Biochemical Pharmacology, Vol. 42, No. 9, pp. 1751–1759, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

INDUCIBILITY OF $P450_{Coh}$ BY PYRAZOLE AND ITS DERIVATIVES

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(*Received* 9 *November* 1990; *accepted* 16 *June* 1991)

Abstract—Pyrazole and several of its derivatives increase the hepatic microsomal coumarin 7-hydroxylase to a variable extent. The strongest inducers are pyrazole itself and those derivatives which have a hydroxy group or a halogen at the 4-position of the molecule. The increase in coumarin 7-hydroxylase is due to an increase in the microsomal $P450_{Coh}$ and the corresponding mRNA. The increase of $P450_{Coh}$ by pyrazole and 4-hydroxypyrazole is selective because several other mono-oxygenase enzymes and the total P450 content are either not affected or even decreased. These include the testosterone 15α-hydroxylase ($P450_{15\alpha}$), a close structural analogue of $P450_{Coh}$, which is induced only marginally by pyrazole and even decreased by 4-iodopyrazole, and $P450_{ac}$ which is decreased by pyrazole and 4-hydroxypyrazole. Introducing a methyl residue at the 4-position will alter the induction properties of the compound essentially by making it less selective for $P450_{Coh}$. These results demonstrate the special selective action of pyrazole and some of its derivatives on the hepatic microsomal mono-oxygenase complex and the unique mode of regulation of the cytochrome $P450_{Coh}$ even within the same subfamily of cytochromes P450.

We have recently purified and characterized a cytochrome P450 isoenzyme from mouse liver microsomes with a high coumarin 7-hydroxylase activity designated as $P450_{Coh}$† [1] and shown that this protein belongs to the II A subfamily with a high homology (98.3%) to mouse liver testosterone 15α-hydroxylase [2, 3].

The genetic regulation and inducibility of the $P450_{Coh}$ seems to be different from most of the other hepatic cytochromes P450, perhaps the most striking phenomenon being its very strong inducibility by pyrazole with simultaneous decrease of several other microsomal mono-oxygenase activities and up to 40% loss of the total microsomal cytochrome P450 content [4, 5]. The only other enzyme activity known to be increased by pyrazole, in addition to coumarin 7-hydroxylase and 7-ethoxycoumarin *O*-deethylase, is the testosterone 15α-hydroxylase, suggesting not only structural but also regulatory similarities between the two enzymes [2].

Xenobiotics which are strong inducers of the hepatic microsomal cytochrome P450 system are typically lipophilic and in many cases have a characteristic size and shape [6]. Because of its high water solubility and atypical structure it is rather surprising that pyrazole is such a strong inducer.

So far not much is known about the mechanism of induction of coumarin 7-hydroxylase except that both the mRNA and protein levels specific for $P450_{Coh}$ are increased after pyrazole treatment [2, 7]. In order to understand better the induction process and especially which properties of pyrazole are necessary for the induction, in this report we have used pyrazole and several of its derivatives and analysed their effects on the microsomal mono-oxygenase complex.

MATERIALS AND METHODS

P450 nomenclature. In the new proposed P450 nomenclature, the Type I $P450_{15\alpha}$ has been assigned as a product (P450IIA3) of gene locus Cyp 2A3 located at mouse chromosome number 7 [8]. However, it has been clearly shown that the Type I $P450_{15\alpha}$ is testosterone 15α-hydroxylase and the Type II $P450_{15\alpha}$ is coumarin 7-hydroxylase. Consequently, the nomenclature should be changed in this regard. Before the formal recognition of this difference, we have used the term $P450_{Coh/15\alpha}$ to describe these two closely related proteins.

Chemicals. Chemicals were obtained from following sources: benzo(*a*)pyrene, cytochrome *c*, bicinchonic acid, 7-ethoxycoumarin, pyrazole, 5-bromo-4-chloro-3-indolyl phosphate, anti-rabbit IgG conjugated with alkaline phosphatase, anti-mouse IgG conjugated with alkaline phosphatase, 4-iodopyrazole and coumarin (Sigma Chemical Co., St Louis, MO, U.S.A.); 7-pentoxyresorufin (Pierce, Rockford, OR, U.S.A.); 7-hydroxycoumarin and resorufin (Aldrich Chemical Co., Milwaukee, WI, U.S.A.); 4-hydroxypyrazole (The Lilly Research Laboratories, U.S.A.); 4-methylpyrazole (Labkemi

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† Abbreviations: $P450_{Coh}$, mouse liver P450 isozyme catalysing coumarin 7-hydroxylation; $P450_{ac(j)}$, mouse liver P450 isozyme catalysing dimethylnitrosamine demethylation and ethanol oxidation; $P450_{15\alpha}$, mouse liver P450 isozyme catalysing testosterone 15α-hydroxylation; COH, coumarin 7-hydroxylase; ECDE, 7-ethoxycoumarin *O*-deethylase; DMNA, dimethylnitrosamine demethylase.

AB, Götegorg, Sweden); 4-bromopyrazole (Ortho Research, Raritan, NF, U.S.A.); *N*-methylpyrazole (ICN Pharmaceutical, Plainview, NY, U.S.A.); 3,5-dimethylpyrazole and 1,2,4-triazole (Fluka A.G., Buchs, Switzerland); NADPH (Boehringer Mannheim GmbH, Germany), nitrocellulose BA 83 and Nytran NY 13N sheets (Schleicher & Schuell, Feldbach, Switzerland), [α-^{32}P]dCTP (3000 Ci/mmol; Amersham, U.K.). All other chemicals were of the highest grade commercially available.

Production of a polyclonal antibody against $P450_{Coh}$ has been described before [4]. The specificity of the anti-$P450_{Coh}$ antibody was validated by immunoinhibition and immunoblotting techniques [7]. Monoclonal antibody 1-98-1 against rat liver $P450_{ac}$ was a kind gift from Dr Gelboin.

Treatment of animals. Male DBA/2N/Kuo (D2) mice (5–13 weeks old) were obtained from the National Laboratory Animal Center at the University of Kuopio. In the preliminary experiments mice were given pyrazole, 4-hydroxy-, 4-methyl-, *N*-methyl-, 3,5-dimethylpyrazole and 1,2,4-triazole (75 mg/kg), 4-iodopyrazole (125 mg/kg) and 4-bromopyrazole (150 mg/kg) six times every 12 hr i.p. In subsequent experiments the mice were treated as follows: pyrazole (200 mg/kg), 4-hydroxy- and 4-methylpyrazole [10, 20, 50 and 100 mg/kg (=0.12, 0.24, 0.60 and 1.23 mmol/kg, respectively)] and 4-iodopyrazole [25, 50, 125 and 250 mg/kg (=0.12, 0.24, 0.60 and 1.23 mmol/kg, respectively)] i.p. twice a day for 3 consecutive days. Drugs were diluted in physiological saline. Control animals received the same amount of saline. Mice were killed 12 hr after the last injection. During the treatment the animals received water and pelleted food (Hankkija Ltd, Finland) *ad lib.* The microsomal fraction from livers was isolated according to Lang and Nebert [9].

Mono-oxygenase assays. Microsomal protein and cytochrome P450 contents were determined by the methods of Smith *et al.* [10] and Omura and Sato [11], respectively. Coumarin 7-hydroxylase and 7-ethoxycoumarin *O*-deethylase were measured according to Juvonen *et al.* [1], pentoxyresorufin *O*-dealkylase modified by Burke *et al.* [12], benzo(*a*)pyrene hydroxylase, cytochrome *c* reductase and testosterone 15α-hydroxylase were measured by the methods of Nebert and Gelboin [13], Strobel and Dignam [14] and Waxman *et al.* [15], respectively. Dimethylnitrosamine demethylase activity was carried out using a method modified by Juvonen *et al.* [4].

Dot immunobinding analysis. The amount of microsomal $P450_{Coh}$ and $P450_{ac}$ was determined using dot immunobinding assay modified from Domin *et al.* [16]. Pooled microsomal samples (5–2500 ng protein for $P450_{Coh}$ and 12.5–500 ng for $P450_{ac}$) and purified isozyme (5–100 fmol $P450_{Coh}$ and 50–400 fmol $P450_{ac}$) were diluted serially in 20 mM Tris–HCl/500 mM NaCl, pH 7.6 (TBS) containing 0.3 mg/mL bovine serum albumin as a carrier. The samples were applied to a nitrocellulose sheet using Bio-dot SF apparatus (Bio-Rad, Richmont, CA, U.S.A.), sample wells were washed with TBS and the sheet was blocked with 2% (w/v) defatted powdered milk dissolved in TBS. The sheet was incubated successively with anti$P450_{Coh}$ (Mab 1-98-1 for $P450_{ac}$) diluted 1:1000 with blocking solution and secondary antibody (anti-rabbit IgG alkaline phosphatase for $P450_{Coh}$ and anti-mouse IgG alkaline phosphatase for $P450_{ac}$) diluted 1:1000 with blocking solution. The sheets were washed with TBS containing 0.05% (v/v) Tween 80 and twice with TBS. The bands (immunocomplexes) were visualized by 5-bromo-4-chloro-3-indolyl phosphate (0.5 mg/mL) dissolved in 0.1 M $NaHCO_3$, pH 9.1, containing 1 mM $MgCl_2$. The dry sheet was scanned with Shimadzu CS-930 densitometer, and the amount of isozyme was calculated from the standard curve of purified isozyme.

Immunoinhibition of mono-oxygenase activities. Immunoinhibition of COH and ECDE activities were performed by adding anti-$P450_{Coh}$ antibody (0.1–265 μg/pmol cytochrome P450) to the reaction mixtures 15 min prior the start of the reaction by NADPH. Otherwise the assays were carried out as described above. Pooled microsomes from the highest dose group (1.4–16.9 pmol cytochrome P450) from control-, 4-hydroxy-, 4-methyl- and 4-iodopyrazole-treated animals were used. Protein concentration was held constant by the addition of preimmune IgG.

Purification of cytochrome P450 isozymes. Cytochrome $P450_{Coh}$, highly active for the 7-hydroxylation of coumarin was purified from pyrazole-treated DBA/2N mice essentially according to Juvonen *et al.* [1].

Mouse cytochrome $P450_{ac}$ was purified as described for rat $P450_j$ by Terelius and Ingelman-Sundberg [17].

Preparation of cDNA probe. The cloning of cDNA p15α-15 encoding for Type II $P450_{15\alpha}$ has been described previously [18, 19]. Because of the high degree of homology between Type I and Type II $P450_{15\alpha}$ cDNAs (98.3% at the amino acid level), p15α-15 does not discriminate between them [18]. As a control probe, human actin cDNA [20] was used.

Preparation of RNA and slot blots. For hybridization studies mice were treated with pyrazole 200 mg/kg, 4-hydroxy- and 4-methylpyrazole 50 mg/kg (0.60 mmol/kg) and 4-iodopyrazole 125 mg/kg (0.60 mmol/kg) twice a day for 3 consecutive days i.p. The livers were divided into two pieces. One piece was used to isolate microsomes as described previously [9] and a second one for preparation of total RNA. Livers from two mice were pooled for each sample which was quickly immersed in liquid nitrogen and stored at −80°. The samples were subsequently weighed and homogenized in guanidine thiocyanate and RNA was prepared by Chomczynski and Sacchi [21]. Slot blots were performed with a Minifold II apparatus (Schleicher & Schuell, Dassel, Germany). RNA was fixed by baking the Nytran sheets at 80° for 2 hr. The filters were then prehybridized for 2 hr at 42° in prehybridization buffer consisting of 8.6 × SSC (20 × SSC: 3 M NaCl, 0.3 M Na_3citrate, pH 7.0), 5 × Denhardt's solution, 50% (v/v) formamide, 0.1% (v/v) SDS and sonicated salmon sperm DNA (100 μg/mL). The hybridization was done overnight in 20 mL of prehybridization buffer supplemented with 2% (w/v) dextran sulphate

and ^{32}P-labelled p15α-15 probe. The labelling was done with the Pharmacia Oligolabelling kit. This method yielded probes with specific activity of about 510,000 cpm/μg DNA. In slot blot experiments, duplicate filters were prepared and the second filter was hybridized with ^{32}P-labelled actin cDNA. After hybridization, the filters were washed first at room temperature (3 × 30 min) in 2 × SSC, 0.1% (v/v) SDS and subsequently in 0.1 × SSC, 0.1% (v/v) SDS at 42° (3 × 20 min). The filters were exposed for 1 week to Kodak X-OMAT AR-5 Diagnostic film at −80°.

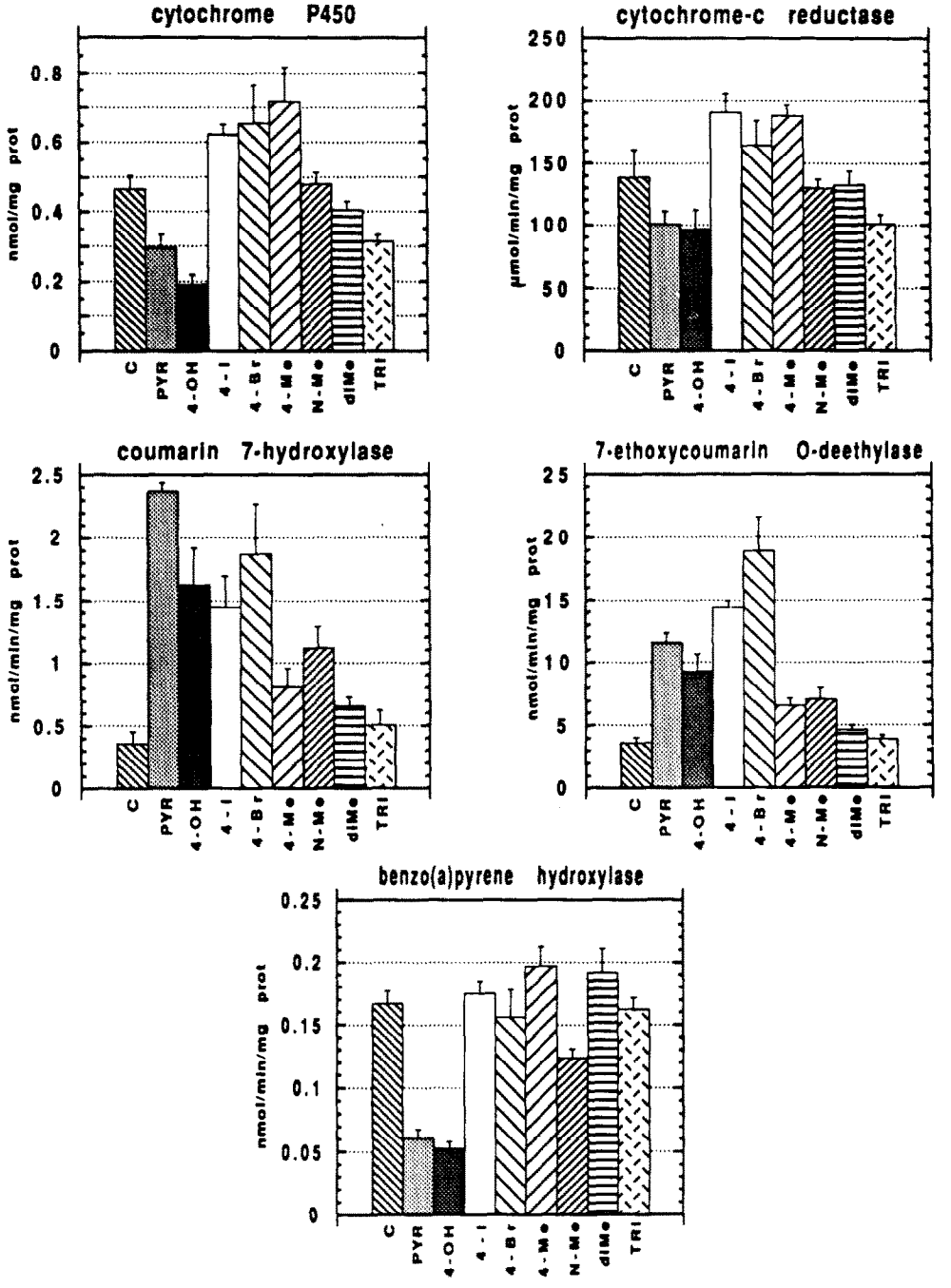

Fig. 1. The amount of liver microsomal cytochrome P450 (nmol/mg protein) and the activities of microsomal mono-oxygenase complex (nmol/min/mg protein or μmol/min/mg protein). In figures the average value of three individuals ± SD is expressed for control (C, ■), pyrazole- (PYR, ▨), 4-hydroxypyrazole- (4-OH, ▩), 4-iodopyrazole- (4-I, □), 4-bromopyrazole- (4-Br, ▧), 4-methylpyrazole- (4-Me, ▨), *N*-methylpyrazole- (N-Me, ▨), 3,5-dimethylpyrazole- (diMe, ▤) and 1,2,4-triazole-treated (TRI, ▧) DBA/2N mice.

RESULTS AND DISCUSSION

The effects on mono-oxygenase parameters

For preliminary experiments we chose pyrazole and several of its derivatives. The amount and time of exposure was based on previous studies giving the maximal induction of $P450_{Coh}$ by pyrazole itself [5]. Figure 1 shows that modification of the molecule changes essentially its effects on the mono-oxygenase complex. Furthermore, depending on the mono-oxygenase parameter the effects are strikingly different. In particular, some of the derivatives have

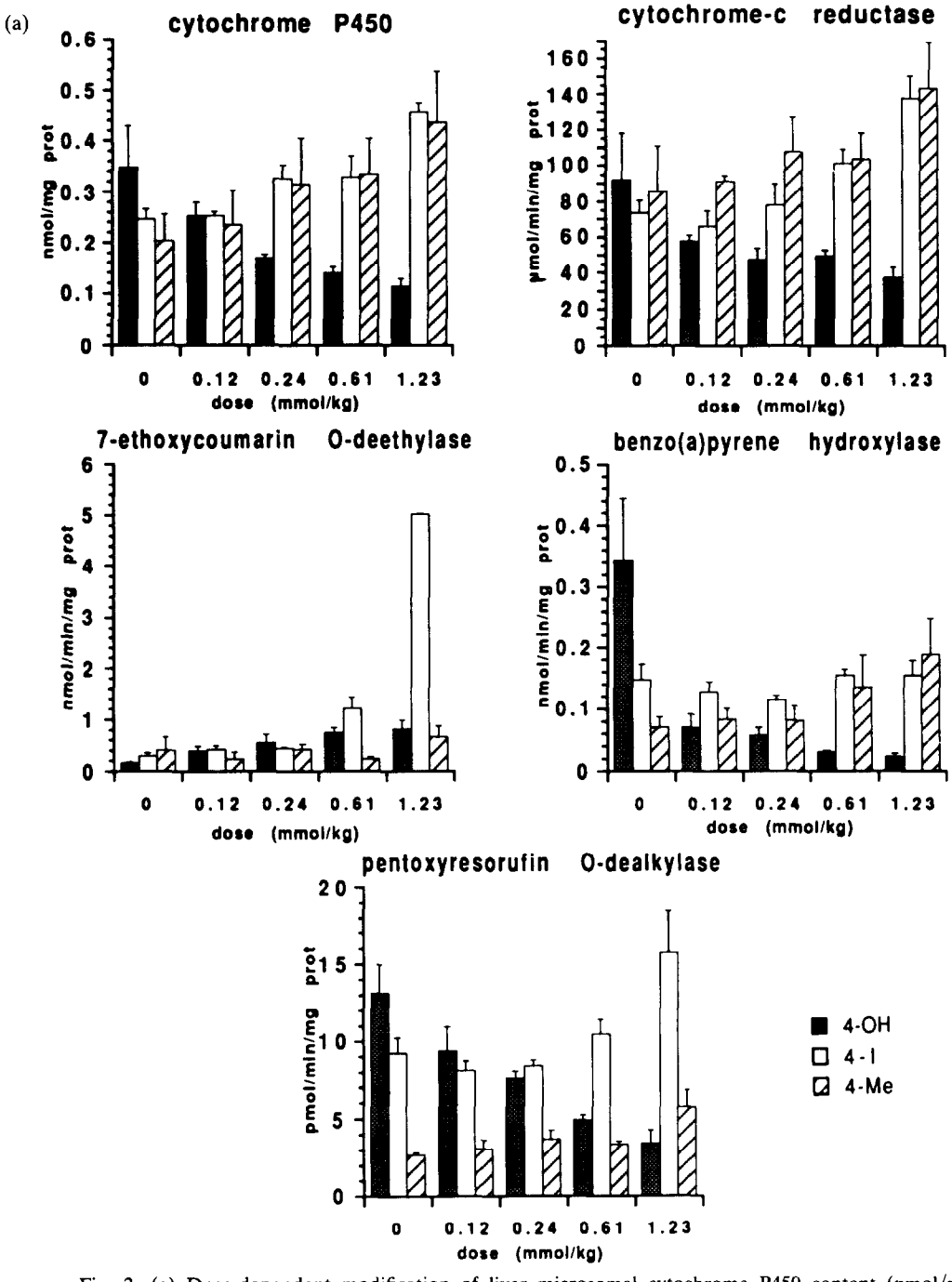

Fig. 2. (a) Dose-dependent modification of liver microsomal cytochrome P450 content (nmol/mg protein) and the activities of the microsomal mono-oxygenase complex (nmol/min/mg protein, μmol/min/mg protein or pmol/min/mg protein). Each bar represents mean ± SD of four animals. Liver microsomes of 4-hydroxy- (4-OH, ▩), 4-methyl- (4-Me, ▨) and 4-iodopyrazole-treated (4-I, □) DBA/2N mice were used. (b) Dot immunobinding analysis of purified $P450_{Coh}$ (●) with anti-$P450_{Coh}$ antibody (nmol $P450_{Coh}$/mg protein) and the microsomal coumarin 7-hydroxylase (COH) activity (□, nmol/min/mg protein). Pooled liver microsomes (four samples) of 4-hydroxy-, 4-iodo- and 4-methylpyrazole-treated DBA/2N mice were used. The assays were performed as described in Materials and Methods.

a very strong increasing effect on coumarin 7-hydroxylase and 7-ethoxycoumarin *O*-deethylase while on other activities the effects are generally weak. However, two of the compounds, pyrazole itself and the 4-hydroxypyrazole, being among the strongest inducers of the coumarin 7-hydroxylase and the 7-ethoxycoumarin *O*-deethylase have a strong but reducing effect on other parameters measured.

Based on the preliminary results three derivatives were chosen for more detailed studies: (i) 4-hydroxypyrazole because it seems to have similar effects as pyrazole and also because this compound is the major metabolite of pyrazole found both *in vivo* and after incubation of pyrazole with hepatocytes or hepatic microsomal fraction [22–24]; (ii) 4-iodopyrazole, where the hydroxy group has been replaced by a large and somewhat less hydrophilic group. This compound still seems to be a very strong inducer of the $P450_{Coh}$ but does not reduce, unlike the 4-hydroxy derivative, the other activities. (Figure 1 and other experiments not shown here demonstrate that 4-iodopyrazole and 4-bromopyrazole have almost identical effects. Therefore only one of them was chosen.); and (iii) 4-methylpyrazole, substituting the hydroxy group or halogen (I^- or Br^-) in the pyrazole ring by a methyl group seems to alter the induction properties of the compound essentially. The 4-methylpyrazole seems to have lost most of its inducing potency towards the $P450_{Coh}$, at the same time having some increasing effects on other mono-oxygenase parameters such as the total microsomal P450 content or benzo(*a*)pyrene hydroxylase activity.

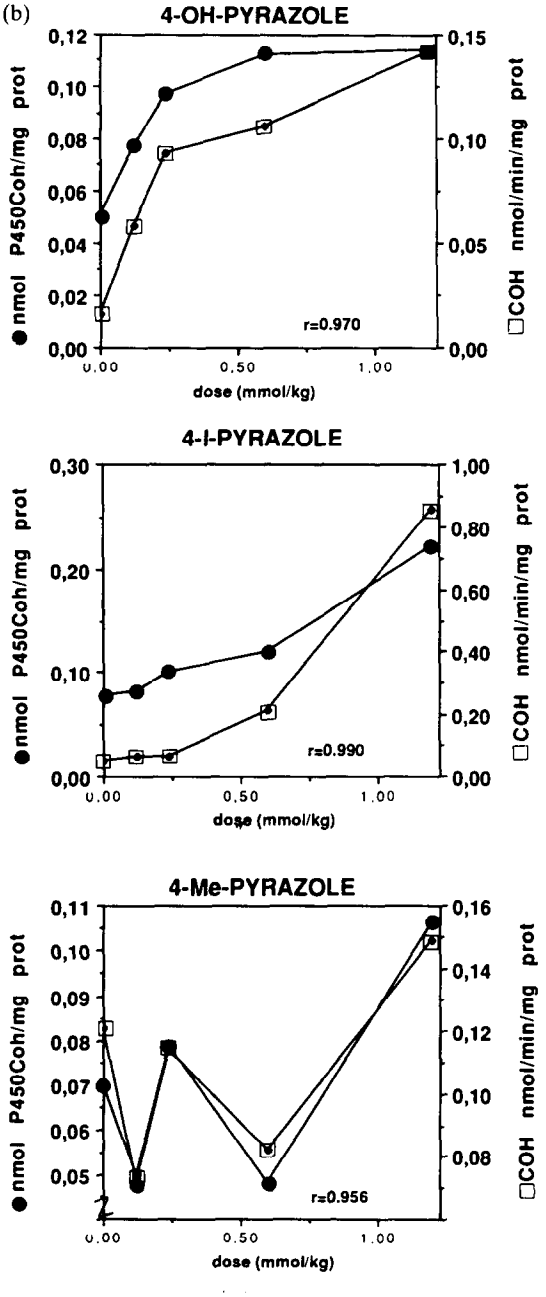

Fig. 2. (b).

With the three compounds a dose–response study was carried out and the results are presented in Fig. 2a and b. As may be expected from preliminary experiments the 4-hydroxypyrazole reduces the microsomal P450 content as well as the activities of cytochrome *c* reductase, benzo(*a*)pyrene hydroxylase and pentoxyresorufin *O*-dealkylase in a dose-dependent manner. At the same time the 7-ethoxycoumarin *O*-deethylase and especially the coumarin 7-hydroxylase are increased. These effects of 4-hydroxypyrazole are almost identical to those of pyrazole, reported earlier by us [5], suggesting that pyrazole and 4-hydroxypyrazole act through the same mechanism.

Based on visual estimation the higher doses of pyrazole and 4-hydroxypyrazole are hepatotoxic. It may therefore be possible that the reducing effects of the two compounds on the mono-oxygenase complex may be due to their hepatotoxicity, as is known for other hepatotoxic compounds [25]. Given this it is very interesting to observe the strong increase in coumarin 7-hydroxylase suggesting that $P450_{Coh}$ is regulated differently from the other P450 isozymes and persists the tissue denaturation better than the others.

4-Iodopyrazole has a very strong, dose-dependent, increasing effect on both coumarin 7-hydroxylase and 7-ethoxycoumarin *O*-deethylase. In fact with the highest dose given, this effect seems to be clearly stronger than with pyrazole itself [5] or 4-hydroxypyrazole (Fig. 2a and b). So far we do not know whether this is due to the lower toxicity of 4-iodopyrazole as compared to pyrazole or 4-hydroxypyrazole but the fact that other mono-oxygenase parameters are not affected, or even slightly increased, by 4-iodopyrazole may suggest that this is the case.

The 4-methylpyrazole seems to have an effect similar to the 4-iodopyrazole on some of the mono-oxygenase parameters, such as the total P450 content, the cytochrome *c* reductase and pentoxyresorufin *O*-deethylase activity. However, there is a striking difference between the two compounds in their effects on 7-ethoxycoumarin *O*-deethylase and coumarin 7-hydroxylase. The replacement of iodine with a methyl group leads to a significant loss of the selective induction potency.

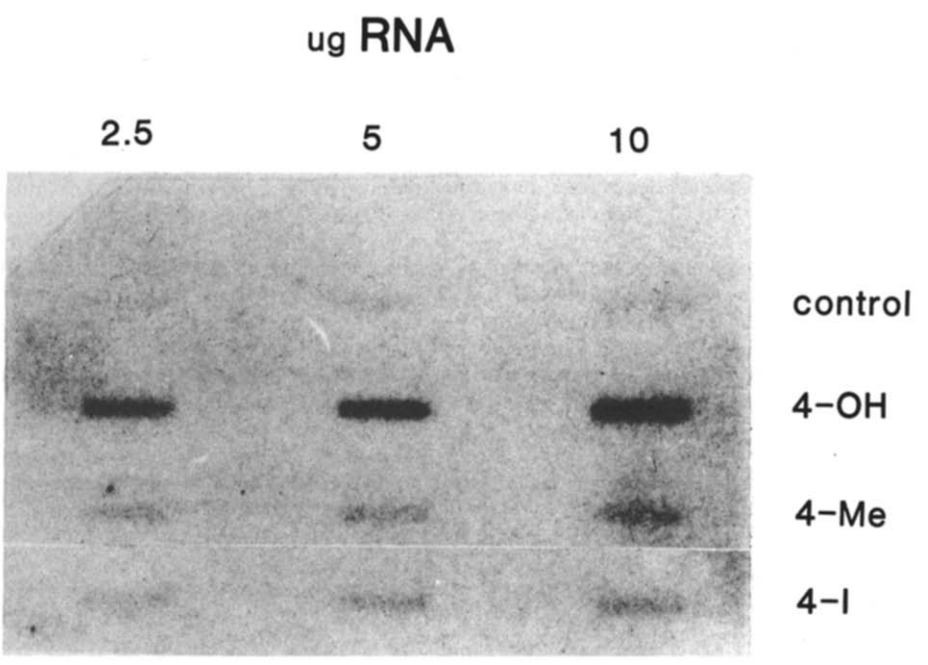

Fig. 3. Slot blot hybridization analysis of RNA extracted from livers of control, 4-hydroxy- (4-OH), 4-iodo- (4-I) and 4-methylpyrazole (4-Me)-treated DBA/2N mice. Total cellular RNA from each sample was spotted onto Nytran filter in three different concentrations. The filter was hybridized with [^{32}P]-p15α-15 cDNA. For the preparation of RNA samples see Materials and Methods.

What is the reason for the different induction properties of pyrazole, 4-hydroxy-, 4-iodo- (4-bromo-) and 4-methylpyrazole? This is not known and to reveal the mechanism of induction requires further studies. However a hydrophilic group in the 4-position of the pyrazole ring seems to be essential for the selective induction of $P450_{Coh}$. On the other hand the size of this group may not be critical because both hydroxy-, bromo- and iodopyrazole seem to be potent inducers (Fig. 1).

Coumarin 7-hydroxylase ($P450_{Coh}$) and testosterone 15α-hydroxylase ($P450_{15\alpha}$) belong to the same subfamily (IIA) and have only 11 amino acid differences (out of 494). So far $P450_{15\alpha}$ is the only other protein, in addition to the $P450_{Coh}$, which is known to be induced by pyrazole in mice [2, 26]. Nevertheless, their regulation may not be identical. In the liver, testosterone 15α-hydroxylase is induced only weakly by pyrazole as compared to coumarin 7-hydroxylase [2] and in the kidney where coumarin 7-hydroxylase is strongly induced, testosterone 15α-hydroxylase is not increased at all.*

Further proof for the different regulation of $P450_{Coh}$ and $P450_{15\alpha}$ is given in Fig. 1 and Table 1, respectively, where we can see that the dose of 4-hydroxy- and 4-iodopyrazole causing about a 3–4 times induction of coumarin 7-hydroxylase does not affect the testosterone 15α-hydroxylase at all or in the case of 4-iodopyrazole even decreases it.

These data, together with the fact that the catalytic properties of $P450_{Coh}$ and $P450_{15\alpha}$ are different [2], stress the unique mode of regulation of the $P450_{Coh}$ and suggest that even highly homologous isozymes belonging to the same subfamily may neither share the catalytic properties nor the same regulatory system.

Dot immunobinding and slot blot analyses

Inducibility of $P450_{Coh}$ by 4-hydroxy-, 4-methyl- and 4-iodopyrazole was further studied by dot blot analysis using the specific anti-$P450_{Coh}$ antibody [1, 7] and slot blot analysis using the specific cDNA probe for $P450_{Coh}$ mRNA [2]. The data are shown in Figs 2b and 3, respectively. As can be seen, the amount of microsomal $P450_{Coh}$ correlates well with the corresponding enzyme activity suggesting that increase in the microsomal enzyme activity is due to increased amount of microsomal $P450_{Coh}$.

Table 1. Effects of pyrazole (pyr), 4-hydroxy- (4-OH), 4-methyl- (4-Me) and 4-iodopyrazole (4-I) on testosterone 15α-hydroxylase activity

Treatment	Testosterone 15α-hydroxylase (pmol/min/mg protein)
Control	141
pyr	264
4-OH	139
4-Me	65
4-I	92

The activity was measured from liver microsomes pooled from three individual DBA/2N (D2) mice. Mice were treated with pyrazole (pyr, 200 mg/kg), 4-hydroxy- (4-OH) and 4-methylpyrazole (4-Me, 50 mg/kg) and 4-iodopyrazole (4-I, 125 mg/kg) six times every 12 hr.

* Lang and Negishi, unpublished observations.

Figure 3 shows that $P450_{Coh}$ cDNA recognizable mRNA is strongly increased after 4-hydroxypyrazole treatment of mice. Whether this is due to stimulated transcription or stabilization of mRNA we do not know and this should be studied further. This result is similar as obtained previously by pyrazole [2]. On the other hand 4-iodopyrazole does not seem to increase the amount of mRNA as much as 4-hydroxypyrazole suggesting that there may be some differences in their acting as inducers. 4-Methylpyrazole seems to have a similar effect on the amount of mRNA as 4-iodopyrazole.

Immunoinhibition of COH and ECDE

In our previous work we have suggested that $P450_{Coh}$ accounts for most, if not all, of the microsomal coumarin 7-hydroxylase activity [3, 7]. Table 2 shows that this seems to be the case also after treating the mice with the three pyrazole derivatives, since in each case the inhibition by anti-$P450_{Coh}$ antibody is almost 100%. 7-Ethoxycoumarin *O*-deethylase, on the other hand, is inhibited about 100% after 4-hydroxypyrazole treatment and only 80% after 4-methylpyrazole treatment. This is consistent with the view that although the main P450 isozyme metabolizing the 7-ethoxycoumarin is the $P450_{Coh}$, others do it too [7] which are, however, destroyed by 4-hydroxypyrazole and induced by 4-methylpyrazole.

Effects on DMNA activity and cytochrome P450ac

Figure 4 shows the correlation between microsomal dimethylnitrosamine demethylase activity and the amount of immunorecognizable $P450_{ac}$ in control mice and after treatment with pyrazole and three of its derivatives (A) and the correlation between DMNA and COH activities in the same samples (B).

Levin and his co-workers [27] purified the $P450_{ac(j)}$ from rat liver microsomes and showed that it is the principal enzyme metabolizing dimethylnitrosamine and also that it is inducible by pyrazole [28]. This cytochrome P450 belongs to the ethanol-inducible P450IIE subfamily. Using a monoclonal antibody against the $P450_{ac}$ we have shown with western blot and immunoinhibition studies that a similar protein also exists in mouse

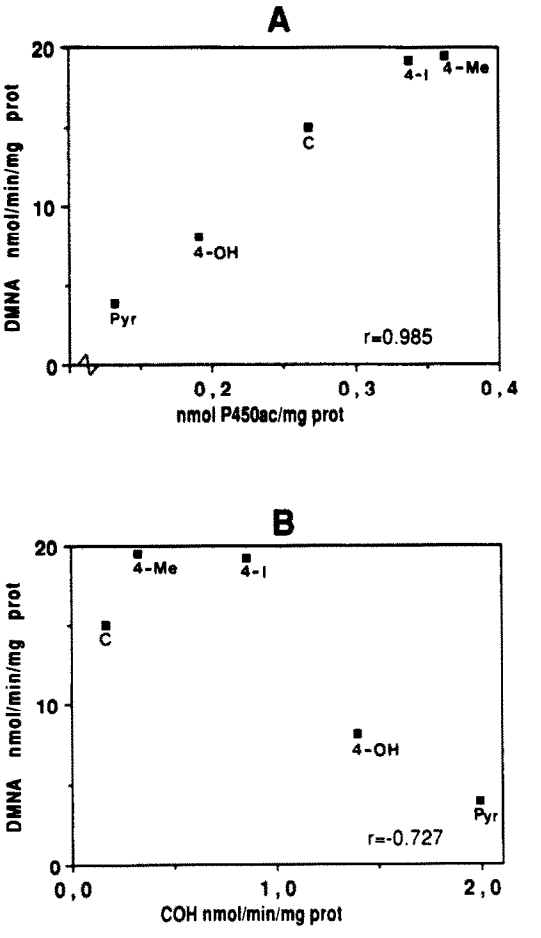

Fig. 4. (A) Correlation of liver microsomal cytochrome $P450_{ac}$ content (nmol/mg protein) and dimethylnitrosamine demethylase (DMNA) activity (nmol/min/mg protein). (B) Correlation of dimethylnitrosamine demethylase (DMNA) and coumarin 7-hydroxylase (COH) activity (nmol/min/mg protein). Pooled liver microsomes (three samples) of control (C), pyrazole- (Pyr), 4-hydroxy- (4-OH), 4-methyl- (4-Me) and 4-iodopyrazole (4-I)-treated DBA/2N mice were used. Mice were treated as follows: pyrazole (200 mg/kg), 4-hydroxy- and 4-methylpyrazole (50 mg/kg) and 4-iodopyrazole (125 mg/kg) i.p six times every 12 hr.

Table 2. Immunoinhibition of coumarin 7-hydroxylase (COH) and 7-ethoxycoumarin *O*-deethylase (ECDE) activities by anti-$P450_{Coh}$ antibody in liver microsomes from control-, 4-hydroxy- (4-OH), 4-methyl- (4-Me) and 4-iodopyrazole (4-I) treated DBA/2N (D2) mice

	Mono-oxygenase reaction	
Microsomes	COH	ECDE
Control	97.9	91.3
4-OH	97.0	99.2
4-Me	94.2	80.4
4-I	98.6	94.6

Pools of microsomes from four livers were used for the determinations. The values indicate the percentage inhibition in the presence of antibody.

liver microsomes and, like in rats, is mainly responsible for the DMNA activity [29]. As shown in Fig. 4A there is indeed a high correlation between the amount of immunorecognizable $P450_{ac}$ and the DMNA activity in mouse liver suggesting similar catalytic properties between the rat and mouse $P450_{ac}$. Contrary to rats, however, pyrazole does not seem to induce this enzyme in mouse but rather decreases it (Fig. 4A). Furthermore Fig. 4B shows that there is no correlation between COH and DMNA activities. In our earlier work we have shown that the $P450_{ac}$ and $P450_{Coh}$ have essentially different structures and do not cross-react immunologically [29].

These data suggest that mouse and rat $P450_{ac}$ are differently regulated. And further, that the regulation of mouse $P450_{Coh}$ is different from mouse $P450_{ac}$.

CONCLUSION

In conclusion we propose that pyrazole and some of its derivatives are strong and selective inducers of mouse liver coumarin 7-hydroxylase. The selectivity of the induction and the unique mode of regulation of the $P450_{Coh}$ amongst the P450s is demonstrated by the fact that even the $P450_{15\alpha}$ belonging to the same subfamily as the $P450_{Coh}$, and the $P450_{ac}$, induced in rats by pyrazole are induced differently from the $P450_{Coh}$. The mechanism of action of the inducers remains largely unknown and requires further study. It seems though that at least some of the compounds act by increasing the amount of the mRNA. Furthermore, for the potency of the inducers the nature of the substitute at the 4-position of the pyrazole molecule seem to be critical.

Acknowledgements—The financial support from the Academy of Finland Medical Research Council (Research Contract 04/320), the North Savo Fund of the Finnish Cultural Foundation and The Finnish Cultural Foundation is acknowledged.

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