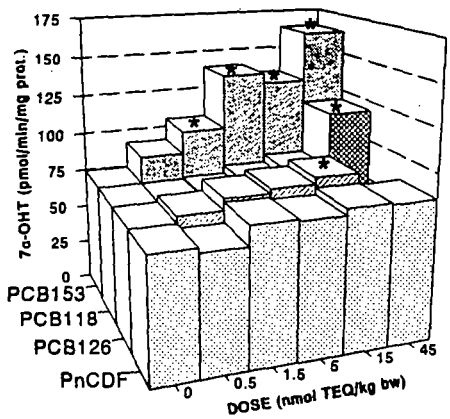
Table 1: Statistically significant hepatic P450 induction in 16 week-old male rats perinatally exposed to PCB 126, PCB 118, PCB 153 or 2,3,4,7,8-PnCDF (highest dose groups)

OH-testosterone	PnCDF	PCB 126	PCB 118	PCB 153
16β-OHT (CYP2B1)	=	↑ (1.5x)	=	↑ (1.7x)
2α-OHT (CYP2C11)	=	=	=	=
6β-OHT (CYP3A1/2)	=	=	=	↑ (1.5x)
7α-OHT (CYP2A1)	=	=	=	=

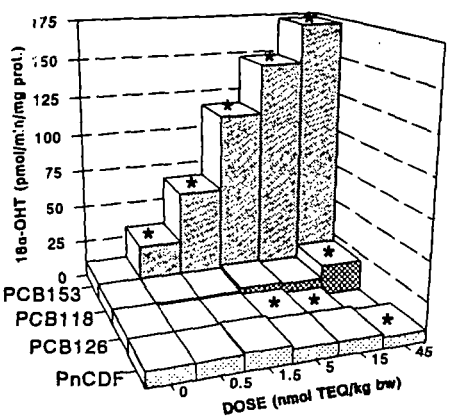
Numbers in parentheses indicate fold induction

Fig. 1: CYP2A1 (A), CYP2B1 (B) and CYP3A1/2-related activities (C) in livers of 3 week-old rats perinatally exposed to PCB 126, PCB 118, PCB 153 or 2,3,4,7,8-PnCDF

(A)



(B)



(C)

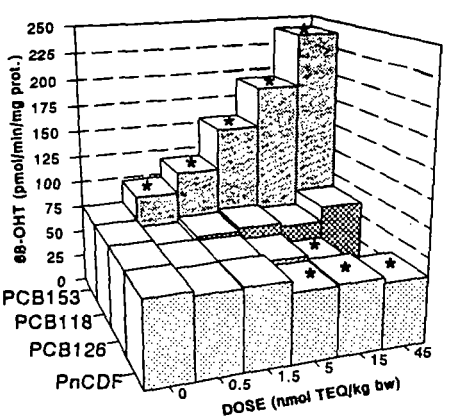


Table 2: Hepatic concentrations of PCB 126, PCB 118 and PCB 153 in 16 week-old rats after perinatal exposure

Congener		lowest dose	low dose	mid dose	high dose	highest dose
PCB 126	male	≤ 0.01[a]	≤ 0.01	≤ 0.01	0.75 ± 0.34	-
	female	≤ 0.01	0.17 ± 0.05	0.53 ± 0.07	1.04 ± 0.36	-
PCB 118	male	1.2 ± 0.3	3.9 ± 0.7	12.5 ± 3.4	23.8 ± 13.0	45.7 ± 15.5
	female	1.9 ± 0.4	5.9 ± 1.3	13.3 ± 4.5	34.1 ± 9.8	71.2 ± 23.7
PCB 153	male	65 ± 15	104 ± 24	268 ± 86	451 ± 81	1,053 ± 346
	female	92 ± 23	163 ± 38	289 ± 52	542 ± 94	1,395 ± 296

[a] in ng per gram liver; group means and standard deviations
Data of PnCDF will be presented at the symposium

Plasma testosterone concentrations No significant effects on plasma testosterone concentrations were found in 16 week-old male offspring (data not shown).

Liver concentrations At PND 112 dose-dependent disposition of PCB 126, PCB 118, PCB 153 and PnCDF were still measurable in the livers of the offspring (table 2).

4. Discussion and conclusions

Perinatal exposure to PCB 126 and PnCDF resulted in induction of female-predominant CYP2A1 activity and reduction of male-predominant P450 activities in 3 week-old rats. While in PCB 153 and PCB 118-exposed offspring both female and male-predominant P450s were (dose-dependently) induced. At PND 112 after raising the offspring to adulthood, only slight induction was measurable in rats exposed to the highest doses[5]. Most likely the observed induction is caused by low concentrations of the congeners that are still present in the livers of the offspring.

From the results of these studies lifelong alterations in hepatic P450 expression due to perinatal interference of PCBs with the imprinting of the P450s is doubtful and unlikely as a mechanism of action for the reproduction failures. This conclusion is supported by the observations on reproduction effects in the offspring which are in contrast with the results on P450 induction at PND 112. The most severe reproduction effects were observed in PCB 126-exposed offspring, followed by PnCDF and finally PCB 118 exposure[5]. In the offspring exposed to PCB 118 no P450 induction was found again at PND 112. With PCB 153 the only effect found was a decrease in epididymal sperm counts in the male offspring[5]. Yet, in PCB 153-exposed offspring induction of P450 activities was most pronounced compared to all other offspring. In addition, no differences in plasma testosterone concentrations were found in exposed male offspring at PND 112.

In view of the different effects of perinatal exposure to PCBs on sex-dependent P450s in 3 week-old offspring, it might be worthwhile to study the effects of P450 induction during early development, in connection with steroid hormone concentrations and reproduction failure later in life.

5. References

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