0026-895X/88/030272-07$00.00/0

MOLECULAR PHARMACOLOGY, 33:272-278

Induction of Rat Cytochrome P-450 3 and Its mRNA by 3,4,5,3′,4′,5′-Hexachlorobiphenyl

HEATHER N. YEOWELL, DAVID J. WAXMAN, GERALD A. LEBLANC, PATRICIA LINKO, and JOYCE A. GOLDSTEIN

Laboratory for Biochemical Risk Analysis, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 (H.N.Y., P.L., J.A.G.) and Department of Biological Chemistry and Molecular Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115 (D.J.W., G.A.L.B.)

Received May 5, 1987; Accepted November 30, 1987

SUMMARY

Rat cytochrome P-450 3 (P-450 3) is a constitutive hepatic steroid hormone 7α-hydroxylase which is relatively unresponsive to a number of monooxygenase-inducing agents. The present study demonstrates that a polyhalogenated aromatic hydrocarbon inducer, 3,4,5,3′,4′,5′-hexachlorobiphenyl (HCB), induces P-450 3 in livers of adult male rats, and that the increase is the result of an increase in the mRNA for this enzyme. Cytochrome P-450 3 and its mRNA were increased more slowly and to a lesser extent than cytochrome P-450c (P-450c) and its mRNA, indicating that these enzymes are not regulated coordinately in liver. The maximum increase in P-450 3 and P-450 3-dependent androstenedione 7α-hydroxylase activity (2- to 3-fold) occurred 7 days after administration of HCB, in contrast to the increase in P-450c (>200-fold) which was maximal by 3–5 days. The rate of induction of P-450 3 mRNA was also slower [maximum increase (9-fold) at 5 days after HCB administration] than that of P-450c mRNA [maximum increase (30-fold) at 2–3 days]. Moreover, a higher dose of HCB was required to produce maximum induction of P-450 3 (50 mg/kg) than that required to produce maximum induction of P-450c (10 mg/kg). P-450 3 was not detected on Western blots of lung, kidney, or prostate microsomes isolated from control or HCB-treated rats (≤2% of that found in livers of HCB-treated rats). Moreover, P-450 3-dependent steroid 7α-hydroxylase activity was not detected in these extrahepatic tissues of control or HCB-treated rats (≤1% of that found in the corresponding liver microsomes of untreated or HCB-treated rats). In contrast, P-450c was increased dramatically by HCB in lung, kidney, and prostate tissues, indicating differential expression of P-450c and P-450 3 in extrahepatic tissues.

The P-450[1] monooxygenases comprise a family of closely related enzymes which catalyze the oxidative metabolism of drugs, environmental pollutants, and endogenous compounds such as steroids and fatty acids (1). A number of forms of P-450 can be induced by xenobiotics. For example, cytochromes P-450c and P-450d are present at relatively low levels in uninduced livers of rats (~0.5 and 5%, respectively, of the total hepatic P-450) but are induced dramatically by polycyclic aromatic hydrocarbons such as 3-MC, whereas P-450b and P-450e are minor constitutive forms that can be induced dramatically by phenobarbital (2, 3). In contrast to these cytochromes, P-450 3 is expressed constitutively in rat liver (comprising ~5–10% of total P-450) and is relatively resistant to induction (4). However, modest increases in P-450 3 and its associated testosterone and androstenedione 7α-hydroxylase activities have been observed in response to 3-MC and some polyhalogenated biphenyls (4–10). The largest increase in P-450 3 is produced by administration of those coplanar halogenated biphenyls that induce P-450c and P-450d rather than those that induce the phenobarbital-inducible cytochromes P-450b and P-450e (5, 6), suggesting a possible common mode of induction for P-450 3, P-450c, and P-450d. Induction of P-450s c and d has been shown to be the result of increases in their mRNAs (11). However, the mechanism of the increase in P-450 3 has received considerably less study.

The present study examines whether the increase in the hepatic content of P-450 3 after administration to adult male rats of the potent 3-MC-type inducer, HCB, is due to an increase in the hepatic content of its mRNA. In addition, the time course and dose response for the increase in P-450 3, its associated (9) steroid hormone 7α-hydroxylase activity, and its mRNA are compared with that of P-450c and its mRNA. Finally, since 3-MC-type inducers have been shown to induce P-450c but not P-450d in extrahepatic tissues (12), we compared the effects of HCB on the microsomal content of P-450 3 and P-450c in several extrahepatic tissues.

This work was supported in part by National Institutes of Health Grant DK33765 (to D. J. W.). A preliminary report of this work was presented at the 78th Annual Meeting of the American Society of Biological Chemists [*Fed. Proc.* 46:2141 (1987)].

[1] The nomenclature used for individual forms of P-450 is detailed under Materials and Methods.

ABBREVIATIONS: P-450, cytochrome P-450; 3-MC, 3-methylcholanthrene; HCB, 3,4,5,3′,4′,5′-hexachlorobiphenyl; ELISA, enzyme-linked immunosorbent assay; *Ah*, aromatic hydrocarbon; RIA, radioimmunoassay; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Materials and Methods

Treatment of rats

Adult male Sprague-Dawley rats (8 weeks of age, Charles River Breeding Laboratories) were dosed intraperitoneally with 50 mg/kg HCB in corn oil and were sacrificed by decapitation 1, 2, 3, 5, or 7 days later (4 rats/group). Control rats received corn oil alone and were sacrificed on day 7. Microsomes were prepared as previously described (13). Prostate microsomes from four rats were pooled at each time point to give sufficient tissue for analysis.

Purification of Cytochromes and Antibodies

Cytochrome P-450c was purified as previously described (13). Antibody to P-450c was obtained from rabbits and immunopurified as previously described (2). Cytochrome P-450 3 was purified from phenobarbital-induced immature male rats as described (7). Antibody to P-450 3 was raised in 6-month-old female Lupus mice (*NZB/NZW*) immunized intraperitoneally with 2 μg of antigen [emulsified in Freund's complete adjuvant (1:4, v/v) at 0.1 ml/mouse]. Animals were boosted ip with 1 μg of antigen [emulsified in Freund's complete adjuvant (1:9, v/v; 0.05 ml/mouse)] 2 weeks and again 4 weeks later. Ascites fluids were tapped as required beginning 3–4 weeks after the second boost, clarified, and then stored at −20°. The antibody thus obtained was judged specific for P-450 3 by ELISA (8), with <3% cross-reactivity observed versus nine other purified rat liver P-450s (i.e., P-450s PB-1, PB-2a, 2c, 2d, PB-4, PB-5, PB-6, c, and d; Refs. 4 and 8). The rat hepatic P-450 designations used in this and previous reports (4) can be related to the standardized gene designations (14) and alternative protein nomenclatures used by other investigators as follows: P-450 3 (gene *IIA1*) = P-450a (3) or P-450 UT-F (15); P-450 2c (gene *IIC11*) = P-450h (16), P-450 male (17), P-450 UT-A (15), or P-450 RLM5 (18); P-450 PB-2a (gene *IIIA1*) = P-450 PCN-E (15) or P-450p (19). P-450b (form PB-4; gene *IIB1*), P-450c (gene *IA1*), and P-450d (gene *IA2*) are classified according to the terminology of Ryan *et al.* (20).

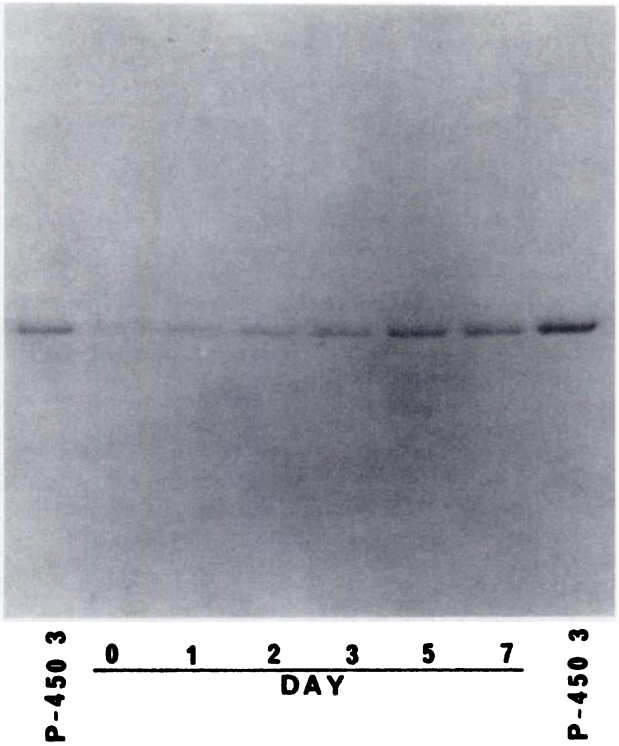

Fig. 1. Western blot of the time course for induction of P-450 3 in liver microsomes of male rats 1–7 days after administration of HCB (50 mg/kg). Lanes contained 10 μg of microsomal protein or 2 pmol (*left lane*) or 4 pmol (*right lane*) of purified P-450 3.

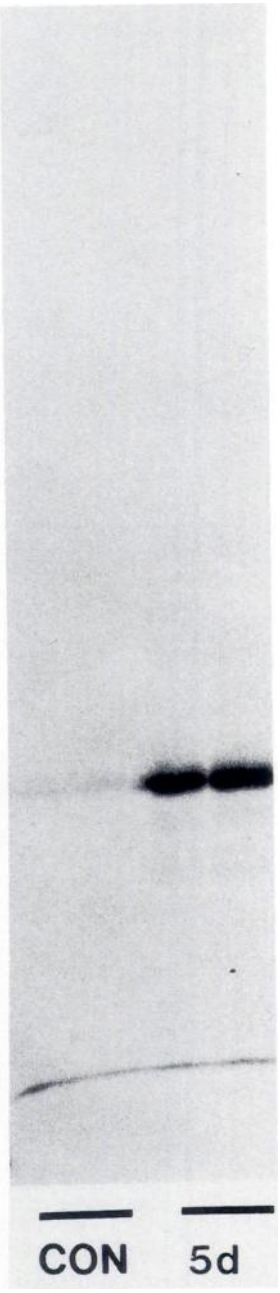

Fig. 2. Autoradiograph of sodium dodecyl sulfate gel analyzing immunoprecipitates of translation products of liver polysomes from untreated rats (*CON*) and rats 5 days (*5d*) after HCB treatment (50 mg/kg). Aliquots of 2×10^6 dpm from each translation mixture were immunoprecipitated with anti-P-450 3, and an equivalent volume of each sample was electrophoresed and autoradiographed.

P-450 Protein Quantitation

Western blots. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (7.6% acrylamide) was performed as described by Laemmli (21). The proteins were transferred to nitrocellulose sheets by the method of Towbin *et al.* (22). The sheets were immunostained for P-450 3 using peroxidase-coupled second antibody as outlined previously for P-450s b, c, and d (13).

Radioimmunoassay (RIA). Cytochrome P-450c content of microsomes was assayed by RIA (2). Cytochrome P-450 3 was radioiodinated using Bolton-Hunter reagent (25 mCi/mg), and microsomal P-450 3

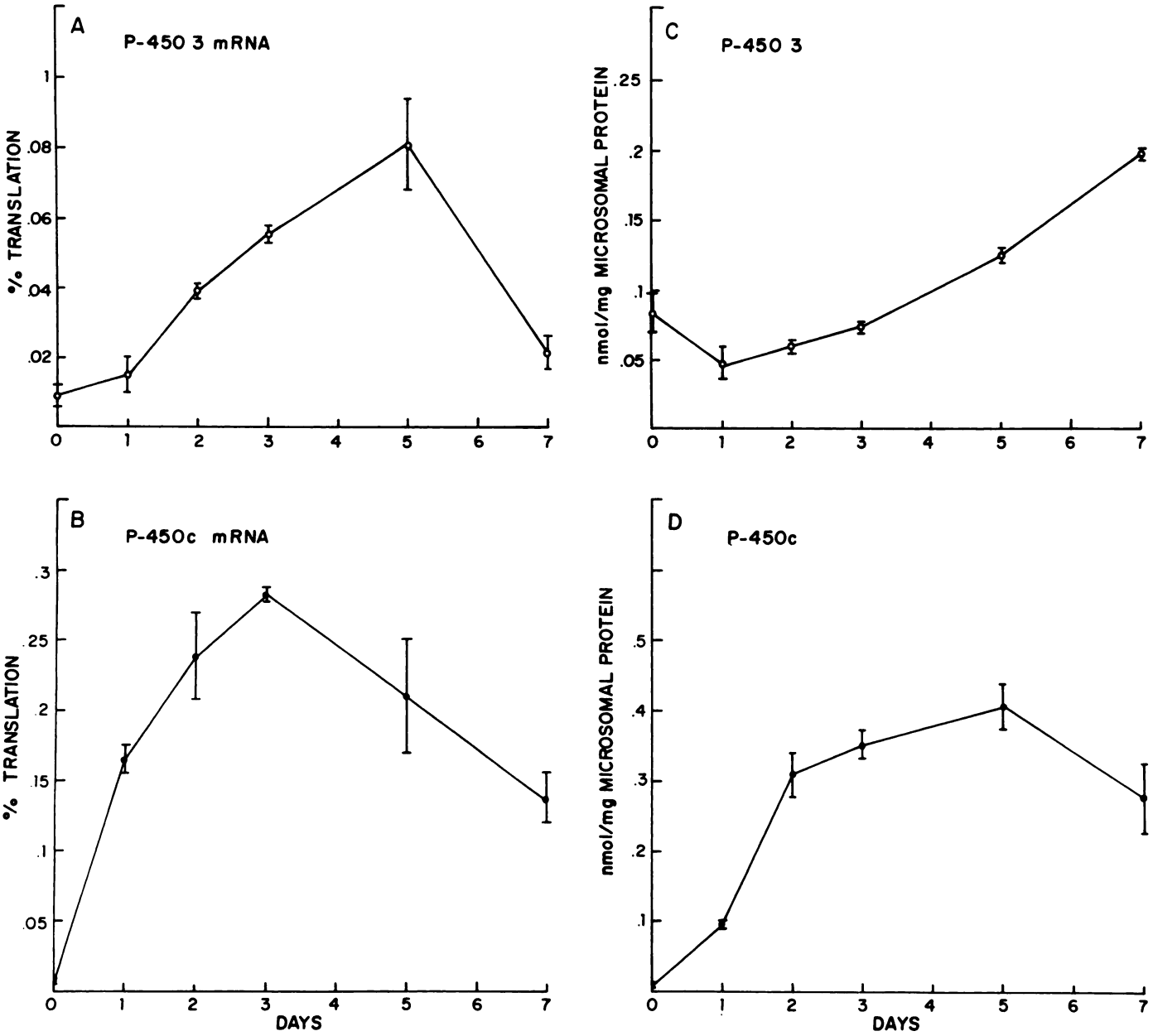

Fig. 3. Induction of P-450 3 mRNA and P-450 3 protein (A and C) as compared to P-450c mRNA and P-450c protein (B and D) in livers of adult male rats sacrificed 0–7 days after administration of HCB at 50 mg/kg. A and B. mRNAs for P-450 3 and P-450c were measured by translation in a reticulocyte lysate system followed by analysis as described under Materials and Methods. Results are expressed as per cent of total translation and represent the mean ± standard deviation of duplicate determinations on three individual animals. C and D. P-450 3 and P-450c protein levels were determined by RIA. Values represent the mean ± standard deviation of duplicate determinations on four individual animals.

content also was estimated by RIA in a manner similar to that described for P-450c and P-450d (2).

Quantitation of P-450 mRNA. Since no cDNA probe was available for P-450 3 at the time of these studies, total liver polysomes were isolated and translated in the presence of [^{35}S]methionine using a cell-free reticulocyte lysate system. Quantitation of translatable mRNAs for both P-450 3 and P-450c was carried out in a manner similar to the procedure described previously for P-450c mRNA (23). Translation products were precipitated by the addition of antibody to P-450 3 or P-450c followed by 100 μl of *Staphylococcus aureus* cells (10% suspension) and the immunoprecipitates were then analyzed on sodium dodecyl sulfate gels. Incorporation of radioactivity into a band equivalent in electrophoretic mobility to purified P-450 3 was quantitated following electrophoresis and autoradiography of the immunoprecipitates. The bands were excised, incubated overnight with H_2O_2 (30%)/concentrated NH_4OH (19:1,v/v), neutralized with glacial acetic acid, and counted. A duplicate area was cut from electrophoresed samples to which preimmune IgG had been added instead of antibody to P-450 3. The radioactivity in these slices was subtracted from that of the actual samples. Values were expressed as percentage of total translation (dpm in the corrected specific polypeptide band divided by total trichloroacetic acid-precipitable counts × 100). The amount of antibody required to precipitate the maximum radioactivity in a polypeptide equivalent in electrophoretic mobility to purified P-450 3 was determined in preliminary experiments, and this amount of anti-P-450 3 was used in all subsequent experiments.

Catalytic assays. Androstenedione hydroxylase and 5α-reductase activities were measured in microsomal samples using [4-^{14}C]androstenedione (7 mCi/mmol) as described previously (8), except that the substrate concentration was increased to 50 μM and the assays were

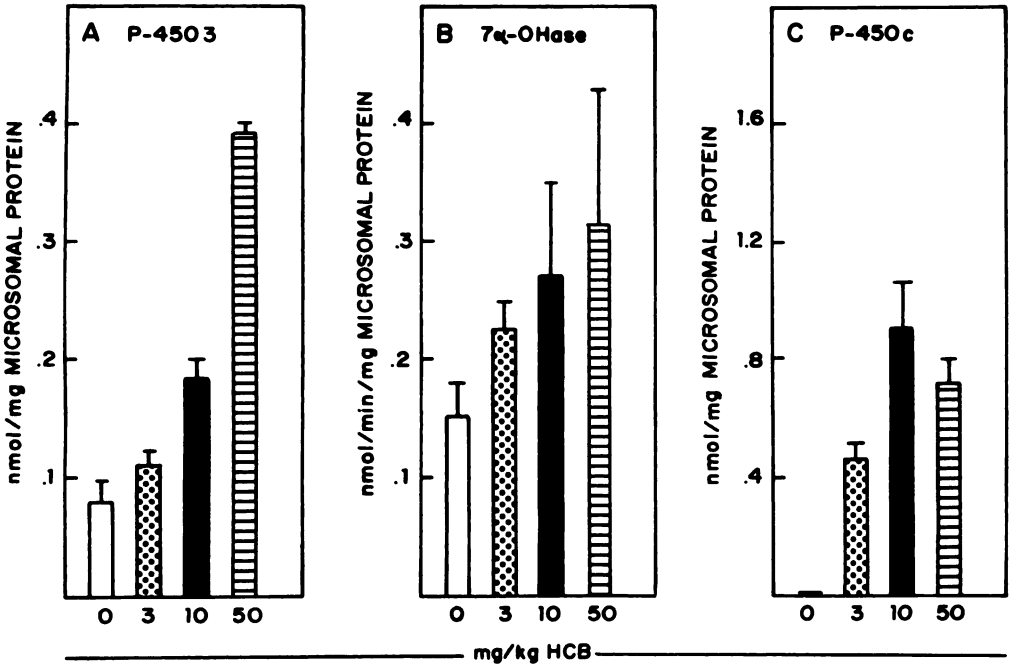

Fig. 4. Dose response for P-450 3, its associated androstenedione 7α-hydroxylase activity, and P-450c in livers isolated from adult male rats 7 days after administration of HCB at 3, 10, and 50 mg/kg. P-450 3 and P-450c levels (A and C) were determined by RIA (mean ± standard deviation for duplicate determinations on four individual animals) and androstenedione 7α-hydroxylation (B) was measured as described under Materials and Methods.

TABLE 1

Metabolism of androstenedione in liver microsomes from control and HCB-treated male rats

Hepatic microsomes were isolated from adult male rats at various times after administration of HCB at 50 mg/kg and assayed for androstenedione metabolism as described under Materials and Methods. Values represent mean catalytic activities for four individual rats ± standard error for each of the indicated metabolites.

Days	7αOH-A[a]	6βOH-A	16αOH-A	5αH-A[b]
	nmol/min/mg			
0	0.35 ± 0.07	0.68 ± 0.13	1.71 ± 0.07	2.17 ± 0.93
1	0.21 ± 0.02	0.28 ± 0.06	1.03 ± 0.15	1.74 ± 0.65
2	0.19 ± 0.01	0.25 ± 0.09	0.61 ± 0.35	1.42 ± 0.21
3	0.32 ± 0.06	0.17 ± 0.03	0.23 ± 0.05	1.18 ± 0.11
5	0.47 ± 0.05	0.14 ± 0.03	0.14 ± 0.06	1.72 ± 1.10
7	0.54 ± 0.14	0.09 ± 0.02	0.12 ± 0.02	1.47 ± 0.54

[a] A, androstenedione.
[b] Androstenedione 5α-reductase activity.

performed in 0.1 M HEPES buffer, pH 7.5, to optimize microsomal 7α-hydroxylation. Extrahepatic microsomes were assayed using 150 μg of protein and [^{14}C]androstenedione at 58 mCi/mmol. 7-Ethoxycoumarin *O*-deethylase activity was measured fluorimetrically as described previously (7).

Other methods. Protein was determined by the method of Lowry *et al.* (24).

Results

Antibody to P-450 3, judged specific for P-450 3 by ELISA (see Materials and Methods), recognized primarily a single band on immunoblots of liver microsomes from uninduced male rats (Fig. 1). This band co-migrated with purified P-450 3 and the intensity of the band was increased by HCB treatment. A single radiolabeled electrophoretic band with a mobility identical to that of P-450 3 was observed in autoradiographs of translational products of polysomes from livers of adult male rats precipitated with anti-P-450 3 (Fig. 2). HCB increased hepatic P-450 3 content 2- to 3-fold and increased the translatable mRNA P-450 3 approximately 9-fold (Fig. 3). The magnitude of the increase in P-450 3 and its mRNA was much smaller than that of P-450c (>200-fold) and P-450c mRNA (30-fold). The rate of P-450 3 induction was also much slower than that of P-450c and its mRNA. P-450c mRNA was increased dramatically 24 hr after HCB treatment, and the maximum increase occurred at 2–3 days. In contrast, increases in P-450 3 mRNA were first observed 2 days after HCB treatment, and the maximum increase occurred at 5 days. P-450 3 protein levels appeared to be slightly suppressed by HCB at 24–48 hr, and a significant increase was first observed at 5 days. Maximum induction of P-450 3 occurred at 7 days. In contrast, immunoreactive P-450c was induced maximally 2–5 days after HCB administration. A higher dose of HCB was also required to produce maximum induction of P-450 3 (50 mg/kg) than that required to maximally induce P-450c (10 mg/kg) (Fig. 4).

Liver microsomal P-450 3-dependent steroid hormone 7α-hydroxylase activity was also decreased 1–2 days after HCB administration, followed by an increase at days 5–7 (Table 1). The time course and magnitude of the changes in 7α-hydroxylase activity (2-fold) were parallel with the changes in P-450 3 content (Figs. 3 and 4). This increase in P-450 3-dependent steroid 7α-hydroxylase activity contrasted with a >85% decrease in microsomal steroid 6β-hydroxylase [P-450 2a-dependent (9)] and 16α-hydroxylase (P-450 2c-dependent (8)) activities 7 days after HCB administration (Table 1). The decrease in steroid 16α-hydroxylase activity has been shown to reflect a decrease in P-450 2c and its mRNA (25).

No polypeptide band with a mobility identical to that of P-450 3 could be detected in Western blots of lung, kidney, and prostate microsomes isolated from untreated rats or HCB-induced rats (Fig. 5) (limit of detection <7 pmol of P-450 3/mg of microsomal protein). Somewhat surprisingly, when high concentrations of microsomal protein (>70 μg) were electrophoresed and immunoblotted, anti-P-450 3 did detect a polypeptide band with a distinctly higher mobility than that of P-450 3 in lung and prostate microsomes from control rats. This polypeptide appeared to be decreased by HCB treatment and could conceivably represent another form of P-450 immuno-

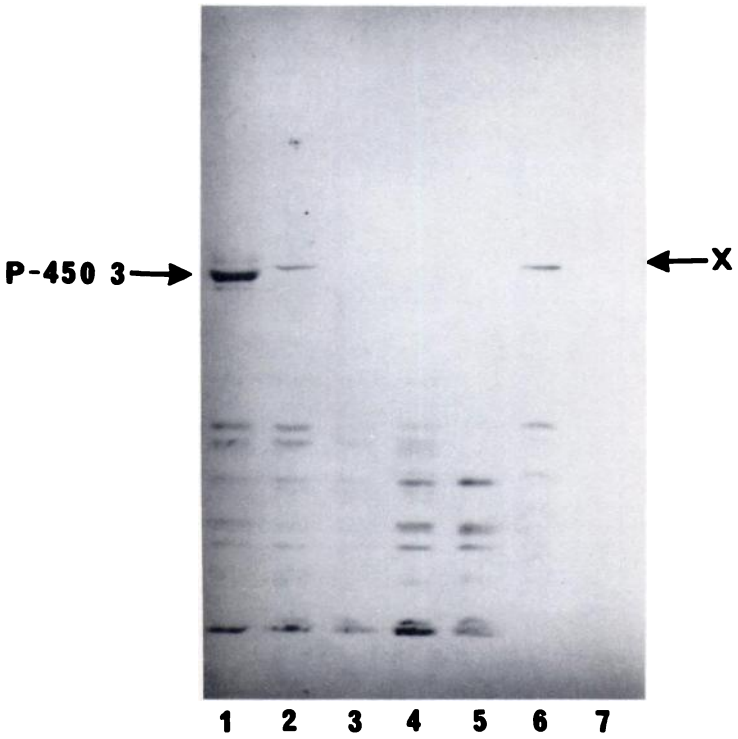

Fig. 5. Western blot showing the absence of a band with the mobility of P-450 3 in microsomes of lung, kidney, and prostates from control and HCB-treated (50 mg/kg) rats compared to liver from control rats. Lanes contain 70 μg of microsomal protein from liver of a control rat (*lane 1*), lungs of control (*lane 2*), and HCB-treated (*lane 3*) rats, kidneys of control (*lane 4*) and HCB-treated (*lane 5*) rats, and prostates of control (*lane 6*) and HCB-treated (*lane 7*) rats. ←X, the mobility of the higher molecular weight protein seen in lung and prostate of control rats. Mobility of purified P-450 3 electrophoresed on the same gel was as indicated on the left. Nonspecific binding of the antibody to lower molecular weight proteins was only observed on immunoblots of relatively high microsomal protein concentrations (≥70 μg) (cf. Fig. 1).

TABLE 2

Induction of cytochrome P-450c, P-450c-dependent catalytic activity, and P-450 3-dependent catalytic activity in extrahepatic versus hepatic tissues

Male rats were dosed intraperitoneally with corn oil (controls) or HCB (50 mg/kg) in corn oil and killed 2–7 days later. Values (except for prostate) represent mean of four determinations ± standard error.

Tissue	Cytochrome P-450c[a]			
	Control	2 Days	5 Days	7 Days
	pmol/mg protein			
Lung	5 ± 1	38 ± 3	48 ± 3	64 ± 1
Kidney	0.2 ± 0.0	44 ± 6	60 ± 7	70 ± 9
Prostate[b]	0.3	120	120	170
Liver	0.3 ± 0.0	309 ± 27	407 ± 31	273 ± 51

	Catalytic activities			
	P-450 3-dependent 7α-hydroxylase[c]		P-450 c/d-dependent O-deethylase[d]	
	Control	7 Days	Control	7 Days
	pmol/min/mg			
Lung	<2	5.2 ± 2.7	60 ± 35	400 ± 150
Kidney	<2	<2	70 ± 70	575 ± 480
Prostate	<2	<2	20 ± 20	710 ± 50
Liver	350 ± 70	540 ± 140	180 ± 50	1280 ± 290

[a] Isozyme content was determined by RIA.
[b] Each value represents triplicate determinations on pooled prostate microsomes from four animals.
[c] Androstenedione 7α-hydroxylase activity.
[d] 7-Ethoxycoumarin *O*-deethylase activity.

chemically related to P-450 3. The extrahepatic content of P-450 3 in both control and HCB-treated rats was determined to be, at most, 2% of the level found in HCB-induced liver and 9% of that found in uninduced liver. Consistent with these observations, P-450 3-dependent steroid 7α-hydroxylase activity was not detected in kidney or prostate microsomes from either control or HCB-treated rats (<2 pmol of 7α-hydroxyandrostenedione/min/mg) (Table 2). In lung, however, a low level steroid 7α-hydroxylase activity was detectable after induction with HCB (Table 2). P-450 3-dependent steroid 7α-hydroxylase activity is thus expressed in uninduced and in HCB-induced extraphepatic tissues at ≤1% of the level found in the corresponding liver microsomes. In contrast, P-450c was dramatically induced by HCB in microsomes of lung, kidney, and prostate to a specific content of 60–300 pmol of P-450c/mg of microsomal protein, with the greatest induction occurring in prostate (>500-fold increase over control at 7 days) (Table 2). P-450c was thus readily detected in immunoblots of extrahepatic microsomes from HCB-induced rats (Fig. 6).

Discussion

HCB is a polychlorinated biphenyl isomer which binds to the *Ah* receptor (26) and subsequently induces P-450c and P-450d in liver microsomes of rats (2, 5). HCB also increases hepatic levels of P-450 3; however, it is uncertain whether the increases in P-450 3 are mediated by the HCB-*Ah* receptor complex. The induction of P-450c and P-450d is the consequence of increases in the hepatic content of their respective mRNAs (11, 27), presumably as the result of increases in the rate of transcription as has been shown for the orthologous enzymes (P_1-450 and P_3-450) in mice (28). The present study demonstrates that HCB increases hepatic microsomal content of cytochrome P-450 3 by increasing the hepatic content of the translatable mRNA for this enzyme. Since the completion of this study, Nagata *et al.* (29) reported the isolation of a cDNA clone for P-450a (P-450 3). Using this cDNA, they demonstrated that 3-MC, another compound which binds to the *Ah* receptor, also increases P-450 3 mRNA in rat liver.

Differences in the time course and the magnitude of the increases in P-450c and P-450 3 after administration of a polychlorinated biphenyl mixture, Aroclor 1254, to rats led Parkinson *et al.* (30) to suggest that these enzymes might be regulated differently. Alternatively, these authors (31) also suggested that the slower and less dramatic response of P-450

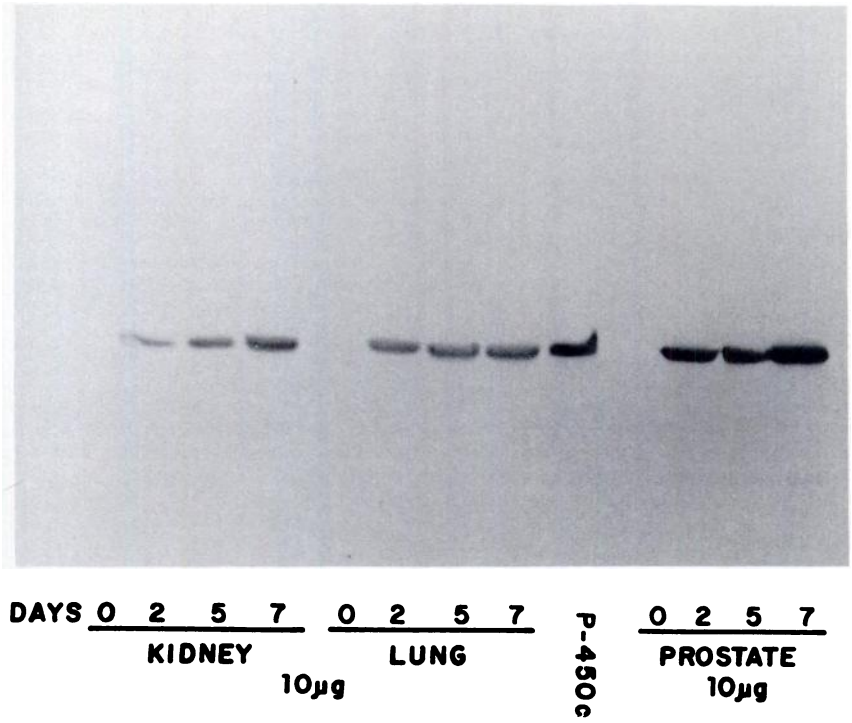

Fig. 6. Western blot showing induction of P-450c in kidney, lung, and prostate microsomes (10 μg/well) at 0–7 days after HCB treatment. A standard of 0.1 μg of purified P-450c was electrophoresed for reference.

3 to Aroclor 1254 compared to that of P-450c might reflect the slower turnover of P-450 3, since the relative rates of induction of these and other P-450 cytochromes were inversely correlated with their half-lives. The present study indicates that the slower induction of P-450 3 is the consequence of differences in the time course for the increases in the mRNAs for P-450 3 as compared to P-450c.

Measurements of P-450 proteins and translatable mRNA such as those reported in the present studies are only as reliable as the specificity of the antibodies employed. Our antibody to P-450 3 recognized only one polypeptide band in the translation products of polysomes from HCB-treated rats, and we found no evidence for cross-reactivity of anti-P-450 3 to nine purified P-450 forms as analyzed by ELISA. However, the recent studies of Nagata *et al.* (29) provide evidence for a P-450 form closely related to P-450 3 that is *not* inducible by 3-MC and whose potential reactivity with our antibodies cannot be established. If our antibody to P-450 3 does recognize this or another gene product related to P-450 3 in liver microsomes of control males, the magnitude of the increase in P-450 3 and its mRNA might be underestimated in our studies. However, the magnitude of the increase in P-450 3-dependent steroid 7α-hydroxylase activity (2-fold) agreed with the increase in P-450 3 (2- to 3-fold), suggesting that these potential contributions of related proteins do not substantially influence the findings reported in this study.

Our studies suggest some quantitative discrepancies between the magnitude of the increase in P-450 3 and its mRNA. However, factors such as efficiency of translation *in vivo* and differences in the half-lives of the protein and its mRNA could influence the relative induction of the protein and its mRNA. For example, Kawajiri *et al.* (11) found smaller increases in the P-450c mRNA (50-fold) than in those reported for P-450c itself (~500-fold) (23, 27).

Previous studies have demonstrated the differential regulation of cytochrome P-450c and P-450d in liver and extrahepatic tissues of rats (12). Both P-450c and P-450d are induced in livers of rats by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, but only P-450c is induced in extrahepatic tissues. The present study demonstrates that the constitutively expressed levels of P-450 3 and its associated steroid 7α-hydroxylase activity were not detectable in kidney or prostate microsomes and that these levels were not detectably induced by HCB in these tissues (extrahepatic levels <2–3% of levels in HCB-induced liver). In HCB-induced lung, steroid 7α-hydroxylase activity was detected at only about 1% of the level found in HCB-induced liver. This contrasts with induction of P-450c in extrahepatic tissues to a level which reaches ~25–50% of the levels found in induced livers. Thus, the liver-specific constitutive expression and inducibility of P-450 3 by polyhalogenated aromatic hydrocarbons are analogous to that found previously for P-450d (12, 27).

In summary, the present study demonstrates that HCB induces P-450 3 in liver microsomes of adult male rats by increasing the hepatic content of the mRNA for this enzyme. The differential time course for induction of P-450 3 and P-450c by HCB was shown to result from differential increases in the mRNAs for the two enzymes. In addition, there is a difference in the regulation of P-450 3 and P-450c in hepatic as compared to extrahepatic tissues. Cytochrome P-450c is readily induced by HCB in both liver and extrahepatic tissues. In contrast, P-450 3 was detectable only in liver. P-450 3 does not appear to be constitutively expressed at significant levels in lung, kidney, or prostate, and could not be detected in these extrahepatic tissues after HCB administration.

References

1. Conney, A. H. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons. *Cancer Res.* **42**:4875–4917 (1982).
2. Luster, M. I., L. D. Lawson, P. Linko, and J. A. Goldstein. Immunochemical evidence for two 3-methylcholanthrene-inducible forms of cytochrome P-448

in rat liver microsomes using a double-antibody radioimmunoassay procedure. *Mol. Pharmacol.* **23**:252–257 (1983).
3. Thomas, P. E., L. M. Reik, D. E. Ryan, and W. Levin. Induction of two immunochemically related rat liver cytochrome P-450 isozymes, P-450c and P-450d, by structurally diverse xenobiotics. *J. Biol. Chem.* **258**:4590–4598 (1983).
4. Waxman, D. J. Rat hepatic cytochrome P-450: comparative study of multiple isozymic forms, in *Cytochrome P-450* (P. R. Ortiz de Montellano, ed.) Plenum Publishing Corp., New York, 525–539 (1986).
5. Parkinson, A., S. H. Safe, L. W. Robertson, P. E. Thomas, D. E. Ryan, L. M. Reik, and W. Levin. Immunochemical quantitation of cytochrome P-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. *J. Biol. Chem.* **258**:5967–5978 (1983).
6. Dannan, G. A., F. P. Guengerich, L. S. Kaminsky, and S. D. Aust. Regulation of cytochrome P-450. Immunochemical quantitation of eight isozymes in liver microsomes of rats treated with polybrominated biphenyl congeners. *J. Biol. Chem.* **258**:1282–1288 (1983).
7. Waxman, D. J., A. Ko, and C. Walsh. Regioselectivity and stereoselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isozymes purified from phenobarbital-induced rat liver. *J. Biol. Chem.* **258**:11937–11947 (1983).
8. Waxman, D. J. Rat hepatic cytochrome P-450 isoenzyme 2c: identification as a male-specific, developmentally-induced steroid 16α-hydroxylase and comparison to a female-specific isozyme. *J. Biol. Chem.* **259**:15481–15490 (1984).
9. Waxman, D. J., G. A. Dannan, and F. P. Guengerich. Regulation of rat hepatic cytochrome P-450: age dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isoenzymes. *Biochemistry* **24**:4409–4417 (1985).
10. Nagata, K., T. Matsunaga, P. Buppodom, M. Ishimatsu, H. Yamato, S. Yoshihara, and H. Yoshimura. Unique induction of cytochrome P-450 isozymes in rat liver microsomes by treatment with 3,4,5,3′,4′-pentachlorobiphenyl and its effect on testosterone metabolism. *J. Pharmacobio-Dyn.* **8**:948–957 (1985).
11. Kawajiri, K., O. Gotho, Y. Tagashira, K. Sogawa, and Y. Fujii-Kuriyama. Titration of mRNAs for cytochrome P-450c and P-450d under drug-inductive conditions in rat livers by their specific probes of cloned DNAs. *J. Biol. Chem.* **259**:10145–10149 (1984).
12. Goldstein, J. A., and P. Linko. Differential induction of two 2,3,7,8-tetrachorodibenzo-*p*-dioxin-inducible forms of cytochrome P-450 in extrahepatic versus hepatic tissues. *Mol. Pharmacol.* **25**:185–191 (1983).
13. Goldstein, J. A., P. Linko, M. I. Luster, and D. W. Sundheimer. Purification and characterization of a second form of hepatic cytochrome P-448 from rats treated with a pure polychlorinated biphenyl isomer. *J. Biol. Chem.* **257**:2702–2707 (1982).
14. Nebert, D. W., M. Adesnik, M. J. Coon, R. W. Estabrook, F. J. Gonzalez, F. P. Guengerich, I. C. Gunsalus, E. F. Johnson, B. Kemper, W. Levin, I. R. Phillips, R. Sato, and M. R. Waterman. The P-450 gene superfamily. Recommended nomenclature. *DNA* **6**:1–24 (1987).
15. Guengerich, F. P., G. A. Dannan, S. T. Wright, M. V. Martin, and L. S. Kaminsky. Purification and characterization of liver microsomal cytochromes P-450: electrophoretic, spectral, catalytic, and immunochemical properties and inducibility of eight isozymes isolated from rats treated with phenobarbital or β-napthoflavone. *Biochemistry* **21**:6019–6030 (1982).
16. Ryan, D. E., S. Iida, A. W. Wood, P. E. Thomas, C. S. Lieber, and W. Levin. Characterization of three highly purified cytochromes P-450 from hepatic microsomes of adult male rats. *J. Biol. Chem.* **259**:1239–1250 (1984).
17. Kamataki, T., M. Shimada, K. Maeda, and R. Kato. Pituitary regulation of sex-specific forms of cytochrome P-450 in liver microsomes of rats. *Biochem. Biophys. Res. Commun.* **130**:1247–1253 (1985).
18. Cheng, K.-C., and J. B. Schenkman. Purification and characterization of two constitutive forms of rat liver microsomal cytochrome P-450. *J. Biol. Chem.* **257**:2378–2385 (1982).
19. Wrighton, S. A., P. Maurel, E. G. Schuetz, P. B. Watkins, B. Young, and P. S. Guzelian. Identification of the cytochrome P-450 induced by macrolide antibiotics in rat liver as the glucocorticoid responsive cytochrome P-450p. *Biochemistry* **24**:2171–2178 (1985).
20. Ryan D. E., P. E. Thomas, and W. Levin. Hepatic microsomal cytochrome P-450 from rats treated with isosafrole. *J. Biol. Chem.* **255**:7941–7955 (1980).
21. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* **277**:680–685 (1970).
22. Towbin, H., T. Staehelin, and J. Gordon. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350–4354 (1979).
23. Yeowell, H. N., P. Linko, E. Hodgson, and J. A. Goldstein. Induction of specific cytochrome P-450 isozymes by methylenedioxyphenyl compounds and antagonism by 3-methylcholanthrene. *Arch. Biochem. Biophys.* **243**:408–419 (1985).
24. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. R. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
25. Yeowell, H. N., D. J. Waxman, A. Wadhera, and J. A. Goldstein. Suppression of the constitutive, male-specific rat hepatic cytochrome P-450 2c and its mRNA by 3,4,5,3′,4′,5′-hexachlorobiphenyl and 3-methylcholanthrene. *Mol. Pharmacol.* **32**:340–347 (1987).
26. Bandiera, S., S. Safe, and A. B. Okey. Binding of polychlorinated biphenyls classified as either phenobarbitone-, 3-methylcholanthrene-, or mixed-type inducers to cytosolic *Ah* receptor. *Chem. Biol. Interact.* **39**:259–277 (1982).
27. Hardwick, J. P., P. Linko, and J. A. Goldstein. Dose response for induction of two cytochrome P-450 isozymes and their mRNAs by 3,4,5,3′,4′,5′-hexachlorobiphenyl indicating coordinate regulation in rat liver. *Mol. Pharmacol.* **27**:676–682 (1985).
28. Gonzalez, F. J., R. H. Tukey, and D. W. Nebert. Structural gene products of the Ah locus. Transcriptional regulation of cytochrome P_1-450 and P_3-450 mRNA levels by 3-methylcholanthrene. *Mol. Pharmacol.* **26**:117–121 (1984).
29. Nagata, K., T. Matsunaga, J. Gillette, H. V. Gelboin, and F. J. Gonzalez. Rat testosterone 7α-hydroxylase. Isolation, sequence and expression of cDNA and its developmental regulation and induction by 3-methylcholanthrene. *J. Biol. Chem.* **262**:2787–2793 (1987).
30. Parkinson, A., P. E. Thomas, D. E. Ryan, L. M. Reik, S. H. Safe, L. W. Robertson, and W. Levin. Differential time course of induction of rat liver microsomal cytochrome P-450 isozymes and epoxide hydrolase by Aroclor 1254. *Arch. Biochem. Biophys.* **225**:203–215 (1983).
31. Parkinson, A., P. E. Thomas, D. E. Ryan, and W. Levin. The *in vivo* turnover of rat liver microsomal epoxide hydrolase and both the apoprotein and heme moieties of specific cytochrome P-450 isozymes. *Arch. Biochem. Biophys.* **225**:216–236 (1983).

Send reprint requests to: Dr. Joyce A. Goldstein (B3-02), NIEHS, P. O. Box 12233, Research Triangle Park, NC 27709 or Dr. David J. Waxman, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115.