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Absence of hepatic microsomal pentyl- or benzyl-resorufin *O*-dealkylase induction in rainbow trout (*Salmo gairdneri*) treated with phenobarbitone

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Phenobarbitone (PB) induces hepatic microsomal mono-oxygenases, particularly *N*-demethylases and aldrin epoxidase, in mammals [1–3] but does not seem to do so in fish [4, 5]. However, acrivities of these enzymes in fish are low, and even in mammals PB induces a relatively small (2- to 3-fold) increase in activity [1, 3]; thus, the inference that fish hepatic mono-oxygenases are not inducible by PB is based on measurements of enzyme activities that are not very sensitive to induction, and which may be assayed near their detection limits. Pentyl- and benzyl-resorufin (alkoxyphenoxazone) *O*-dealkylase (PROD and BROD respectively) activities in mammalian liver microsomes are selectively induced by PB [6, 7], often to many times control values, and are, therefore, sensitive indicators of PB induction. We now report that using these assays we found no change in hepatic microsomal mono-oxygenase activity in trout treated with PB.

Materials and methods

Cultured immature rainbow trout (*Salmo gairdneri*) weighing approximately 100–200 g were kept in flowing fresh water at 20°. In two experiments, trout were randomised by weight into groups of six fish, kept separately in fiberglass tanks. In Expt. 1 control fish were not treated; polychlorinated biphenyl (PCB)-treated fish received a single i.p. injection of Aroclor 1254 at 100 mg/kg. A third group was kept in a recirculating system with a sand filter in approximately 500 liters of fresh water containing phenobarbitone sodium (BDH) at 80 mg/l to provide a constant supply of PB to the fish. After 5 days the fish were killed by a blow on the head, and livers were removed and analysed essentially as described previously [8].

Experiment 2 was carried out as described above, but treatments consisted of β-naphthoflavone (β-NF: 100 mg/kg i.p., in 0.1 ml olive oil), phenobarbitone sodium in water at 50 mg/l, and controls (0.1 ml olive oil, i.p.), for 7 days.

Microsomal protein was determined by the method of Lowry *et al.* [9]. *O*-Dealkylase activities were determined at 20° as described for ethylresorufin *O*-de-ethylase (EROD; [10]) in 2 ml of 0.1 M phosphate buffer, pH 8.0, containing NADPH (Sigma) at 150 μM and 1–5 mg microsomal protein. Ethylresorufin was used at 2.5 μM; pentylresorufin and benzylresorufin [6] were used at 5 and 10 μM respectively. Under these conditions, the rate of resorufin production was linear for at least 2 min.

PCB residues were analysed [11] in samples of lateral muscle. Residues of unchanged PB in water were measured by u.v. absorbance of the lactim tautomer [12] at 260 nm, pH 10.2 [13].

Data were analysed by analysis of variance and by Student's *t*-test.

Results and discussion

Table 1 summarises the results of both experiments. EROD activity in control fish was similar to that reported elsewhere in rainbow trout (e.g. Ref. 14). PROD and BROD activities in control fish were much lower than those of EROD and were near the limit of detection of the reaction (2 pmol resorufin formed·min^{-1}·mg protein^{-1}). (PROD and BROD activities in untreated rats are also lower than those of EROD [6, 15].) PCB treatment in Expt. 1 approximately doubled EROD activity but did not affect BROD or PROD. However, EROD was not induced as much as in other studies (e.g. Ref. 8) even though tissue PCB concentrations increased from 2.09 ± 2.00 μg/g wet wt (mean ± SD, N = 6) in controls to 50.5 ± 30.1 μg/g (mean ± SD, N = 6) in treated fish. In Expt. 2, β-NF increased liver size and protein content significantly and induced EROD about 60-fold but did not change BROD or PROD. PB treatment did not affect BROD or PROD

Table 1. Indices of hepatic MFO activity in trout treated with PCBs (Aroclor 1254), β-naphthoflavone or phenobarbitone

Treatment	Fish wt (g)	Liver (% body wt)	Microsomal protein (mg/g liver)	EROD (pmol/min/mg protein)	PROD (pmol/min/mg protein)	BROD (pmol/min/mg protein)
Experiment 1						
Control (N = 6)	160 ± 33	0.73 ± 0.12	15.9 ± 2.33	32.4 ± 22.0	4.9 ± 3.8	5.3 ± 4.4
PCB (N = 6)	142 ± 47	0.81 ± 0.13	18.1 ± 3.46	64.1 ± 30.6*	2.8 ± 2.6	2.5 ± 4.1
Phenobarbitone (N = 5)	146 ± 32	0.80 ± 0.06	15.0 ± 2.75	20.3 ± 5.2	2.8 ± 3.5	7.2 ± 7.8
Experiment 2						
Control (N = 6)	148 ± 26	1.10 ± 0.18	16.9 ± 1.8	13.0 ± 9.5	1.0 ± 2.5	2.8 ± 3.0
β-Naphthoflavone (N = 6)	126 ± 38	1.31 ± 0.15*	20.6 ± 4.2*	797 ± 332†	6.5 ± 7.6	3.4 ± 5.2
Phenobarbitone (N = 6)	125 ± 15	1.09 ± 0.15	17.2 ± 2.2	15.8 ± 12.8	0.6 ± 1.4	1.7 ± 2.9

Trout were treated with PCBs (Aroclor 1254), β-naphthoflavone or phenobarbitone as described in the text. Data are mean ± SD. Abbreviations: EROD, ethylresorufin *O*-de-ethylase; PROD, pentylresorufin *O*-dealkylase and BROD, benzylresorufin *O*-dealkylase.

* Significantly different from control value by Student's *t*-test ($P < 0.05$).

† Significantly different from control value by Student's *t*-test ($P < 0.01$).

in either experiment, although PB concentrations in the tank water remained constant throughout the exposures (83.7 ± 1.6 mg/l, mean ± SD of four daily estimations in Expt. 1, and 49.3 ± 1.9 mg/l, mean ± SD of six daily determinations in Expt. 2). (Control water had a u.v. absorbance corresponding to <1 mg/l PB.) In contrast, mice injected i.p. with three doses of PB (80 mg/kg) showed 4-fold induction of PROD and 8-fold induction of BROD in our hands [16].

Induction of EROD activity by PCBs and β-NF is, therefore, consistent with previous results and, in the case of PCBs, can be attributed to the planar congeners present in the mixture. The absence of PROD or BROD induction either by PB or by the non-planar PCB congeners in Aroclor 1254 cannot be attributed to either insufficient time for induction or failure of the fish to accumulate the compounds. In other studies (e.g. Ref. 17), trout hepatic mono-oxygenases were induced within approximately 3 days of treatment with various inducers, so the exposures used here should have allowed ample time for induction. Appreciable amounts of PCB residues (including non-planar components identifiable by capillary column GC) were accumulated by the fish, and in other studies [16] water concentrations of PB similar to those used here produced serum PB concentrations (measured by HPLC [18]) in the 50–150 mg/l range.

In summary, treatment of rainbow trout with PCBs and with β-NF induced hepatic microsomal EROD activity. However, neither the PCBs (accumulated to tissue concentrations of around 50 μg/g) nor PB administered in water at constant concentrations of around 50 or 80 mg/l for 5–7 days affected BROD or PROD activity, even though these enzymes have been shown to be sensitive to PB induction in mammals.

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