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Species and congener specific induction of hepatic cytochrome P4504A by polychlorinated biphenyls

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Cytochrome P450 is a large gene family of haemoproteins responsible for the metabolism of a variety of substrates ranging from highly toxic environmental pollutants to biologically important endogenous substances, such as fatty acids in both man and experimental animals [1]. The cytochrome P4504AI isoenzyme is responsible for fatty acid 12-hydroxylation [2], and is a constitutive isoenzyme, which can be induced by treatment with hypolipidaemic agents [3], phthalate ester plasticizers [4] and by the pathophysiological state of diabetes [5]. Induction of cytochrome P4504AI by the ubiquitous environmental pollutants, polychlorinated biphenyls (PCBs*), was first reported in rats by Borlakoglu *et al.* [6] after treatment with Aroclor 1254. However, Aroclor 1254 is a technical grade mixture of PCBs, containing many different PCB congeners and isomers and previous work has demonstrated that different sub-families and particular cytochrome P450 isoenzymes are inducible by these isomers [7, 8]. We now report on the PCB-congener specific induction of the cytochrome P4504A sub-family as assessed by its associated lauric acid hydroxylase activity in the rat and the guinea pig, the latter species being chosen because of our recent arachidonic acid hydroxylase induction studies by a PCB in this species [9].

Materials and Methods

2,4,2′,4′-Tetrachlorobiphenyl (TCB) (99% pure) and 3,4,5,3′,4′-pentachlorobiphenyl (PENCB) (99% pure) were purchased from the Greyhound Chromatography & Allied Chemical Co. (Birkenhead, Merseyside, U.K.). Although it is unlikely because of the high purity of these chemicals, we cannot exclude the possibility that contaminants may be present which may have biological activity. All other chemicals were obtained from commercial sources and were of the highest purity available.

Male Wistar albino rats (100–130 g initial body weight, University of Surrey Breeders) were administered a single i.p. injection of either TCB (88 mg/kg in corn oil) or PENCB (5 mg/kg in corn oil), the control rats receiving an equivalent amount of the corn oil vehicle. Male Dunkin Hartley guinea pigs (130–190 g initial body weight, University of Surrey Breeders) were given a single i.p. injection of either a high dose (88 mg/kg) or low dose (29 mg/kg) of TCB in corn oil. PENCB was given to guinea pigs by a single i.p. injection at the dose levels of 0.025, 0.05, 0.05, 0.1, 0.5 and 1.0 mg/kg. These dose levels in the rat and guinea pig were chosen because they caused maximal induction of cytochrome P450 in a previous study [9]. The animals were maintained in a 12 hr light/12 hr dark cycle with feed provided *ad lib.* and killed 5 days after dosing by cervical dislocation. The livers were immediately removed and perfused with 0.9% (w/v) ice-cold saline, blotted dry, weighed and scissor-minced in 0.25 M ice-cold sucrose and a 25% (w/v) homogenate prepared. Liver microsomal fractions were isolated by ultracentrifugation as described previously [9], and microsomal protein [10], total cytochrome P450 [11] and lauric acid 11- and 12-hydroxylase activities [12] determined as indicated.

Results and Discussion

TCB and PENCB are representatives of the phenobarbital-type and 3-methylcholanthrene-type of PCB inducers, respectively [7, 8], and both significantly increased the total hepatic cytochrome P450 content, but differentially influenced laurate 11- and 12-hydroxylase activities in the rat (Table 1). At a dose of 88 mg/kg, TCB significantly

* Abbreviations: PCB, polychlorinated biphenyls; TCB, 2,4,2′,4′-tetrachlorobiphenyl; PENCB, 3,4,5,3′,4′-pentachlorobiphenyl.

Table 1. PCB congener-specific induction of lauric acid 11- and 12-hydroxylase activities in the rat

	Total P450 specific content (nmol/mg)	Lauric acid hydroxylase (nmol/min/mg)	
		11-Hydroxylase	12-Hydroxylase
Control	0.78 ± 0.10	1.18 ± 0.22	2.57 ± 0.69
TCB (88 mg/kg)	1.27 ± 0.05†	1.81 ± 0.34*	4.69 ± 0.23*
PENCB (5 mg/kg)	2.59 ± 0.15‡	0.42 ± 0.22†	0.74 ± 0.37*

Results are expressed as mean ± SD from three animals. The assay was carried out in duplicate at 37°.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ as compared to the control value (Student's *t*-test).

induced the 11- and 12-hydroxylase activities to 153% and 182% of the corresponding control values when expressed as nmol product formed/min/mg of protein. By contrast, PENCB treatment (5 mg/kg) resulted in significant inhibition of both 11- and 12-hydroxylase activities of lauric acid to 35% and 29% of the corresponding control values. The results presented in Table 1 clearly indicate that cytochrome P4504AI inducibility by PCBs is strictly congener specific in rat.

Table 2. Dose-dependent induction of laurate hydroxylase activities by PENCB treatment in the guinea pig

PENCB dose (mg/kg)	11-Hydroxylase (nmol/min/mg)	12-hydroxylase (nmol/min/mg)
0	2.35 ± 0.26	3.54 ± 0.48
0.025	2.70 ± 0.62	3.35 ± 0.51
0.05	3.88 ± 0.67*	4.94 ± 0.67*
0.1	4.20 ± 0.45‡	6.02 ± 0.39†
0.5	3.51 ± 0.37†	6.25 ± 0.56†
1.0	4.06 ± 0.27‡	7.14 ± 0.58‡

Guinea pigs were treated with a single i.p. injection of PENCB at different dose levels and killed 5 days after treatment. The activities of lauric acid 11- and 12-hydroxylases were determined from the liver microsomal fractions as described in Materials and Methods.

Results are expressed as the mean ± SD from three animals.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ as compared to the control value (Student's *t*-test).

Table 2 shows the dose dependency of PENCB induction of laurate 11- and 12-hydroxylase activities in the guinea pig. PENCB treatment resulted in a dose-dependent increase in 12-hydroxylase activity in guinea pig liver microsomal fractions. The maximum induction of laurate 12-hydroxylase activity was obtained at 1.0 mg/kg, the highest dose used in the study. The 11-hydroxylase activity was also significantly induced by PENCB at doses above 0.05 mg/kg. The maximum induction of this enzyme activity was observed at a dose of 0.1 mg/kg (1.79-fold) and higher doses did not cause any further induction of the 11-hydroxylase activity. These PENCB-dependent increases in lauric acid hydroxylation in the guinea pig are mirrored by similar increases in both total cytochrome P450 specific content and ELISA-based determinations using an antibody to P4504AI, as reported previously [9].

Compared to the rat (Table 1), TCB treatment of guinea pigs had no effect on either the induction of hepatic microsomal cytochrome P450 content or the lauric acid 11- and 12-hydroxylase activities in either low or high doses (Table 3). The above-noted changes in lauric acid hydroxylase activities are consistent with PENCB-dependent changes in Western blot analyses using a P4504AI antibody, as described previously [9].

In conclusion, results presented in the present study provide preliminary evidence that the induction of hepatic cytochrome P4504A-related isoenzymes by PCB treatment is both species dependent and congener specific. PENCB significantly suppressed cytochrome P4504A expression in the rat, but significantly induced its activity in the guinea pig, whereas TCB treatment significantly induced the activity in the rat but had no effect in the guinea pig. Which particular isoenzyme(s) of the cytochrome P4504A family [13] is modulated by these PCBs awaits further resolution.

Table 3. Effects of TCB treatment on lauric acid 11- and 12-hydroxylase activities in the guinea pig

	Total P450 specific content (nmol/mg)	Lauric acid hydroxylase activity	
		11-Hydroxylase	12-Hydroxylase
Control (5)	0.94 ± 0.08	2.16 ± 0.42	3.58 ± 0.68
29 mg/kg TCB (3)	0.87 ± 0.18	2.32 ± 0.68	2.89 ± 0.51
88 mg/kg TCB (3)	0.96 ± 0.09	2.10 ± 0.53	3.67 ± 0.07

Guinea pigs were treated once with low (29 mg/kg) or high (88 mg/kg) doses of TCB and killed 5 days after dosing. The 11- and 12-lauric acid hydroxylase activities were determined as described in Materials and Methods from the liver microsomal fractions, and expressed as nmol product/min/mg. Statistically, no difference was observed in either the total cytochrome P450 content or lauric acid 11- and 12-hydroxylase activities between control and treated groups. The numbers in brackets represent the numbers of animals in each group.

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Differential inhibition of long-chain acyl-CoA hydrolases by hypolipidemic drugs *in vitro*

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Abstract—The effect of *in vitro* addition of three hypolipidemic drugs (clofibric acid, bezafibrate and gemfibrozil) on rat palmitoyl-CoA hydrolases has been studied, by using a spectrophotometric method (Berge RK, *Biochim Biophys Acta* **574**: 321–333, 1979) optimized for valoration of crude enzyme preparations. Mitochondrial and microsomal hepatic palmitoyl-CoA hydrolase activities were inhibited by the three drugs in a concentration-dependent fashion. The order of inhibitory potency was gemfibrozil > bezafibrate > clofibric acid, irrespective of the enzyme activity tested. Cytosolic rat brain palmitoyl-CoA hydrolase activity was not affected. Kinetic studies with gemfibrozil on the solubilized microsomal palmitoyl-CoA hydrolase activity point to a mixed non-competitive type of inhibition.

Long-chain acyl-CoA hydrolases are enzymes that are widely distributed in mammalian tissues [1]. They catalyse the hydrolysis of fatty acyl-CoA thioesters [2] and play an important role in controlling the chain length of synthesized fatty acids and modifying the product specificity of fatty acid synthetase [3]. Several researchers have shown the existence of at least three different long-chain fatty acyl-CoA hydrolases, two located in membranous subcellular