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Polychlorinated Biphenyls: A New Type of Inducer of Cytochrome P-448 in the Liver

(cytochrome P-450/rats aryl hydrocarbon hydroxylase/enzyme induction/microsomal enzymes)

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ABSTRACT **The CO-difference spectrum of microsomes from rats treated with the polychlorinated biphenyls mixture, Aroclor 1254, has an absorption maximum at 448 nm. With ethylisocyananide as the ligand for reduced microsomes, Aroclor 1254 treatment causes a shift in the 455-nm peak to 453 nm and increases the ratio of absorbance of 455 nm to that at 430 nm from 0.53, obtained with untreated rats, to 1.24. These findings are similar to those seen in rats treated with the polycyclic hydrocarbon, 3-methylcholanthrene, but differ from those that characterize cytochrome P-450 in control or phenobarbital-treated rats. Aroclor 1254 treatment results in a tripling of cytochrome P-448 content and a 10-fold increase in benzo[a]pyrene hydroxylation. However—unlike 3-methylcholanthrene, but like the phenobarbital type of inducing agents—Aroclor 1254 treatment causes a significant enhancement of ethylmorphine N-demethylase. These data suggest that Aroclor 1254-induced cytochrome P-448 may be catalytically different from the 3-methylcholanthrene-induced P-448 or that the hemoprotein(s) induced by Aroclor 1254 may be a mixture of cytochromes P-448 and P-450 exhibiting catalytic properties of both cytochromes.**

Polychlorinated biphenyls are industrial chemicals used as lubricants, heat-exchange fluids, insulators, and as plasticizers in paints, synthetic resins, and plastics used in a wide variety of commercial and domestic products. Residues of polychlorinated biphenyls have been widely found in tissues of fish and wildlife (1–4). Contamination of human adipose tissue and of bovine and human milk has been reported (5–8). The polychlorinated biphenyl mixtures manufactured in the United States are marketed under the trade name Aroclor. The Aroclors are designated by four-digit numbers, the last two digits of which define the percent of chlorine content by weight, e.g., Aroclor 1254 contains 54% chlorine.

Previous studies have shown that polychlorinated biphenyls are inducers of steroid hydroxylases in birds (1, 9), and of drug-metabolizing enzymes in animals (10, 11). The microsomal hemoprotein, cytochrome P-450, has been implicated as the terminal oxidase in the hepatic enzyme systems that metabolize many drugs, other foreign compounds, and normal body constituents. Inducers of drug-metabolizing enzymes in liver have been categorized into two groups (12, 13). One groups of inducers, of which phenobarbital is a prototype, enhances the metabolism of a large variety of substrates by liver cells; a second group stimulates the metabolism of only a few substrates. Polycyclic hydrocarbon carcinogens, such as benzo[a]pyrene and 3-methylcholanthrene (3-MeChol) comprise the second group of compounds. In addition, the effects of these two groups of inducers on cytochrome P-450 differ. Treatment of rats with phenobarbital or 3-MeChol increases the concentration of the hemoprotein present in liver microsomes (14, 15). However, 3-MeChol induces the formation of cytochrome P-448, a hemoprotein that differs in spectral and catalytic activities from cytochrome P-450 present in untreated rats or in rats treated with phenobarbital (14–17). Cytochrome P-448 has also been referred to as cytochrome P_1-450 (15).

Drugs and steroids interact with microsomal hemoprotein to give a type-I difference spectrum, characterized by a peak at about 385 nm and a trough at about 420 nm, or a type-II difference spectrum, characterized by a peak at about 430 nm and a trough at about 390 nm (18, 19). We showed (10) that polychlorinated biphenyls stimulated the metabolism of ethylmorphine, a type-I substrate, and of aniline, a type-II substrate. In addition, treatment with the polychlorinated biphenyls resulted in a tripled content of cytochrome P-450 of liver microsomes (10).

In the present study, we have further investigated the properties of the polychlorinated biphenyl-induced cytochrome. The results of these studies indicate that polychlorinated biphenyls are a new type of inducer, causing the formation of polycyclic hydrocarbon-induced cytochrome P-448, while eliciting the more general enzyme induction response evoked by drugs such as phenobarbital.

METHODS

Male Sprague–Dawley rats weighing 100–120 g were used. The polychlorinated biphenyl mixture used was Aroclor 1254, supplied by Monsanto Chemical Co. St. Louis, Mo. Aroclor 1254, dissolved in corn oil, was administered intraperitoneally at a dosage of 25 mg/kg per day for 6 days, unless otherwise indicated. Control rats received corn oil only. Actinomycin D, dissolved in 10% ethanol, was administered intraperitoneally at a dose of 1 mg/kg 1 hr before and 6 hr after the administration of 100 mg/kg of Aroclor 1254 or corn oil. 3-MeChol was administered by the same route at a dose of 25 mg/kg per day for 4 days and phenobarbital was administered intraperitoneally at a dose of 75 mg/kg per day for 4 days. The animals were killed 24 hr after the injection of the last dose of Aroclor 1254, 3-MeChol, or phenobarbital. Livers were removed and microsomes were prepared (20).

Abbreviation: 3-MeChol, 3-Methylcholanthrene.

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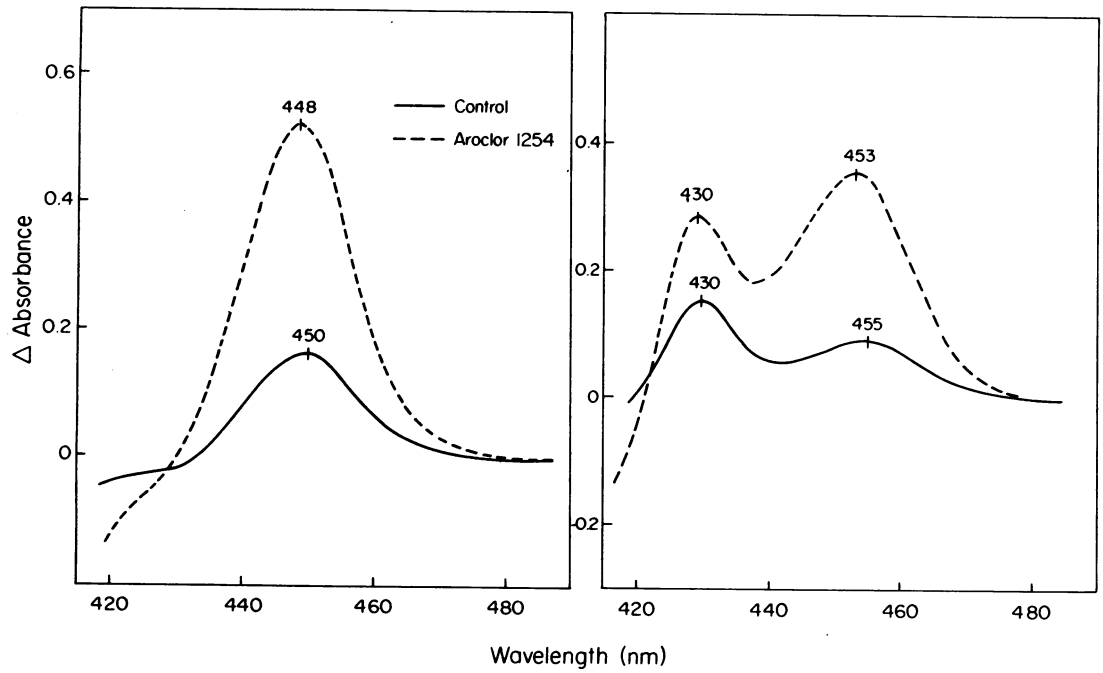

FIG. 1. Carbon monoxide (*left*)- and ethylisocyanaide (*right*)- difference spectra of microsomes from untreated rats and rats treated with Aroclor 1254. Aroclor 1254 was administered at a dosage of 25 mg/kg per day for 6 days.

Ethylmorphine *N*-demethylase activity was determined as described (20). Benzo[a]pyrene hydroxylase activity was determined in a reaction mixture containing 100 μmol of potassium phosphate buffer (pH 7.4), 3 μmol of $MgCl_2$, 1.0 μmol of NADPH, 100 nmol of benzo[a]pyrene (added in 0.05 ml of acetone), and 9000 × *g* supernatant derived from 4 mg of liver, wet weight, in a final volume of 1.0 ml. The mixture was incubated at 37° for 5 min and the product was measured by the method of Nebert and Gelboin (21). In the benzo[a]pyrene hydroxylase assay, 9000 × *g* supernatant was used because previous studies (22) had shown that the Michaelis constant was similar whether 9000 × *g* supernatant or microsomes were used. Cytochrome P-450 content was determined by the method of Omura and Sato (23), from the CO-difference spectrum of dithionite-reduced microsomes, using an extinction coefficient of 91 mM^{-1} cm^{-1} between 450 or 448 and 490 nm. The ethylisocyanide difference spectrum was determined in a similar manner, except that instead of bubbling CO through the solution, ethylisocyanide in a final concentration of 4.5 mM was added to the sample cuvette. The difference in absorption at 430 and 500 nm was used as an estimate of the 430-nm peak, and the difference in absorption at 455 or 453 and 500 nm was used as an estimate of the 455-nm peak. Protein contents were determined by the method of Sutherland *et al.* (24).

RESULTS

Effect of Aroclor 1254 Treatment on the Spectral Properties of Cytochrome P-450 in Liver Microsomes. The CO-difference spectra of liver microsomes from untreated rats and rats treated with Aroclor 1254, are shown in Fig. 1. A difference spectrum with a peak at 450 nm was observed with microsomes from untreated rats, as expected (25, 26). However, when rats were treated with Aroclor, a peak shift occurred and the CO-binding pigment now exhibited an absorption maximum at 448 nm instead of 450 nm. When ethylisocyanide, instead of CO, was used as the ligand for the reduced microsomal hemoprotein, spectral peaks at 430 and 455 nm were observed, as reported (27). The ethylisocyanide difference spectrum of microsomes from rats injected with Aroclor 1254 exhibited a spectral shift from 455 to 453 nm; no such shift occurred with the 430-nm peak (Fig. 1). As can also be seen in Fig. 1, treatment with Aroclor increased the absorbance at 430 nm, as well as at 453 nm. However, the increase in absorbance at 453 nm was greater than the increase observed with the 430-nm peak, resulting in an overall increase in the ratio of the 455-nm to 430-nm peaks. These findings suggested the possibility that Aroclor 1254 or its metabolite(s) may be bound to the microsomal hemoprotein and that this complex could possess spectral properties different from the native cytochrome. This binding could account for the change in the ratio of the 455-nm to 430-nm peaks observed in Aroclor-treated rats. Alternatively, the observed changes could be due to the synthesis of a new hemoprotein having spectral and catalytic activities different from the native cytochrome.

Effect of Actinomycin D on Changes in Properties of Cytochrome P-450 Induced by Aroclor 1254. To test the above alternatives the following experiments were performed: (*i*) addition of Aroclor to microsomes before reduction and bubbling of CO or addition of ethylisocyanide resulted in spectra that were identical to those obtained with microsomes from untreated rats assayed in the absence of Aroclor. (*ii*) When whole homogenate from untreated rats was incubated with Aroclor and an NADPH-generating system for 0.5 hr at 37°, and microsomes were then prepared, these microsomes exhibited spectral properties identical to those of microsomes obtained from homogenate incubated in the absence of Aroclor. Drug-induced increases in microsomal enzyme activity can be prevented by certain inhibitors of protein synthesis

TABLE 1. *Effect of actinomycin D on Aroclor 1254-induced changes in microsomal hemoprotein and benzo[a]pyrene hydroxylase and ethylmorphine N-demethylase activities*

Treatment	Benzo[a]pyrene hydroxylase activity*	Ethylmorphine *N*-demethylase activity†	Cytochrome P-450‡	Ethylisocyanide difference spectra: 455 peak $\Delta A_{455-500}$	Ethylisocyanide difference spectra: 430 peak $\Delta A_{430-500}$	Ethylisocyanide difference spectra: Ratio of 455-nm to 430-nm peaks
Controls	3.90 ± 0.22	0.287 ± 0.018	0.79 ± 0.02	0.062 ± 0.002	0.114 ± 0.006	0.54 ± 0.01
Actinomycin D	3.37 ± 0.28	0.270 ± 0.006	0.73 ± 0.04	0.060 ± 0.005	0.105 ± 0.008	0.57 ± 0.02
Aroclor 1254	22.42§ ± 1.36	0.505§ ± 0.023	1.28§ ± 0.04	0.087§ ± 0.003	0.100 ± 0.003	0.87§ ± 0.03
Actinomycin D + Aroclor 1254	6.29 ± 1.35	0.331 ± 0.028	0.79 ± 0.07	0.057 ± 0.010	0.100 ± 0.007	0.57 ± 0.07

Aroclor 1254, 100 mg/kg, was administered by a single injection. Actinomycin D, 1 mg/kg, was administered 1 hr before and 6 hr after the administration of Aroclor 1254 or corn oil. Animals were killed 24 hr after the administration of Aroclor 1254 or corn oil. Each value represents mean ± SEM from five rats.

* nmol of hydroxybenzpyrine formed per mg of protein per hr.

† μmol of HCHO formed per mg of protein per hr.

‡ nmol of cytochrome P-450 per mg of protein.

§ Value significantly different from respective control value ($P < 0.05$).

or DNA-dependent RNA synthesis. To determine if Aroclor was indeed causing the induction of a spectrally-different hemoprotein, the effect of actinomycin D on the induction of benzo[a]pyrene hydroxylase and ethylmorphine *N*-demethylase, the levels of cytochrome P-450, and the ethylisocyanide difference spectral peaks (455-nm and 430-nm peaks) was studied (Table 1). 100 mg/kg of Aroclor 1254 was administered and the rats were killed 24 hr later. The shift in the 450-nm peak of the CO-difference spectrum or of the 455-nm peak of the ethylisocyanide difference spectrum was not apparent 24 hr after a single injection of Aroclor. However, administration of Aroclor did cause a significant enhancement of benzo[a]pyrene hydroxylase, ethylmorphine *N*-demethylase, and cytochrome P-450 content, as well as an increase in the ratio of the 455-nm to 430-nm peaks from 0.54 to 0.87. Administration of actinomycin D, 1 hr before and 6 hr after the administration of Aroclor, prevented the marked stimulation of enzyme activities, the increase in cytochrome P-450, and the increase in the ratio of the absorbance at the 455-nm to 430-nm peaks. Actinomycin D administered alone had no effect on the basal levels of enzyme activity or the microsomal hemoprotein.

Comparative Effects of Phenobarbital, 3-MeChol, and Aroclor 1254 on Hepatic Microsomal Hemoprotein, Benzo[a]pyrene Hydroxylase, and Ethylmorphine N-Demethylase. As shown in Table 2, Aroclor 1254 caused a 10-fold induction of benzo[a]pyrene hydroxylase, whereas 3-MeChol and phenobarbital caused 13- and 4-fold induction of the hydroxylase, respectively. The three inducers increased the cytochrome P-450 levels; however, the CO difference spectra showed absorption maxima at 448, 448, and 450 nm with Aroclor, 3-MeChol and phenobarbital, respectively. Similarly, like 3-MeChol, Aroclor treatment resulted in a marked increase in the ratio of the 455-nm to 430-nm peaks. The increases in benzo[a]pyrene hydroxylase activity and in the ratio of the 455-nm

TABLE 2. *Comparative effects of Aroclor 1254, 3-MeChol and phenobarbital on hepatic microsomal hemoprotein and benzo[a]pyrene hydroxylase and ethylmorphine N-demethylase activities*

Treatment	Benzo[a]pyrene hydroxylase activity*	Ethylmorphine *N*-demethylase activity†	Cytochrome P-450‡	Ethylisocyanide difference spectra: 455 peak $\Delta A_{455-500}$	Ethylisocyanide difference spectra: 430 peak $\Delta A_{430-500}$	Ethylisocyanide difference spectra: Ratio of 455-nm to 430-nm peaks
Controls	4.21 ± 0.74	0.433 ± 0.026	0.73 ± 0.02	0.062 ± 0.002	0.117 ± 0.008	0.53 ± 0.02
Aroclor 1254	42.08§ ± 3.47	0.951§ ± 0.031	2.01§ ± 0.10	0.230§ ± 0.036	0.192 ± 0.033	1.24§ ± 0.10
3-MeChol	55.84§ ± 1.51	0.475 ± 0.027	1.70§ ± 0.05	0.224§ ± 0.010	0.129 ± 0.009	1.74§ ± 0.05
Phenobarbital	16.38§ ± 0.34	1.586§ ± 0.055	2.32§ ± 0.03	0.211§ ± 0.010	0.354§ ± 0.008	0.59§ ± 0.02

Rats were administered Aroclor 1254, 25 mg/kg per day for 6 days or 3-MeChol, 25 mg/kg per day for 4 days, or phenobarbital, 75 mg/kg per day for 4 days. Each value represents mean ± SEM from five rats.

* nmol of hydroxybenzpyrene formed per mg of protein per hr.

† μmol of HCHO formed per mg of protein per hr.

‡ nmol of cytochrome P-450 per mg of protein.

§ Value significantly different from respective control value ($P < 0.05$).

to 430-nm peaks observed with Aroclor were of a lesser magnitude than those observed with 3-MeChol. In contrast, phenobarbital pretreatment resulted in a 3-fold increase in cytochrome P-450, and the ratio of the 455-nm to 430-nm peaks was similar to that observed with untreated rats. As expected (15, 28), 3-MeChol had no effect on the activity of ethylmorphine *N*-demethylase; however, Aroclor treatment (Table 2) resulted in a significant enhancement of *N*-demethylase activity, though of a lesser magnitude than that observed in rats treated with phenobarbital. The data obtained with 3-MeChol and phenobarbital are similar to those previously reported (14, 15, 28, 29).

DISCUSSION

The possible health hazard to humans presented by the environmental pollutant, polychlorinated biphenyls, is unclear, but recent disclosures of the presence of this contaminant in human adipose tissue (5, 7, 8) and in human milk (6) raise serious questions as to the potential effects of these tissue contaminants on human health. In these studies Aroclor 1254 was used as a representative mixture of polychlorinated biphenyl compounds since it is similar to the polychlorinated biphenyl residues found in some human adipose tissues (7). The data presented in this paper show that Aroclor 1254 is a potent inducer of the microsomal hemoprotein, cytochrome P-448, and of benzo[a]pyrene hydroxylase and ethylmorphine *N*-demethylase.

Barbiturates and polycyclic hydrocarbons appear to stimulate drug-metabolizing enzymes of liver by different mechanisms. The barbiturates induce the metabolism of many substrates. For example, phenobarbital in rats is a potent inducer of cytochrome P-450, and enhances the metabolism of drugs *in vitro* as well as *in vivo*. However, it is a poor inducer of the aryl hydrocarbon hydroxylases. In contrast, polycyclic hydrocarbons, such as 3-MeChol, induce the formation of a spectrally and catalytically different hemoprotein, cytochrome P-448 (14, 16). 3-MeChol is a potent inducer of aryl hydrocarbon hydroxylases (30), but does not enhance the *N*-demethylation of ethylmorphine (28). Polychlorinated biphenyls, as shown in these studies, share the properties of both the polycyclic hydrocarbon and the phenobarbital types of inducer compounds. Aroclor 1254, for example, caused a 3-fold induction of cytochrome P-448, a 2-fold induction of *N*-demethylase activity, and a 10-fold induction of benzo[a]-pyrene hydroxylase. In addition, the hemoprotein induced by Aroclor 1254 showed a typical type-I difference spectrum with hexobarbital, similar to that observed with microsomes from untreated or phenobarbital-treated rats (unpublished observations). Cytochrome P-448 from 3-MeChol-induced rats showed with hexobarbital a modified type-II difference spectrum, as previourly reported (31). Treatment of rats with phenobarbital enhances the metabolism and decreases the duration of action of both zoxazolamine and hexobarbital in the intact animal. On the other hand, polycyclic hydrocarbons accelerate the metabolism of zoxazolamine, but not of hexobarbital (32). We have shown that Aroclor 1254 accelerates the metabolism of hexobarbital and zoxazolamine *in vivo* (10). Thus, these data strongly suggest that the Aroclor 1254-induced cytochrome P-448 is catalytically different from the 3-MeChol-induced cytochrome P-448 or, alternatively, that the hemoprotein(s) induced by treatment with Aroclor 1254 may be a mixture of cytochromes P-448 and P-450 exhibiting catalytic properties of both cytochromes.

Aroclor 1254 is a mixture of polychlorinated biphenyls comprising chemical congeners of both high and low chlorine content. Since Aroclor 1254 shows the inducing properties of both the barbiturate and the polycyclic hydrocarbon classes of inducers, it is possible that the mixture of polychlorinated biphenyls contains one or more compounds having the barbiturate type of inductive properties and compounds having the properties of the polycyclic hydrocarbon class of inducers. The two types of induction produced by 3-MeChol and phenobarbital proceed independently of each other when these compounds are administered simultaneously, as shown by Bidleman and Mannering (33); these independent induction phenomena result in biochemical and spectral findings similar to those obtained after the administration of Aroclor 1254. Our studies show that the ratio of the 455-nm to the 430-nm peaks (1.24) produced with ethylisocyanide in Aroclor 1254-treated animals (Table 2) is almost exactly intermediate between the ratio seen with microsomes from untreated and phenobarbital-treated rats (0.53 and 0.59, respectively) and the ratio seen with microsomes from 3-MeChol-treated rats (1.74). However, a second possibility exists, namely, that a single component of the polychlorinated biphenyl mixture possesses the combined properties of the two classes of inducers and that the characteristic inducing action of Aroclor 1254 described here is intrinsic to the polychlorinated biphenyl moiety itself.

Drug-induced increases in enzyme activity can be prevented by inhibitors of nucleic acid and protein synthesis. In the present studies, actinomycin D, an inhibitor of DNA-dependent RNA synthesis, was administered before Aroclor 1254 and this treatment prevented (*a*) the change in the ratio of the 455-nm to the 430-nm peaks of the ethylisocyanide difference spectrum of reduced microsomes, and (*b*) the increase in benzo[a]pyrene hydroxylase and ethylmorphine *N*-demethylase activities induced by the polychlorinated biphenyls. This inhibitory effect on the Aroclor 1254-induced spectral changes in microsomes supports the view that Aroclor 1254 enhances the synthesis of a distinct microsomal hemoprotein having spectral properties different from that present in the livers of untreated rats.

The aryl hydrocarbon hydroxylase system has come under increasing investigation in the field of oncology. Polycyclic hydrocarbon carcinogens are the most widely occurring environmental chemical carcinogens known and occur as atmospheric pollutants resulting from combustion of fuels and other organic materials; they are also present in cigarette smoke. In addition, they are potent inducers of aryl hydrocarbon hydroxylase in liver and nonhepatic tissues in animals and man, and several of them have been shown to induce cytochrome P-448 in the liver. Since the polychlorinated biphenyls possess certain of the characteristic inducing properties of benzo[a]pyrene and 3-MeChol, and are themselves ubiquitously distributed in the environment, it may be of considerable importance to examine the carcinogenic potentialities of these biphenyl mixtures. In this regard, it is of considerable interest that recent studies by Allen and Norback (34) have shown that ingestion of polychlorinated biphenyls by rhesus monkeys results in hyperplastic, dysplastic, and invasive changes suggestive of eventual neoplastic transformation of gastric mucosa.

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