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Multiple effects of 3,4,5,3′,4′,5′-hexachlorobiphenyl administration on hepatic cytochrome P450 isozymes and associated mixed-function oxidase activities in rainbow trout

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Polychlorinated biphenyls (PCBs) are major environmental pollutants that are potent inducers of hepatic and extrahepatic drug-metabolizing enzymes in mammals [1]. The commercial PCB mixture, Aroclor 1254, simultaneously induces the 3-methylcholanthrene (3-MC)-inducible cytochromes P450IA1 and P450IA2, and the phenobarbital (PB)-inducible cyrochromes P450IIB1 and P450IIB2 [1]. The PCB isomer, 3,4,5,3′,4′,5′-hexachlorobiphenyl (HCB), also induces both cytochromes P450IA1 and P450IA2 [2], but represses rat cytochrome P4502c (UT-A) [3].

In rainbow trout, Aroclor 1254 induces cytochrome P450 LM4b but reduces the level of the constitutive P450 isozyme, LM2 [4]. However, little is known about the effects of individual isomers of PCB on trout P450s. In this report, we examine the effects of HCB administration on the levels and catalytic activities of three previously identified constitutive P450 isozymes from rainbow trout [5], namely LMC1, LMC2 and LMC5, and on mixed-function oxidase (MFO) activities associated with these isozymes. The letters LMC stand for "liver microsomal constitutive" and the numbers 1 to 5 indicate the P450 isozymes in the order of their increasing molecular weight on sodium dodecyl sulfate–polyacrylamide gel electrophoresis [5]. LMC2 is believed to be identical to LM2 previously isolated from β-naphthoflavone (BNF)-treated trout [4, 5].

Methods

Male and female rainbow trout (*Oncorhynchus mykiss*) of the Mt. Shasta strain (14 months old) were injected i.p. with HCB in corn oil, at doses of 1 or 20 mg/kg. A control group for each sex was injected i.p. with corn oil. The fish were killed 5 days after HCB treatment, and livers were removed for tissue fractionation. Liver microsomes were prepared as previously described [5].

Hepatic microsomal protein [6], total cytochrome P450 content [7], and benzo[*a*]pyrene (BaP) hydroxylase activity [8] were measured as described. The NADPH-dependent covalent binding of aflatoxin B_1 (AFB_1) to DNA and progesterone 6β-hydroxylase activity were determined by the method of Yoshizawa *et al.* [9] and Backes *et al.* [10] respectively. All enzyme assays were done at 30° using a 30-min incubation period.

Western blot analysis of cytochrome P450 isozymes in trout liver microsomes was performed according to the method of Miranda *et al.* [11]. The data obtained in this study were analysed by Student's *t*-test.

Results and Discussion

The results of the Western blot analysis of trout liver microsomes demonstrated that HCB produces a selective repression and induction of hepatic P450 isozymes in trout (Table 1). At 20 mg/kg HCB, LMC2 levels were reduced

Table 1. Total hepatic microsomal cytochrome P450 content and individual cytochrome P450 isozyme concentrations in liver microsomes from rainbow trout treated with HCB*

Treatment	Total cytochrome P450 (nmol/mg microsomal protein)	P450 isozymes (nmol/mg microsomal protein)			
		LMC1	LMC2	LMC5	LM4b
Males					
Control	0.27 ± 0.05	33	54	34	2
HCB					
1 mg/kg	0.26 ± 0.02	36	56	36	88
20 mg/kg	0.28 ± 0.03	35	12	32	191
Females					
Control	0.26 ± 0.03	26	49	32	2
HCB					
1 mg/kg	0.29 ± 0.04	27	16	31	90
20 mg/kg	0.24 ± 0.05	25	6	33	163

* Trout (14 months) were killed 5 days after an i.p. administration of HCB or corn oil (control). Values for total cytochrome P450 content determined spectrally are means ± SE of four to six animals, whereas values for individual P450s are means of duplicate or triplicate determinations.

by 78 and 88% in males and females respectively. At 1 mg/kg, LMC2 was reduced by 67% in females and unchanged in males. The concentrations of LMC1 and LMC5 were not affected by HCB treatment. In contrast, the levels of LM4b, represented by protein bands immunostained with anti-LM4b antibody, was increased markedly in microsomes from both males and females treated with HCB, with the greatest increase at a dose of 20 mg/kg. Although the concentrations of the individual P450 isozymes were altered, total P450 content measured spectrally (Table 1) and protein content (data not shown) of trout liver microsomes were unaffected by HCB treatment.

A close examination of the Western blots of liver microsomes of rats treated with 20 mg/kg of HCB revealed that two protein bands are recognized by the anti-LM4b antibody (Fig. 1). One of the bands had the same electrophoretic mobility as LM4b (M_r = 58,000), whereas the other band had a slightly greater mobility than LM4b. These two bands were quantitated as one band representing LM4b in Table 1.

The induction of two trout P450 isozymes with different mobilities as seen in the present study may be a unique finding in fish with HCB as the inducer. Although Heilmann *et al.* [12] found only one gene coding for induced P450 ($P_1$450) in 3-MC-treated rainbow trout, it is possible that a second gene coding for a different P450 isozyme could be expressed in the HCB-treated trout. In rats, HCB induces two hepatic P450 isozymes, $P450_{MC}$ and $P450_{HCB}$ (which are identical to P450IA1 and P450IA2 respectively); they are expressed by two different genes [2]. Further studies are needed to confirm that HCB or other PCB isomers induce two P450 isozymes in rainbow trout.

The catalytic activities of the liver microsomes of HCB-treated trout towards selected substrates are shown in Table 2. The substrates chosen were those that provide a measure of the characteristic activity of LMC2, LMC5 and LM4b found in a previous study [5]. The metabolic activation of AFB_1 as measured by DNA adduct formation which is catalysed almost exclusively by LMC2 [5] or LM2 [4] was decreased significantly in male and female trout dosed with

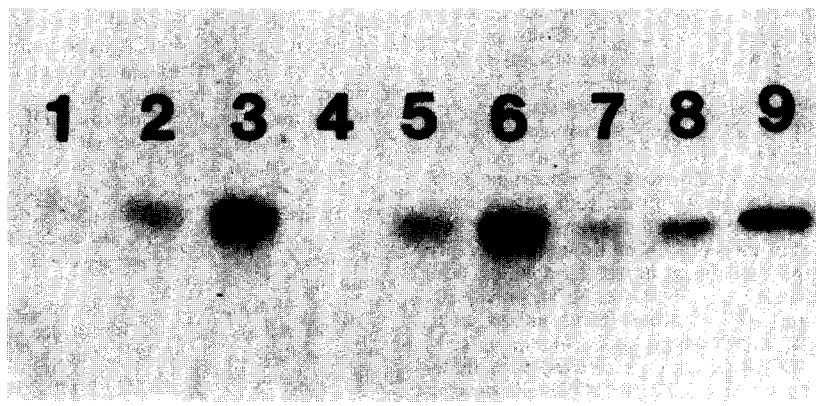

Fig. 1. Western blotting of rainbow trout liver microsomes (15 mg/well) and purified LM4b and immunostaining for LM4b. The lanes represent: microsomes from control male (lane 1); male, 1 mg/kg HCB (lane 2); male, 20 mg/kg HCB (lane 3); control female, (lane 4); female, 1 mg/kg HCB (lane 5); female, 20 mg/kg HCB (lane 6); LM4b, 0.5 pmol (lane 7); LM4b, 1.0 pmol (lane 8); and LM4b, 2.0 pmol (lane 9).

Table 2. Effect of HCB on the *in vitro* binding of aflatoxin B_1 metabolites to DNA and on progesterone 6β-hydroxylase and benzo[*a*]pyrene (BaP) hydroxylase activities of liver microsomes from rainbow trout*

Treatment	DNA binding (pmol/min/mg protein)		Progesterone 6β-hydroxylase (pmol/min/mg protein)		BaP hydroxylase (nmol/min/mg protein)	
	Male	Female	Male	Female	Male	Female
Control	19.3 ± 1.1	24.6 ± 2.7	126 ± 4	137 ± 14	0.03 ± 0.003	0.03 ± 0.002
HCB						
1 mg/kg	20.2 ± 1.8	13.3 ± 1.4‡	181 ± 18†	215 ± 13†	0.23 ± 0.06†	0.20 ± 0.04†
20 mg/kg	7.7 ± 3.1†‡	8.1 ± 1.1†‡	59 ± 9†‡	39 ± 6†‡	0.11 ± 0.04†‡	0.10 ± 0.05†‡

* Trout (14 months) were killed 5 days after an i.p. administration of HCB or corn oil (control). Values are means ± SE of four to six animals.

† $P < 0.05$ vs control of the same sex.

‡ $P < 0.05$ vs HCB, 1 mg/kg, of the same sex.

20 mg/kg of HCB. These HCB-induced changes in AFB_1 bioactivation are consistent with the reduction of LMC2 in trout liver microsomes (Table 1). These findings suggest that this PCB isomer has the potential to modulate AFB_1-induced carcinogenesis like Aroclor 1254 [13].

Microsomal hepatic 6β-hydroxylation of progesterone which is catalysed by LMC5 [5] was decreased significantly by HCB at a dose of 20 mg/kg (Table 2). However, immunodetectable LMC5 was not reduced at this dose of HCB in male and female trout. These findings indicate that the reduction in enzyme activity is not due to a repression in the synthesis of LMC5. At 20 mg/kg, HCB may bind and inactivate or inhibit the enzyme similar to the binding of 3,4,5,3′,4′,5′-hexabromobiphenyl (HBB) to rat P450IA1 [14].

HCB induced the activity of microsomal BaP hydroxylase in an unexpected manner. In both male and female trout, BaP hydroxylase activity was lower at 20 mg/kg than at 1 mg/kg of HCB (Table 2). However, the protein bands stained with LM4b antibody were much greater at 20 mg/kg than at 1 mg/kg (Table 1), suggesting that there could be inhibition of the P450 isozymes induced at the higher dose of HCB. These findings are analogous to the stronger induction of hepatic ethoxyresorufin *O*-deethylase activity at lower doses than at higher doses of 3,4,3′,4′,-tetrachlorbiphenyl in scup (*Stenotomus chrysops*) [15]. The direct inhibitory effect of HCB on BaP hydroxylase activity of trout liver microsomes is being investigated currently in our laboratory.

An alternative explanation for the decrease in progesterone 6β-hydroxylase and BaP hydroxylase activities of liver microsomes from trout dosed with 20 mg/kg of HCB is a reduction in microsomal NADPH–cytochrome *c* reductase activity. However, the latter was unchanged after HCB treatment (data not shown). The lack of effect of the 20 mg/kg dose of HCB on hepatic NADPH–cytochrome *c* reductase activity as well as on total cytochrome P450 content, microsomal protein or liver weights (data not shown) indicates that HCB was not overtly hepatotoxic at the dose used. Thus, hepatotoxicity is excluded as an explanation for the decrease in xenobiotic metabolism in trout liver microsomes following HCB treatment.

In summary, the results of these studies demonstrate that the administration of HCB produces a selective repression, inhibition and induction of hepatic cytochrome P450 isozymes in rainbow trout without any change in total P450 content of liver.

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Effects of indomethacin and prostaglandin E_2 on amylase secretion by rat parotid tissue

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There is much evidence that prostaglandins (PGs) modulate sympathetic [1, 2] and parasympathetic [3–7] neuronal activity. The salivary glands are innervated by both autonomic nervous systems, and excitations of both systems enhance their secretory responses. $PGF_{2\alpha}$ [8–11] and PGE_2 [12] were reported to induce salivary secretion by the submandibular gland of dogs, and PGE_1 [13] to induce secretion by the parotid gland of rats. Results have suggested that $PGF_{2\alpha}$ induces salivation by exciting the parasympathetic neurons [8–11], and that PGE_1 acts as a modulator of the secretory response to cholinergic stimulation [14]. There is also a negative report on the role of endogenous PGs during parasympathetic nerve stimulation of rat salivary glands [15]. However, little is known about whether PGs have primary or modulatory roles in the amylase secretory response of the parotid gland. In the present study we examined the effects of PGE_2 on amylase secretion from rat parotid tissue *in vitro* induced by adrenergic agonists.

Materials and Methods

Parotid glands were obtained from male Wistar rats (250–350 g), and small pieces of the tissue were prepared as described previously [16]. Before experiments, Krebs–Ringer Tris (KRT) solution, consisting of 120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgCl_2$, 3.0 mM $CaCl_2$, 16 mM Tris–HCl buffer (pH 7.4) and 5 mM glucose, was aerated with O_2 gas, and pieces of parotid tissue were equilibrated with the solution for 20 min at 37° with shaking. Samples of about 30 mg of tissue pieces were incubated in 10 mL of KRT solution at 37°, and cumulative secretion of amylase into the medium was measured as described by Bernfeld [17]. Activity was assayed at 20° for 5 min with amylase as substrate and expressed as the amount of maltose liberated into the medium in mg per 100 mg tissue.

Indomethacin was dissolved in ethanol and added to the incubation medium at a final concentration of 14 μM with 0.5% ethanol. This concentration of ethanol did not affect

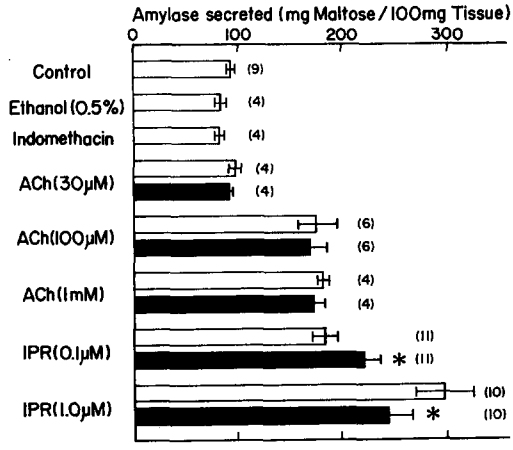

Fig. 1. Effects of indomethacin on amylase secretion from rat parotid tissue. Tissues were incubated at 37° for 10 min with the indicated agonists in the absence (open columns) and presence (closed columns) of 14 μM indomethacin. Columns and bars are means and standard errors; the numbers of experiments are shown in parentheses. Significantly different from the value in the absence of indomethacin by Student's paired *t*-test, $^*P < 0.01$.