Biochemical Pharmacology, Vol. 37, No. 21, pp. 4141–4147, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

MOUSE HEPATIC CYTOCHROME P-450 ISOZYME INDUCTION BY 1,4-BIS[2-(3,5-DICHLOROPYRIDYLOXY)]BENZENE, PYRAZOLE, AND PHENOBARBITAL

HANNU RAUNIO,*† ANNELI KOJO,‡ RISTO JUVONEN,‡ PAAVO HONKAKOSKI,‡ PÄIVI JÄRVINEN,‡ MATTI A. LANG,‡ KIRSI VÄHÄKANGAS,* HARRY V. GELBOIN,§ SANG-SHIN PARK§ and OLAVI PELKONEN*

* Department of Pharmacology, University of Oulu, 90220 Oulu, Finland, ‡ Department of Pharmacology and Toxicology, University of Kuopio, P.O.B. 6, 70211 Kuopio, Finland, and § Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD 20892, U.S.A.

(*Received* 16 *February* 1988; *accepted* 21 *May* 1988)

Abstract—The effects of 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) and pyrazole on mouse hepatic cytochrome P-450 isozyme expression were compared to the P-450 induction pattern elicited by phenobarbital. TCPOBOP and PB administration caused a similar induction profile by increasing microsomal protein and cytochrome P-450 content and the catalytic activities of several monooxygenases in DBA/2N and AKR/J mice. There were, however, several quantitative and some qualitative differences in the induction profile caused by phenobarbital and TCPOBOP. A few strain-related differences were also observed. Immunoblot analysis with polyclonal anti-coumarin hydroxylase ($P\text{-}450_{Coh}$) antibody and epitope-specific monoclonal antibodies 1-7-1 and 2-66-3 showed that both phenobarbital and TCPOBOP increase the amount of P450IIB and $P\text{-}450_{Coh}$. TCPOBOP caused a more pronounced increase in the amount of P-450IIB than phenobarbital, and TCPOBOP also caused an increase in the amount of P-450IA2. These data suggest that in the mouse, TCPOBOP increases mainly the expression of P-450 isozymes responsive to phenobarbital. The effects of pyrazole differed greatly from those caused by TCPOBOP and phenobarbital. In the DBA/2N mice, pyrazole increased coumarin 7-hydroxylation 9.4-fold, whereas in the AKR/J mice the activity was induced only to a level equivalent to the DBA/2N basal level. In immunoblot experiments with anti-$P\text{-}450_{Coh}$ antibody, the amount of $P\text{-}450_{Coh}$ was considerably higher in DBA/2N mice treated with phenobarbital, TCPOBOP, or pyrazole in comparison with the AKR/J mice, indicating a strain specificity in the inducibility of coumarin 7-hydroxylase by pyrazole.

The cytochrome P-450 isozymes catalyze the metabolism of numerous exogenous and endogenous compounds [1]. The mammalian P-450 gene and gene product superfamily comprises at least eight families, each with one to five subfamilies [2]. Members of the P-450 families exhibit distinct but broad and overlapping substrate specificities [3].

3-Methylcholanthrene (MC)‖ and other polycyclic aromatic hydrocarbons induce two P-450 isozymes, P450IA1 and P450IA2 [2]. Catalytic activities associated for most part with class IA P-450s include aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin *O*-deethylase (ECOD) [3]. Phenobarbital induces class IIB P-450s [2]. Some catalytic activities typically increased by the inducers of this class are benzphetamine or ethylmorphine *N*-demethylation [4], coumarin 7-hydroxylation [5], and pentoxyresorufin *O*-deethylation (PROD) [6].

Recently, two compounds structurally unrelated to phenobarbital have been shown to be potent inducers of some P-450IIB-associated activities in the mouse. First, Poland and associates [7, 8] showed that 1,4-bis[2-(3,5,-dichloropyridyloxy)]benzene (TCPOBOP) is 650 times more potent than phenobarbital in eliciting a phenobarbital-type pleiotypic response in mouse liver with well-defined structure–activity relationship [9]. Second, Juvonen *et al.* [10] showed that pyrazole is a potent and selective inducer of coumarin 7-hydroxylase in the DBA/2N mouse liver.

This study was undertaken to compare the effects of phenobarbital, TCPOBOP and pyrazole on P-450IIB-linked and some unlinked catalytic activities in DBA/2N and AKR/J mice, which have previously been shown to differ greatly from each other in responsiveness to inducers of this class [5, 11, 12].

MATERIALS AND METHODS

P-450 nomenclature. Recently, a new common nomenclature for the P-450 genes and their products has been proposed [2]. According to the new nomenclature, the P-450I family contains polycyclic

†To whom all correspondence and requests for reprints should be addressed.

‖Abbreviations used: AHH, aryl hydrocarbon (benzo(*a*)pyrene) hydroxylase; ECOD, ethoxycoumarin *O*-dealkylase; Mab, monoclonal antibody; MC, 3-methylcholanthrene; $P\text{-}450_{Coh}$, purified P-450 isozyme highly active in the 7-hydroxylation of coumarin; PROD, pentoxyresorufin *O*-dealkylase; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene.

aromatic hydrocarbon-inducible mouse isozymes P_1-450 and P_3-450 which are orthologous to rat isozymes P-450$_c$ and P-450$_d$, respectively. The subfamily P-450IIB contains the phenobarbital-inducible isozymes P-450$_b$ and P-450$_e$ and the P-450IIE subfamily contains the alcohol-inducible P-450$_j$. P-450$_{a-j}$ are designations used by Levin and associates [13]. Since this report deals with orthologous P-450 isozymes from the mouse and rat, the trivial names indicating the source of the isozyme will be used concomitantly with the new proposed nomenclature.

Chemicals. Pyrazole, aniline, 7-ethoxycoumarin, and ethylmorphine were purchased from Sigma (St Louis, MO). 7-Pentoxyresorufin was purchased from Pierce and coumarin from Aldrich. Phenobarbital was from Merck. TCPOBOP was a generous gift from Prof. Urs Meyer (Basel University, Switzerland).

Treatment of animals. Adult male DBA/2N/Kuo and AKR/J/Kuo mice were obtained from the National Laboratory Animal Center, University of Kuopio, Finland. Maximally inducing doses of the agents were used: phenobarbital was administered to the mice i.p. in physiological saline at a dose of 80 mg/kg daily for 4 consecutive days. Pyrazole (in physiological saline) was injected i.p. at a daily dose of 200 mg/kg for 3 consecutive days. TCPOBOP was injected i.p. in olive oil as a single dose of 3 mg/kg. Reference male Sprague–Dawley rats were treated with either phenobarbital (0.5 g/l in drinking water for one week) or MC (a single 50 mg/kg i.p. injection). All animals were killed 24 hr after the last treatment except those treated with TCPOBOP, which were killed 9 days after the injection.

Enzyme assays. Liver microsomal fractions were isolated by differential centrifugation [14]. Cytochrome P-450 content was measured according to Omura and Sato [15]. ECOD (100 μM substrate concentration) was determined as described by Aitio [16]. Determination of coumarin 7-hydroxylase activity was done by the same method using 100 μM coumarin as the substrate. AHH activity (80 μM substrate concentration) was measured according to Nebert and Gelboin [17]. Ethylmorphine *N*-demethylase [18], aniline hydroxylase [19], and PROD [20] with substrate concentrations of 5 mM, 4 mM and 2 μM, respectively, were assayed by the cited methods. Protein determinations were done according to Lowry *et al.* [21] or Bradford [22].

Purification of P-450$_{Coh}$ and preparation of antibodies. Pyrazole-inducible P-450 associated with high coumarin 7-hydroxylase activity was purified to apparent electrophoretic homogeneity from livers of DBA/2N mice as described in detail elsewhere [23]. The purified protein was used as the antigen to raise polyclonal antiserum in rabbits. The specificity of the anti-P-450$_{Coh}$ antibody was validated by immunoinhibition and immunoblotting techniques ([24], Honkakoski *et al.*, unpublished). Monoclonal antibodies were raised against rat MC-inducible P-450s (Mab 1-7-1) and PB-inducible P-450s (Mab 2-66-3) as described earlier [25, 26].

Immunoinhibition studies. The contribution of individual P-450s to some of the catalytic activities studied here was analyzed by enzyme immunoinhibition. The general inhibition profile of polyclonal anti-P-450$_{Coh}$ [24], Mab 1-7-1 and Mab 2-66-3 [25–27] have been reported previously. A saturating concentration of the antibodies (10 μg of IgG protein/pmol P-450) was used in the immunoinhibition assays.

SDS-PAGE and immunoblots. SDS-PAGE was done in gels containing 7.5% polyacrylamide by the method of Laemmli [28]. The electrophoresis was run at 15–20 mA/gel until the dye front reached gel bottoms. Proteins were transferred from the gels to nitrocellulose sheets (Schleicher & Schuell) according to Towbin *et al.* [29]. Gels with molecular weight markers (Pharmacia) were also run and stained with Coomassie Brilliant Blue. For P-450 isozyme detection, the nitrocellulose sheets were first blocked with 3% (w/v) gelatin in TBS (50 mM Tris–200 mM NaCl, pH 7.4) for 1 hr at 37°. The sheets were subsequently reacted with (a) the antibodies (1:200 to 1:400 dilutions in TBS–1% gelatin–0.05% Tween 20), (b) biotinylated Protein A or anti-mouse IgG (1:400 dilutions), and (c) streptavidin-biotinylated horseradish peroxidase complex in TBS (all from Amersham). The sheets were developed with 4-chloro-1-naphthol (Bio-Rad) and hydrogen peroxide as substrates.

Statistical analysis. The enzyme activity data were evaluated by the computerized non-parametric Kruskal–Wallis test [30].

RESULTS

Effects of phenobarbital, TCPOBOP and pyrazole on monooxygenase activities in DBA/2N mice

As listed in Table 1, the content of liver microsomal protein in the DBA/2N mice was increased to a similar degree by phenobarbital and TCPOBOP, while pyrazole failed to affect it. Also cytochrome P-450 content was increased similarly by phenobarbital and TCPOBOP whereas pyrazole decreased it.

Coumarin 7-hydroxylase was increased to a comparable extent by phenobarbital and TCPOBOP (6.5- and 4.8-fold induction, respectively), whereas pyrazole caused a 9.4-fold increase in coumarin 7-hydroxylase activity. In the ethylmorphine *N*-demethylase activity, an approximately 2-fold increase was observed after treatment with both phenobarbital and TCPOBOP. In contrast, pyrazole significantly reduced this activity to 20% of the control value. PROD activity was substantially increased by both phenobarbital (16-fold) and TCPOBOP (62-fold), whereas pyrazole decreased PROD activity to 60% of the control value.

Of the catalytic activities partly associated with the P-450IA family, ECOD was induced to a similar extent by phenobarbital, TCPOBOP, and pyrazole in DBA/2N mice (5–6-fold increase compared with control mice). AHH activity was increased 4.2-fold by phenobarbital and 2.2-fold by TCPOBOP, but pyrazole reduced AHH activity to 30% of the control value.

Aniline hydroxylase, an activity mostly associated with P-450IIE1, was virtually unaffected by the compounds. Only TCPOBOP caused a slight, but significant increase (1.4-fold, $P < 0.001$) in aniline hydroxylase activity, while the enzyme activity was unaffected by phenobarbital and pyrazole.

Table 1. Effect of PB, TCPOBOP and pyrazole on liver microsomal monooxygenase activities in DBA/2 mice

Treatment	Microsomal protein (mg/g liver)	Total P-450 (nmol/mg protein)	Coumarin 7-hydroxylase	Ethylmorphine *N*-demethylase	7-Pentoxyresorufin *O*-dealkylase	7-Ethoxycoumarin *O*-deethylase	Aryl hydrocarbon hydroxylase	Aniline hydroxylase
Control	10.84 ± 0.97*	0.55 ± 0.07	0.14 ± 0.05	9.81 ± 5.30	0.009 ± 0.001	1.24 ± 0.28	0.12 ± 0.05	2.03 ± 0.33
Phenobarbital	16.96 ± 2.03†	1.30 ± 0.17†	0.91 ± 0.52†	18.37 ± 2.27†	0.144 ± 0.075†	7.30 ± 1.31†	0.50 ± 0.08†	2.29 ± 0.28
TCPOBOP	14.96 ± 2.52‡	1.75 ± 0.24†	0.67 ± 0.11†	20.28 ± 3.11†	0.562 ± 0.123†	6.24 ± 0.78†	0.26 ± 0.10†	2.92 ± 0.14†
Pyrazole	12.82 ± 4.42	0.42 ± 0.05‡	1.32 ± 0.33	2.28 ± 0.57‡	0.005 ± 0.003§	6.92 ± 1.03†	0.04 ± 0.01†	2.29 ± 0.91

The treatment of mice and the assays were carried out as described in the Materials and Methods section. All enzymatic activities are given in nmoles/mg protein/min.

* The numbers are means ±SD of 4–10 animals.

† Significantly different from control value, $P < 0.001$.

‡ Significantly different from control value, $P < 0.01$.

§ Significantly different from control value, $P < 0.05$.

Table 2. Effect of PB, TCPOBOP, and pyrazole on liver microsomal monooxygenase activities in AKR/J mice

Treatment	Total protein (mg/g liver)	Total P-450 (nmol/mg protein)	Coumarin hydroxylase	Ethylmorphine *N*-demethylase	7-Pentoxyresorufin *O*-dealkylase	7-Ethoxycoumarin *O*-deethylase	Aryl hydrocarbon hydroxylase	Aniline hydroxylase
Control	13.31 ± 2.53*	0.85 ± 0.13	0.04 ± 0.01	11.07 ± 2.58	0.021 ± 0.009	1.47 ± 0.94	0.21 ± 0.12	2.58 ± 0.68
Phenobarb.	20.85 ± 0.95†	1.54 ± 0.14‡	0.05 ± 0.02	9.47 ± 0.64	0.043 ± 0.003†	6.16 ± 0.74†	1.27 ± 0.06‡	2.28 ± 0.44
TCPOBOP	23.80 ± 1.27‡	2.13 ± 0.31‡	0.05 ± 0.01	13.79 ± 0.85†	0.963 ± 0.436‡	4.17 ± 0.44‡	0.39 ± 0.10†	1.45 ± 0.15‡
Pyrazole	12.71 ± 2.49	0.46 ± 0.05‡	0.18 ± 0.07‡	4.56 ± 0.90‡	0.022 ± 0.010	3.04 ± 0.83‡	0.08 ± 0.01‡	2.25 ± 0.18

The treatment of mice and the assays were carried out as described in Materials and Methods.
All enzymatic activities are given in nmoles/mg protein/min.

* The numbers are means ±SD of 4–10 mice.

† Significantly different from control value, $P < 0.01$.

‡ Significantly different from control value, $P < 0.001$.

Table 3. Immunoinhibition by anti-P-450$_{Coh}$ antibody and Mab 2-66-3 of coumarin 7-hydroxylase and PROD activities in DBA/2N mouse liver microsomes

Treatment	Anti-P-450$_{Coh}$		Mab 2-66-3	
	Coumarin 7-hydroxylase	PROD	Coumarin 7-hydroxylase	PROD
Control	98*	<10	<10	40
Pyrazole	98	<10	<10	40
Phenobarbital	98	<10	<10	80
TCPOBOP	98	<10	<10	90

The assays were carried out by adding 10 μg of IgG/pmol P450 to the reaction mixture.
* The numbers denote per cent inhibition in the presence of the antibody.

Effects of phenobarbital, TCPOBOP and pyrazole on monooxygenase activities in AKR/J mice

In the AKR/J mice, microsomal protein and P-450 content responded similarly to the treatments as in the DBA/2N mice (Table 2). In the AKR/J mice in comparison with the DBA/2N mice, 3.5 times lower constitutive activities and no inducibility of coumarin 7-hydroxylase was observed after phenobarbital and TCPOBOP treatment. Pyrazole did cause a 4.5-fold increase in coumarin 7-hydroxylase activity, but to a level of approximately eight times lower than the pyrazole-induced level in DBA/2N mice.

Ethylmorphine *N*-demethylation was not appreciably affected by phenobarbital or TCPOBOP. As in the DBA/2N strain, pyrazole decreased this activity. PROD activity was induced only 2-fold in response to phenobarbital but 46-fold by TCPOBOP. ECOD was induced to a variable extent (2.1- to 4.2-fold) by all three compounds, which is slightly less than in the DBA/2N strain. AHH activity responded similarly in the AKR/J mice as in the DBA/2 mice.

Phenobarbital and pyrazole caused no marked effect on aniline hydroxylase activity. Opposite to the DBA/2N mice, aniline hydroxylase activity was decreased 44% by TCPOBOP in the AKR/J mice.

Immunoinhibition studies

Immunoinhibition assays were carried out to determine the contribution of P-450$_{Coh}$ (neutralized by anti-P-450$_{Coh}$ antibody), and P-450IIB (neutralized by Mab 2-66-3) to the activities of coumarin 7-hydroxylase and PROD. As shown in Table 3, anti-P-450$_{Coh}$ very effectively blocks coumarin 7-hydroxylase without interfering with PROD. Mab 2-66-3 does not affect coumarin 7-hydroxylase but effectively inhibits PB and TCPOBOP-induced PROD activity. In addition, previous studies have shown that anti-P-450$_{Coh}$ does not affect the activities of aryl hydrocarbon hydroxylase, aniline hydroxylase, ethylmorphine *N*-demethylase or ethoxyresorufin *O*-deethylase [24].

Immunoblot analysis of P-450 isozymes

To delineate the P-450 isozymes induced by phenobarbital, TCPOBOP and pyrazole, hepatic microsomes were electrophoresed in polyacrylamide gels, transferred to nitrocellulose, and probed with polyclonal anti-P-450$_{Coh}$ antibody, Mab 1-7-1 (detects polycyclic aromatic hydrocarbon-inducible P-450s), and Mab 2-66-3. The ability of anti-rat P-450 antibodies to detect orthologous P-450 forms in other species, including the mouse, has also been shown by Thomas *et al.* [13].

A Mab 2-66-3-detectable protein which co-migrated with P-450IIB1 (P-450b) derived from phenobarbital-treated rat liver (Fig. 1, upper panel) was increased strongly in TCPOBOP-treated mice in both strains (panel A, lane 2). This protein band was increased somewhat less by phenobarbital while pyrazole failed to affect it. A distinct band (approx. 40 kDa) was also recognized by Mab 2-66-3 in mouse samples. The nature of this immunodetectable band is unknown.

Anti-P-450$_{Coh}$ antibody detected in both strains a 50-kDa protein which co-migrated with purified P-450$_{Coh}$ (Fig. 1, middle panel). This protein could not be detected by Mabs 2-66-3 or 1-7-1 or in phenobarbital-treated rat liver microsomes. Staining of this protein was increased markedly by treatment with phenobarbital, TCPOBOP, and pyrazole in the DBA/2N strain. The staining was much weaker in the AKR/J strain.

In both strains, Mab 1-7-1 detected from control and treated mice a protein corresponding to P-450IA2 (P-450$_d$) in MC-treated rat liver microsomes (Fig. 1, lower panel). Only TCPOBOP increased markedly the amount of this immunodetectable protein in both species. No proteins corresponding to P-450IA1 could be detected in any of the microsomal preparations.

DISCUSSION

In this study we have compared the effects of maximally inducing doses of TCPOBOP and pyrazole, two recently reported inducers of P-450IIB-associated enzyme activities, to the classic inducer phenobarbital. TCPOBOP is attracting interest because of its potency in eliciting a phenobarbital-type pleiotypic response in the liver [7], curious species specificity [8], and tumor promoting properties [31].

In agreement with previous studies [7, 32], microsomal protein and P-450 content were increased to a comparable extent after treatment with phenobarbital and TCPOBOP in both mouse strains. There was a clear strain difference between DBA/2N and

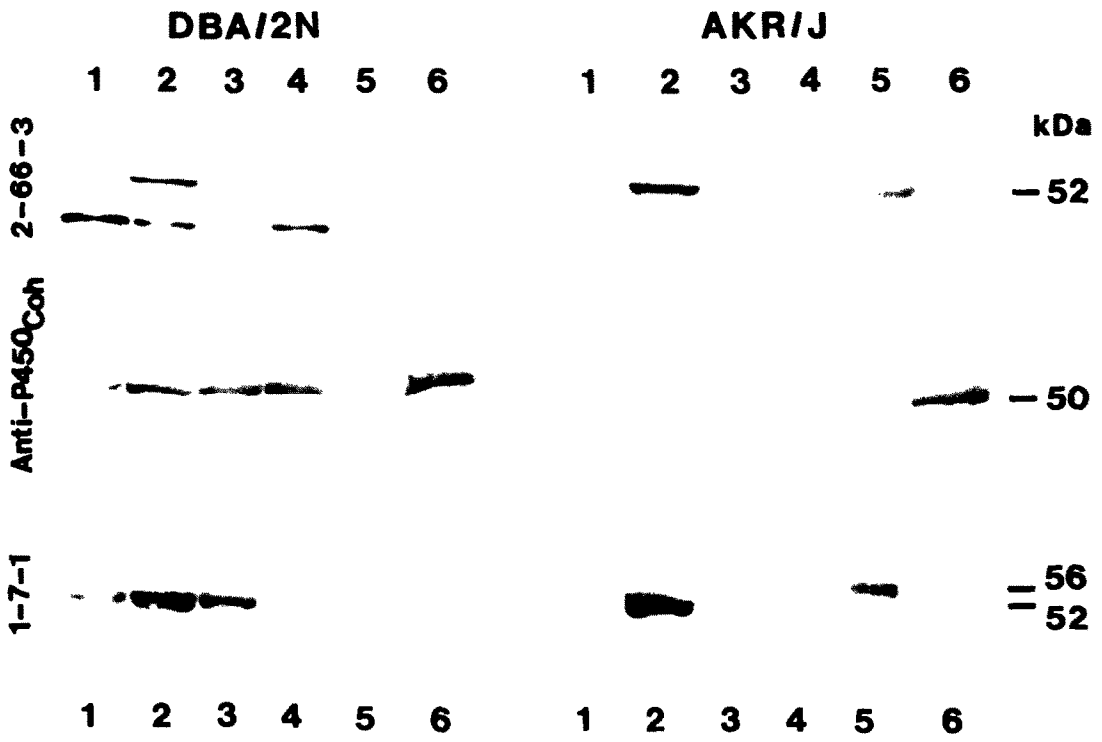

Fig. 1. Immunoblots with Mab 2-66-3, polyclonal anti-P-450$_{Coh}$, and Mab 1-7-1 of hepatic microsomal preparations from DBA/2N and AKR/J mice treated with phenobarbital, TCPOBOP or pyrazole. Lanes 1–4 contained 10 μg of microsomal protein from control (lane 1), TCPOBOP-treated (lane 2), phenobarbital-treated (lane 3) and pyrazole-treated (lane 4) mice. Lane 5 contained 10 μg of microsomal protein from phenobarbital-treated rats (upper and middle panel) or MC-treated rats (lower panel). Lane 6 contained 2 pmol of purified P-450$_{Coh}$ from pyrazole-treated DBA/2N mice in each panel. The appropriate minimum M_rs are given in kilodaltons (kDa).

AKR/J mice in the effect of the inducers on coumarin 7-hydroxylase activity. In DBA/2N mice phenobarbital, pyrazole and TCPOBOP caused a similar increase in coumarin 7-hydroxylase activity, but only pyrazole was able to elevate the activity in AKR/J mice. This is consistent with results obtained by others in phenobarbital-treated DBA/2 and AKR/J mice [11] and also with our own results with phenobarbital and pyrazole-treated mice [12].

Of the two other P-450IIB-linked activities studied, ethylmorphine *N*-demethylase activity was increased by both phenobarbital and TCPOBOP in the DBA/2N strain, but not in the AKR/J strain. PROD was strongly induced by TCPOBOP and phenobarbital in both mouse strains.

Immunoinhibition experiments revealed a clear distinction between coumarin 7-hydroxylase and PROD activities. The inability of anti-P-450$_{Coh}$ antibody to inhibit PROD and Mab 2-66-3 to inhibit coumarin 7-hydroxylase strongly suggests that these two catalytic activities are supported by distinct P-450 isozymes. It appears that in the mouse, phenobarbital and pyrazole induce distinct P-450s both capable of coumarin 7-hydroxylation. Interestingly, pyrazole-inducible P-450 in the DBA/2N mouse seems to be also distinct from the pyrazole and ethanol-inducible P-450j (P-450IIE) in the rat (Honkakoski *et al.*, unpublished).

In agreement with the catalytic activity data, immunoblot experiments with anti-P-450$_{Coh}$ showed that all three inducers caused a marked increase in the amount of immunodetectable P-450$_{Coh}$ in the DBA/2N mice, whereas in the AKR/J mice a much more subdued increase was observed in response to the inducers.

We have previously shown that microsomal coumarin 7-hydroxylase inducibility by pyrazole in the DBA/2N strain is inherited additively as a single autosomal trait like the inducibility by phenobarbital [24]. Experiments with cDNA containing rat P-450IIB1 sequence have shown that the genes for P-450IIB family in the mouse are linked with the *Coh* (coumarin hydroxylase) locus in chromosome 7 [33, 34]. Based on the present immunoinhibition and immunoblot data pyrazole-inducible mouse coumarin 7-hydroxylase does not seem to be immunologically related to P-450IIB. To date, mouse P-450s orthologous to rat P-450IIB have not been characterized [2].

Of the three P-450IIB-associated activities studied here, the dealkylation of pentoxyresorufin (PROD) is clearly the most specific for phenobarbital-induced forms [6, 20]. Two findings in the present study support this notion. First, phenobarbital and especially TCPOBOP elicit a substantial increase in PROD activity. Second, immunoblot analysis with Mab 2-66-3 showed that phenobarbital and TCPOBOP

induce the expression of a P-450 homologous to P-450IIB1 (P-450_b) in both DBA/2N and AKR/J mice. Consistent with the greater increase in PROD activity, the amount of immunodetectable P450IIB1 protein was elevated more in mice treated with TCPOBOP compared with mice treated with phenobarbital.

Of ECOD and AHH, two catalytic activities associated with (but not entirely specific for) P-450IA, ECOD was uniformly induced by all three compounds in both strains, which is consistent with the ability of reconstituted P-450_{Coh} to catalyze also the deethylation of 7-ethoxycoumarin [24]. In immunoblot experiments with Mab 1-7-1 recognizing epitopes common to rat and mouse P-450IA1 and P-450IA2, P-450IA2 was detected in control microsomes and microsomes treated with the inducers. P-450IA1 could not be seen in any microsomes. This is in agreement with Thomas *et al.* [13], who showed that P-450IA1 is immunologically undetectable in DBA/2 mice regardless of treatment. Furthermore, both DBA/2 and AKR strains lack responsiveness in the A*h*-locus governing the inducibility by polycyclic aromatic hydrocarbons [35], implying that the mouse P_1-450 is the homolog of the rat P-450_c [2, 35].

Aniline hydroxylase, a P-450IIE-linked activity, was marginally activated by TCPOBOP in DBA/2N (but not AKR/J) mice. Phenobarbital and pyrazole had no effect on aniline hydroxylase activity, further suggesting that the inducing effects of TCPOBOP and pyrazole are largely restricted to the phenobarbital-inducible class of P-450 linked monooxygenase activities.

In summary, the present data show that phenobarbital and TCPOBOP elicit a similar P-450 isozyme induction profile in the mouse liver with some quantitative and strain-related differences. The induction occurs mainly in class IIB P-450s with some overlap in class IA P-450s. Pyrazole appears to selectively induce a P-450 capable of coumarin 7-hydroxylation which is different from the phenobarbital (and TCPOBOP) induced P-450IIB.

Acknowledgements—The critical comments of Prof. Urs Meyer on the manuscript are gratefully acknowledged. We thank Ms Ritva Tauriainen for expert technical help. This study was supported by the research contract no. 04/320 from the Academy of Finland Council for Medical Research.

REFERENCES

1. Hietanen E, Laitinen M and Hänninen O (Eds), *Cytochrome P-450, Biochemistry, Biophysics and Environmental Implications*. Elsevier Biomedical Press, Amsterdam, 1982.
2. Nebert DW, Adesnick M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR, The P450 gene superfamily: recommended nomenclature. *DNA* **6**: 1–11, 1987.
3. Åström A and DePierre JW, Rat-liver microsomal cytochrome P450: purification, characterization, multiplicity and induction. *Biochim Biophys Acta* **853**: 1–28, 1986.
4. Conney AH, Induction of microsomal cytochrome P450 enzymes: the first Bernard B. Brodie lecture at Pennsylvania State University. *Life Sci* **39**: 2493–2518, 1986.
5. Wood AW and Conney AH. Genetic variation in coumarin hydroxylase activity in the mouse (*mus musculus*). *Science* **185**: 612–614, 1974.
6. Lubet RA, Mayer RT, Cameron JW, Nims RW, Burke T, Wolff T and Guengerich FP, Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P-450 by phenobarbital and other xenobiotics in the rat. *Arch Biochem Biophys* **238**: 43–48, 1985.
7. Poland A, Mak I, Glover E, Boatman RJ, Ebetino FH and Kende AS, 1,4-bis[(2-(3,5-dichloropyridyloxy)]benzene, a potent phenobarbital-like inducer of microsomal monooxygenase activity. *Mol Pharmacol* **18**: 571–580, 1980.
8. Poland AI, Mak I and Glover E, Species differences in responsiveness to 1,4-bis[2-(3,5-dichloropyridyloxy)]-benzene, a potent phenobarbital-like inducer of microsomal monooxygenase activity. *Mol Pharmacol* **20**: 442–450, 1981.
9. Kende AS, Ebetino FH, Drendel WB, Sundaralingam M, Glover E and Poland A, Structure–activity relationship of bispyridyloxybenzene for induction of mouse hepatic aminopyrine N-demethylase activity. Chemical, biological and X-ray crystallographic studies. *Mol Pharmacol* **28**: 445–453, 1985.
10. Juvonen RO, Kaipainen PK and Lang MA, Selective induction of coumarin 7-hydroxylase by pyrazole in D2 mice. *Eur J Biochem* **152**: 3–8, 1985.
11. Wood AW, Genetic regulation of coumarin hydroxylase activity in mice. Biochemical characterization of the enzyme from two inbred strain and their F1 hybrid. *J Biol Chem* **254** 5641–5646, 1979.
12. Juvonen R, Kaipainen PK, Hänninen O and Lang MA, Coumarin 7-hydroxylase in inbred strains of mice: comparison with other microsomal monooxygenase activities. *Arch Toxicol Suppl* **9**: 367–370, 1986.
13. Thomas PE, Reik LM, Ryan DE, Bandiera S and Levin W, Polyclonal and monoclonal antibody probes of the structure and function of hepatic cytochrome P450 isozymes. *Biochem Pharmac Suppl* **1**: 131–139, 1985.
14. Lang MA, Gielen JE and Nebert DW, Genetic evidence for many unique liver microsomal P-450-mediated monooxygenase activities in heterogeneic stock mice. *J Biol Chem* **256**: 12068–12075, 1981.
15. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
16. Aitio A, A simple and sensitive assay of 7-ethoxycoumarin deethylation. *Anal Biochem* **85**: 488–491, 1978.
17. Nebert DW and Gelboin HV, Substrate-inducible microsomal aryl hydrocarbon hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. *J Biol Chem* **243**: 6242–6249, 1968.
18. Anders MW and Mannering GJ, Kinetics of the inhibition of the N-demethylation of ethylmorphine by 2-diethylaminoethyl-2,2-diphenylvalerate HCl (SKF 252 A) and related compounds. *Mol Pharmacol* **2**: 319–327, 1966.
19. Mieyal JJ and Blumer JL, Acceleration of the autooxidation of human oxyhemoglobin by aniline and its relation to hemoglobin catalyzed aniline hydroxylation. *J. Biol Chem* **251**: 3442–3446, 1976.
20. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T and Mayer RT, Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem Pharmacol* **34**: 3337–3345, 1985.
21. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
22. Bradford MM, A rapid and sensitive method for the

quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.

23. Juvonen R, Shkumatov V and Lang M, Purification and characterization of a liver microsomal cytochrome P-450 isoenzyme with a high affinity and metabolic capacity for coumarin from pyrazole treated D2 mice. *Eur J Biochem* **171**: 205–211, 1988.
24. Järvinen P, Juvonen RO, Honkakoski P, Raunio, H and Lang MA, Genetic control of pyrazole-inducible mouse liver coumarin 7–hydroxylase. *Eur J Biochem*, in press.
25. Park SS, Fujino T, West D, Guengerich FP and Gelboin HV, Monoclonal antibodies that inhibit enzyme activity of 3-methyl-cholanthrene-induced cytochrome P450. *Cancer Res* **42**: 1798–1808, 1982.
26. Park SS, Fujino T, Miller H, Guengerich FP and Gelboin HV, Monoclonal antibodies to phenobarbital-induced rat liver cytochrome P-450. *Biochem Pharmacol* **33**: 2071–2081, 1984.
27. Gelboin HV and Friedman FK, Monoclonal antibodies for studies on xenobiotic and endobiotic metabolism. Cytochromes P-450 as a paradigm. *Biochem Pharmacol* **34**: 2225–2234, 1985.
28. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond)* **277**: 680–685, 1970.
29. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose: procedure and some applications. *Proc Natl Acad Sci USA* **79**: 4350–4354, 1979.
30. Theodorsson-Norheim E, Kruskall–Wallis test: BASIC computer program to perform nonparametric one-way analysis of variance and multiple comparisons on ranks of several independent samples. *Comp Meth Prog Biomed* **23**: 57–62, 1986.
31. Dragani TA, Manenti G, Galliani G and Della Porta G, Promoting effects of 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene in mouse hepatocarcinogenesis. *Carcinogenesis* **6**: 225–228, 1985.
32. Romano M, Esteve A, Coccia P, Masturzo P, Galliani G, Ghezzi and Salmona M, Biochemical characterization of the hepatic effects in mice and rats of 1,4-bis 2-(3,5-dichlorodipyridyloxy) benzene, a hepatic neoplasm promoter. *Tox Appl Pharmacol* **83**: 379–385, 1986.
33. Wood AW and Taylor BA, Genetic regulation of coumarin hydroxylase activity in mice. Evidence for single locus control on chromosome 7. *J Biol Chem* **254**: 5647–5651, 1979.
34. Simmons DL and Kasper CB, Genetic polymorphisms for a phenobarbital-inducible cytochrome P-450 map to the coh locus in mice. *J Biol Chem* **258**: 9585–9588, 1983.
35. Negishi M and Nebert DW, Structural gene products of the Ah locus. Genetic and immunochemical evidence for two forms of mouse liver cytochrome P-450 induced by 3-methycholanthrene. *J Biol Chem* **254**: 11015–11023, 1979.