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Enhancement of epoxide-metabolizing enzyme activities by pure PCB isomers

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Introduction

Current evidence suggests that in mammals the metabolism of polychlorinated biphenyls (PCBs), globally distributed environmental pollutants, may proceed via an electrophilic intermediate, arene oxide [1–3]. The further metabolism of arene oxides, or epoxides, is either enzymatic or non-enzymatic. Enzymatic conversion is catalyzed by epoxide hydrolase (E.C. 3.3.2.3) or by glutathione S-transferase. Commercial preparations of PCBs, which are mixtures of several chlorobiphenyl-isomers, are known to increase the activities of epoxide-metabolizing enzymes in rat liver [4, 5]. To find out how the enhancement of enzyme activities depends on the extent and position of chlorination of the PCB molecule, the effects of pure chlorobiphenyl-isomers on mouse liver epoxide hydrolase and glutathione S-transferase activities were studied.

Materials and Methods

Adult male C57BL mice, fed commercial pellets and with free access to water, were used. Mono- and dichlorobiphenyls, as well as 2,4,2′,4′-tetrachlorobiphenyl, were purchased from RFR Corporation, Hope, RI, U.S.A. Other tetrachlorobiphenyls, hexachlorobiphenyls and decachlorobiphenyl were a kind gift from Dr D. J. Ecobichon, McGill University, Montreal, Canada. PCB isomers were used without further purification. The chemicals, suspended or dissolved in corn oil, were administered intraperitoneally as a single dose (0.32 mmol/kg). Control mice received corn oil only. Due to the poor solubility, decachlorobiphenyl was given as a suspension in corn oil, and 3,4,3′,4′-tetrachlorobiphenyl as a warm (about 40°) corn oil solution. Solubility problems were not encountered with other chlorobiphenyls. Phenobarbital (PB) (80 mg/kg × 5) and 3-methylcholanthrene (MC) (20 mg/kg × 3) were administered intraperitoneally. The mice were killed 7 days, 24 hr, or 48 hr after the last injection of PCB isomers, PB, or MC, respectively. The animals were killed by a cervical dislocation, the livers were removed and homogenized in ice-cold 0.25 mol/l sucrose solution. Microsomal fraction and postmicrosomal supernatant were prepared with the calcium aggregation method as described [6]. The activity of epoxide hydrolase was determined in the microsomal fraction by the method of Oesch *et al.* [7]. Glutathione S-transferase activity was measured in the postmicrosomal supernatant as described by James *et al.* [8]. In both assays [^{3}H]styrene oxide was used as the substrate. Statistical evaluation of the results was made using Student's *t*-test.

Results

Figures 1 and 2 show the activities of epoxide-metabolizing enzymes in mouse liver 1 week after a single i.p. dose of chlorobiphenyls. In all, the effects of 17 PCB isomers are listed. Most of the chlorobiphenyl-isomers, from mono- to hexachlorobiphenyls, increased epoxide hydrolase activity. Enhancement was most prominent in mice treated with hexachlorobiphenyls. The rise in epoxide hydrolase activity was of about the same magnitude in mono-, di-, and tetrachlorobiphenyl-treated mice. However, marked differences were found between various isomers within a chlorination level. For example, the enhancement by 2,5-dichlorobiphenyl (DCB) was stronger than by other dichlorinated isomers, and 2,4,2′,4′-tetrachlorobiphenyl (TCB) and 2,4,5,2′4′,5′-hexachlorobiphenyl (HCB) were more potent than other isomers with corresponding chlorine content. Decachlorobiphenyl did not change the epoxide hydrolase activity.

Only hexachlorobiphenyls and two of the five tetrachlorobiphenyl isomers increased the activity of glutathione S-transferase. The rise in epoxide hydrolase activity was also generally higher than that of glutathione S-transferase. The strongest enhancement, within both enzymes in 2,4,5,2′,4′,5′-HCB-treated mice, was 2.5-fold for epoxide hydrolase and 1.7-fold for glutathione S-transferase.

To compare PCB-isomers with classical enzyme inducers, epoxide hydrolase and glutathione S-transferase activities were measured also in mice treated with phenobarbital or with 3-methylcholanthrene. Phenobarbital markedly enhanced the activity of both enzymes. 3-Methylcholanthrene slightly decreased microsomal epoxide hydrolase activity and had no effect on the activity of cytosolic glutathione S-transferase.

Figure 3 shows the activities of epoxide hydrolase and glutathione S-transferase at various time points after a single i.p. administration of either 2,2′-DCB or 2,4,5,2′,4′,5′-HCB. Both isomers brought about maximal levels of epoxide-metabolizing enzyme activities about 7 days after the exposure, whereafter the activities began to decline.

Discussion

Numerous investigations have shown that polychlorinated biphenyls are potent inducers of mixed function oxidases, enzymes catalyzing the first step in the biotransformation of xenobiotics [5, 9–11]. The enhancement of mixed function oxidation is related to the number as well as to the position of chlorines substituted [10–12]. In general, highly chlorinated isomers are strongest inducers of mixed function oxidases.

The results presented here indicate that hexachlorobiphenyls are generally the most potent PCB isomers to enhance the activities of epoxide-metabolizing enzymes. There were, however, only slight differences in the potency of mono, di-, and tetrachlorobiphenyls when compared to each other. Thus, the number of chlorines does not directly determine the magnitude of enhancement of these enzyme activities. The position of chlorine substituents seems to be more important, as seen in different potency of PCB isomers with the same number of chlorines. Yet there seem to be no specific positions the chlorination of which always would lead to a strong enhancement of epoxide-metabolizing enzyme activities.

The individual PCB isomers belong either to 3-methylcholanthrene or to phenobarbital type inducers of mixed function oxidases [12–14]. Evidence is also accumulating

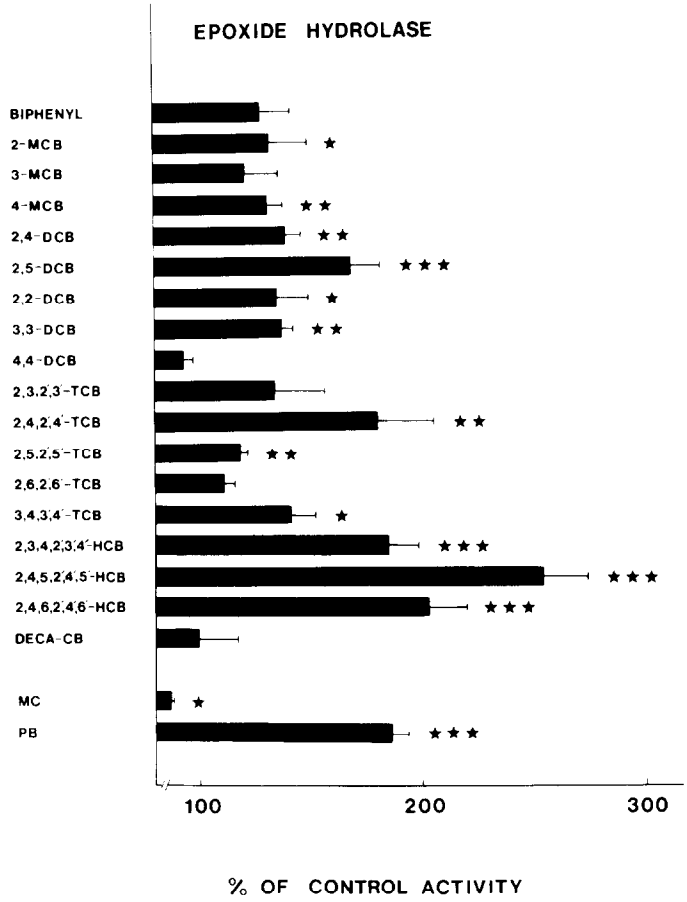

Fig. 1. Hepatic microsomal epoxide hydrolase (styrene oxide) activity of C57BL mice 7 days after a single i.p. dose (0.32 mmol/kg) of PCB isomers. The values represent mean ± S.E.M. from 4–6 animals. Control value was 26.6 ± 1.9 nmol/min per g wet weight. Statistical significance (Student's *t*-test) is expressed as follows: *** = $2P < 0.001$, ** = $2P < 0.01$, * = $2P < 0.05$. Abbreviations: MCB, monochlorobiphenyl; DCB, dichlorobiphenyl; TCB, tetrachlorobiphenyl; HCB, hexachlorobiphenyl; DECA-CB, decachlorobiphenyl; MC, 3-methylcholanthrene, PB, phenobarbital.

to suggest that some PCB isomers have properties of both classical inducers [15, 16]. Previously, 3,4,3',4'-TCB has been shown to be an MC-type inducer [12]. In the present study, however, the effects of this isomer did not much resemble those of 3-methylcholanthrene itself. For example, 3,4,3',4'-TCB and MC had opposite effects on the activity of epoxide hydrolase. All the hexachlorobiphenyls of this study, as well as 2,4,2',4'-TCB, are PB-type inducers [12]. These isomers enhanced the activities of both epoxide hydrolase and glutathione S-transferase, as did phenobarbital itself. The enhancement of epoxide hydrolase activity was greater by the most potent PCB isomer (2,4,5,2',4',5'-HCB) than by PB, whereas the activity of glutathione S-transferase was increased by both chemicals to the same extent.

Goldstein [17] has compared the strength of various PCB isomers as inducers of cytochrome P-450. According to this study 2,4,5,2',4',5'-HCB, 2,3,4,2',3',4'-HCB and 2,4,2',4'-TCB are strong inducers. They were also among the strongest isomers to enhance the activities of epoxide-metabolizing enzymes in the present study. Yet 2,4,6,2',4',6'-HCB which in the list of Goldstein is classified as a weak inducer, caused comparable increases in epoxide-metabolizing enzyme activities as the above-mentioned isomers. Moreover, another weak cytochrome P-450 inducer, 2,5,2',5'-TCB, was one of the strongest isomers of enhance the glutathione S-transferase activity.

Hansell *et al.* [18] have shown that the strength of the induction of hepatic mixed function oxidases (*p*-nitroanisole O-demethylase, aniline hydroxylase) by 2,4,5,2',4',5'-HCB, 2,4,2',4'-TCB, and 2,5,2',5'-TCB, depends on the rate of disappearance of these compounds from the liver. The longer the isomer stays in liver, the stronger is the induction. In case of microsomal epoxide hydrolase, 2,4,5,2',4',5'-HCB, which has the slowest rate of disappearance, caused the strongest enhancement of activity; 2,4,2',4'-TCB, which disappears more rapidly from the liver, increased epoxide hydrolase activity to a lesser extent; 2,5,2',5'-TCB, with the fastest disappearance, caused the smallest enhancement in the activity of epoxide hydrolase. Although parallel, the picture was not as evident for the cytosolic glutathione S-transferase.

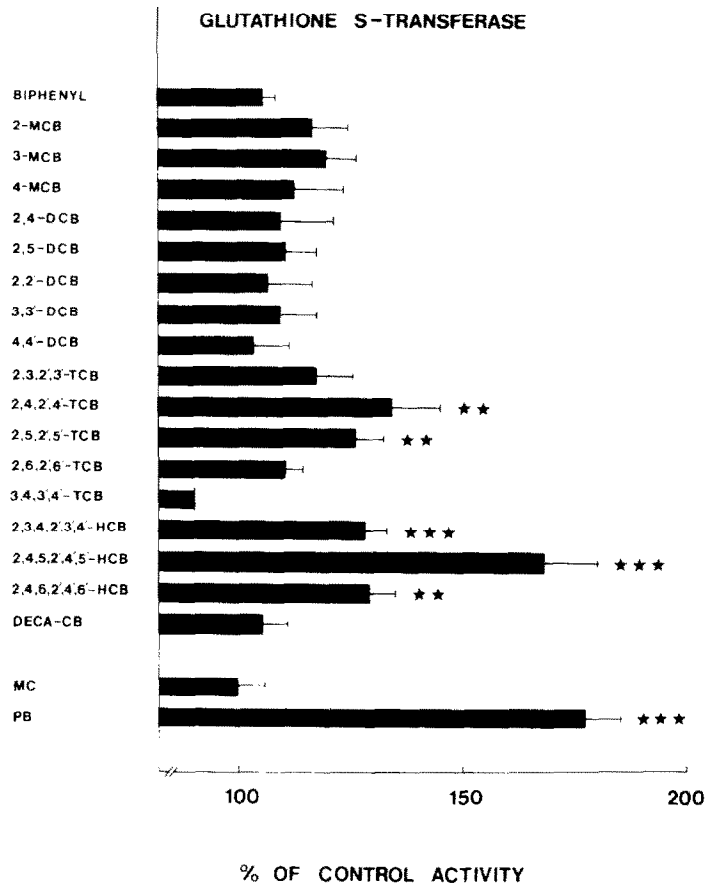

Fig. 2. Hepatic cytosolic glutathione S-transferase activity of C57BL mice 7 days after a single i.p. administration of PCB isomers (0.32 mmol/kg). Mean ± S.E.M. are given. The number of animals was 4–6. Control value was 11,490 ± 790 nmol/min per g wet weight. *** = $2P < 0.001$; ** = $2P < 0.01$; * = $2P < 0.05$ (Student's t-test).

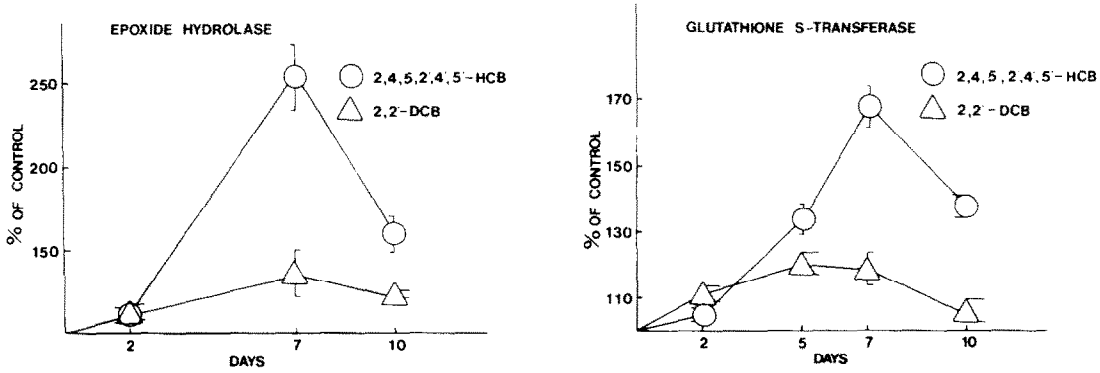

Fig. 3. The activities of hepatic epoxide-metabolizing enzymes at various time points after single doses (0.32 mmol/kg) of 2.4,5,2',4',5'-HCB and 2,2'-DCB. Each point represents the mean (± S.E.M.) enzyme activity of 4–6 mice. Control values were as follows. Epoxide hydrolase: 2 days, 28 ± 1.8; 7 days, 26.6 ± 1.9; 10 days, 42.9 ± 0.6 nmol/min per g wet weight. Glutathione S-transferase: 2 days, 8360 ± 470; 5 days, 8380 ± 550; 7 days, 11,490 ± 790; 10 days, 11,650 ± 650 nmol/min per g wet weight.

One prominent feature of the induction of mixed function oxidases by PCBs is that the enzyme activities, even after a single administration of the inducer, remain for weeks at elevated levels [5, 18]. The activities of epoxide hydrolase and glutathione S-transferase in rat liver, in a similar way, were above control levels still 4 weeks after a single i.p. injection of a commercial PCB mixture [5]. Compared with this, the decline of the enhanced enzyme activities was more rapid in the present study with mice.

To summarize, hepatic epoxide hydrolase and glutathione S-transferase activities of C57BL mice were increased after the i.p. injection of polychlorinated biphenyls (PCBs). The activities reached maximal levels 1 week after a single dose. The rise in epoxide hydrolase activity was generally higher than that of glutathione S-transferase. Enhancement of the activities of epoxide-metabolizing enzymes was most prominent in mice treated with hexachlorobiphenyls. The ability of PCB isomers to enhance the enzyme activities was different for various isomers, but no general rules of structure–activity relationship became apparent.

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Comparative study of three parenteral inhibitors of the angiotensin converting enzyme*

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Over the past few years, inhibition of the angiotensin converting enzyme (ACE, known also as kininase II) has been used for investigational purposes in animal experiments as well as for diagnosis and treatment of human hypertension. At present, two compounds are available for clinical use: teprotide (SQ20881; also known as BPP_{9a}), a nonapeptide suitable for i.v. administration only, and captopril (SQ14225), a modified proline molecule suitable for oral administration. Teprotide can be used for the diagnosis and initial treatment of selected hypertensive emergencies [1]. It has no known side-effects but is expensive and its supplies are limited. Captopril has been used successfully for long-term therapy of hypertension [2, 3] and congestive cardiac failure [4, 5], but it has been associated with a number of adverse reactions that appear to be more frequent and severe in patients with compromised renal function. Therefore, the search continues for new compounds capable of inhibiting the ACE.

Two such compounds have been investigated in the pres-

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