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Evidence for the induction of cytochrome P-452 in rat liver by Aroclor 1254, a commercial mixture of polychlorinated biphenyls

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We have examined the ability of a commercial mixture of polychlorinated biphenyls (Aroclor 1254) to induce hepatic cytochrome P-452-linked enzyme activities in rat and pigeon liver five days after its intraperitoneal injection. The results provide evidence that, at the doses used, Aroclor 1254 induces cytochrome P-452-linked enzyme activities in rats, but not in pigeons. This inductive effect was previously regarded as being specific for hypolipidemic drugs and phthalate ester plasticisers.

Polychlorinated biphenyl; Cytochrome P-450; Lauric acid hydroxylation; Nonradioisotopic assay; (Rat liver, Pigeon liver)

1. INTRODUCTION

Polychlorinated biphenyls (PCBs) are fat-soluble commercial products noted for their chemical stability, miscibility with organic solvents, non-flammability and excellent electrical insulation properties. This has led to their widespread use as industrial fluids, flame retardants and diluents, and hydraulic fluids for capacitors and transformers. Careless disposal practices, e.g. leakage from disused equipment and disposal into sewers, rivers and coastal waters, have led to these compounds becoming one of the most abundant industrial pollutants in the ecosystem. These fat-soluble PCBs accumulate in biological systems by ingestion of PCB-contaminated lipid components of food chains, and PCBs are routinely detected in the fatty tissues of animals, including humans, at the apex of these food chains [1-3].

Commercial mixtures of PCBs (e.g. Aroclor 1254; see section 2) and individual isomers and congeners have the ability to induce several forms of hepatic microsomal cytochrome P-450 (e.g. cytochromes P-450a to P-450e) as well as other enzyme systems involved in hepatic drug metabolism, in a fashion similar to that which occurs after treatment of rodents with phenobarbital (e.g. 'phenobarbital-type' induction), polycyclic aromatic hydrocarbons (e.g. '3-methylcholanthrene-type' induction) or with a combination of both (e.g. 'mixed type' induction) [2].

It is not known whether PCB mixtures have the ability to induce cytochrome P-452, which is specifically induced in hepatic microsomes by hypolipidemic drugs such as clofibrate and by phthalate ester plasticisers [4]. Increased ω-oxidation of the model substrate lauric acid (12:0) to 12-hydroxylauric acid by hepatic microsomes in response to treating rats with clofibrate has been attributed to the induction of cytochrome P-452-linked enzyme activities [4]. In contrast, treating rats with phenobarbital increases the $\omega-1$ oxidation of lauric acid to 11-hydroxylauric acid by hepatic microsomes, but does not increase the ω-

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oxidation of this substrate [5]. Since Aroclor 1254 is known to be very versatile in inducing several forms of cytochrome P-450 in rat hepatic microsomes, it was of interest to investigate whether it was also effective in inducing cytochrome P-452-linked enzyme activities. For comparison between species, the effects of Aroclor 1254 on pigeon hepatic microsomes were also measured.

Two principal methods are available to measure the ω- and $\omega-1$ hydroxylation of radiolabeled lauric acid in vitro: (i) analysis of the resulting mixture by thin-layer chromatography and measurement of the radioactive products by liquid scintillation [6]. Although rapid and simple, this method does not separate 11- and 12-hydroxylauric acids effectively; (ii) an advanced HPLC technique to separate 11- and 12-hydroxylauric acids using a non-isocratic gradient and radiochemical detection [7]. We report here a new method of separating 11- and 12-hydroxyacids by high resolution capillary gas chromatography coupled with flame ionization detection that provides the accuracy and sensitivity required to measure the formation of these hydroxyacids without using radiolabeled lauric acid as substrate. These products were then unambiguously identified by high resolution capillary gas chromatography-mass spectrometry by comparison with authentic standards.

2. MATERIALS AND METHODS

2.1. *Aroclor 1254*

This was a gift from Dr J.P.G. Wilkens, MAFF Harpenden Laboratory, Herts, England. It is a mixture of chlorinated biphenyls, and contains approx. 70 different isomers and congeners with molecular masses ranging from 188 to 430 Da. The average molecular mass for the mixture is 326 Da.

2.2. *Treatment with Aroclor 1254*

Adult female rats were given a single intraperitoneal injection of 600 μmol/kg bodyweight of Aroclor 1254 in corn oil, and pigeons given a single intraperitoneal injection of 1500 μmol/kg of Aroclor 1254 in corn oil. Five days later the animals were killed and hepatic microsomes prepared from control and PCB-treated animals.

2.3. *Incubation conditions and extraction of hydroxylauric acids*

Microsomes (1 mg protein/ml) were incubated for 5 min in 50 mM Tris-HCl buffer, pH 7.4, 150 mM $MgCl_2$, 500 mM nicotinamide, 12 mM $NADP^+$, 12 mM glucose 6-phosphate, glucose 6-phosphate dehydrogenase (60 units) and 1 μM lauric acid (final volume 3 ml). The reaction was stopped with 7.5 ml methanol, 100 mg of methyl arachidate (methyl 20:0) as internal standard and chloroform (16 ml) were added and the mixture vortexed. The aqueous phase was removed, the chloroform extract dried over anhydrous sodium sulphate, and evaporated to 0.5 ml.

2.4. *Separation by capillary gas chromatography and quantitation*

The hydroxy-fatty acids were silylated with Trisil (Pierce reagent), separated on a Chrompak CP Sil 5CB column (50 m × 0.22 mm i.d.) using a Packard 436 capillary gas-liquid chromatograph, and identified by comparison with the relative retention times of authentic standards. Quantitation was based on an internal standard method with prior calibration using known amounts of hydroxylauric acids. Samples were injected in the split mode with a split ratio of 55:1 at a carrier gas flow rate of 0.84 ml/min. Hydrogen was supplied to the flame ionization detector at 30 ml/min, as was nitrogen, with an air flow of approx. 220 ml/min. The temperature was programmed at 200°C for 15 min, raised at 30°C/min to 270°C and maintained at 270°C. The capillary gas chromatograph was interfaced with a Digital Micro PDP 11/23 computer and 'Multichrom' software (V.G. Laboratories, England) to quantify the hydroxy-fatty acids against the known weight of 20:0 methyl ester as internal standard. The detection limit was estimated to be 0.1 pmol.

2.5. *Identification of hydroxylauric acids*

The silylated hydroxy-fatty acids were identified unambiguously by high resolution capillary gas chromatography-mass spectrometry using a Jeol JMS-DX 300 double-focussing mass spectrometer and an experimental protocol as described above. Samples were ionized in the electron impact mode with an accelerating voltage of 70 eV. The formation of hydroxy-fatty acids was linear with microsomal protein and incubation time.

3. RESULTS AND DISCUSSION

Table 1 summarizes the effects of a single injection of Aroclor 1254 on the ability of rat and pigeon hepatic microsomes to metabolize lauric acid to the corresponding 11- and 12-hydroxylauric acids.

With female rats, there was a 5.2-fold increase in the formation of 12-hydroxylauric acid. This hepatic ω-oxidation of lauric acid is specifically enhanced in animals in which cytochrome P-452 has been induced, and lauric acid is known to be an excellent substrate to assay the induction of this cytochrome [4]. Previous work [5] had shown that treatment with the hypolipidemic drug clofibrate resulted in a specific 28-fold induction of hepatic microsomal ω-oxidation of lauric acid in CD-1 mice and marmoset monkeys, whereas phenobarbital treatment increased 11-hydroxylation of lauric acid 2-fold but depressed 12-hydroxylation

Table 1

Effect of polychlorinated biphenyl (PCB)-mediated induction of hepatic cytochrome P-450 on ω- and $\omega-1$ hydroxylation of lauric acid five days after treatment with Aroclor 1254

Species	Treatment	nmol/mg microsomal protein per min	
		12-Hydroxy lauric acid formed	11-Hydroxy lauric acid formed
Rat	None	0.36 ± 0.12	0.41 ± 0.17
	Aroclor (600 μmol/kg)	1.85 ± 1.0**	0.90 ± 0.37*
Pigeon	None	0.12 ± 0.06	0.15 ± 0.04
	Aroclor (1500 μmol/kg)	0.10 ± 0.01	0.30 ± 0.07**

* $p<0.05$; ** $p<0.01$; significant differences between control ($n = 4$) and treated ($n = 5$) rats, and between control ($n = 4$) and treated ($n = 5$) pigeons

of lauric acid to 75% of control values. Hence our observation of a 2-fold increase in rat hepatic microsomal 11-hydroxylation of lauric acid is likely to be due to the known effects of Aroclor 1254 in inducing forms of cytochrome P-450 that catalyse the $\omega-1$ hydroxylation of lauric acid. Unlike rats, pigeons did not respond to treatment with Aroclor 1254 by specifically increasing the hepatic ω-oxidation of lauric acid. The 2-fold increase observed with pigeons in the formation of 11-hydroxylauric acid in response to Aroclor 1254 treatment is similar to that observed with phenobarbital-treated rats [4], and is therefore likely to be due to the induction of several forms of cytochrome P-450 that have the ability to hydroxylate lauric acid at the $\omega-1$ position [2].

In conclusion, the results presented provide evidence for the induction of hepatic cytochrome P-452 in rats treated with Aroclor 1254. This suggests that Aroclor mixtures and individual isomers and congeners may produce a unique pattern of induction of different forms of hepatic cytochrome P-450 that is observed after treating rodents with phenobarbital, 3-methylcholanthrene, and a combination of both of these, as well as after treatment with hypolipidemic drugs and certain plasticisers. The results however also emphasize differences between species in their response to treatment with PCB mixtures.

The induction of cytochrome P-452 may have important biochemical consequences. For example, there is evidence that arachidonate is metabolized by purified cytochrome P-452 at the ω and $\omega-1$ positions [4]. In addition, immunochemical evidence for the induction of a number of different forms of cytochrome P-450 in animals in response to environmental levels of PCB pollution [8] raises the question of whether high levels of contamination with PCBs in humans could alter their response to treatment with hypolipidemic drugs.

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