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Effect of pyrazole, cobalt and phenobarbital on mouse liver cytochrome *P*-450 2a-4/5 (Cyp2a-4/5) expression

Birger HAHNEMANN,*‡ Pirkko SALONPÄÄ,* Markku PASANEN,* Jukka MÄENPÄÄ,* Paavo HONKAKOSKI,† Risto JUVONEN,† Matti A. LANG,†§ Olavi PELKONEN* and Hannu RAUNIO*‖

*Department of Pharmacology and Toxicology, University of Oulu, SF-90220 Oulu, and †Department of Pharmacology and Toxicology, University of Kuopio, SF-70220 Kuopio, Finland

Pyrazole, cobalt and phenobarbital increase the activity of coumarin 7-hydroxylase (COH) in mouse liver. To study the mechanism of this increase, we measured the expression of the cytochrome *P*-450 2a-4/5 (Cyp2a-4/5) complex, which mediates testosterone 15α-hydroxylase and COH activities, as a function of dose and time after the treatment of C57BL/6 (B6) and DBA/2 (D2) male mice with the inducers. COH activity and Cyp2a-4/5 steady-state mRNA levels were increased in both strains in response to the inducers. No marked effect occurred with testosterone 15α-hydroxylase or activities associated with Cyp1a-1 or Cyp2e-1. A 2–7-fold increase in response to the inducers was seen in the amount of *P*-450Coh (cytochrome *P*-450 isoenzyme catalysing coumarin 7-hydroxylation) protein in Western immunoblots. PCR amplification of a 1 kb region in Cyp2a-4/5-mRNA-derived cDNA, followed by cutting at the diagnostic *Pst*I site, showed that most of the steady-state mRNA consisted of Cyp2a-5, which is also the form most affected by pyrazole. Nuclear run-off analysis revealed no increase in the transcription rate of Cyp2a-4/5 after pyrazole or cobalt treatment, whereas a 2–3-fold increase occurred after phenobarbital pretreatment in B6 mice. Together with previous reports [Aida & Negishi (1991) Biochemistry **30**, 8041–8045], the current data suggest that both pyrazole and cobalt increase COH catalytic activity by affecting Cyp2a-5 by post-transcriptional mechanisms in mice.

INTRODUCTION

The cytochrome *P*-450 multigene superfamily encodes a considerable number of isoforms catalysing the metabolism of numerous exogenous and endogenous compounds (Adesnik & Atchison, 1986; Nebert & Gonzalez, 1987). A typical feature of xenobiotic-metabolizing *P*-450s is their inducibility by a large number of different agents, each showing a typical pattern of response in terms of specific isoenzymes and activities induced (Conney, 1982; Okey, 1990). In many cases increased transcription is the cause of increased enzyme activities, although other mechanisms also exist (Gonzalez, 1989).

Earlier work has demonstrated that coumarin 7-hydroxylase (COH) activity is increased in mouse liver by widely disparate types of compounds, including phenobarbital (Wood & Conney, 1974; Wood, 1979), 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) (Raunio *et al.*, 1988*a*), pyrazole and other heterocyclic compounds (Juvonen *et al.*, 1985, 1987; Hahnemann *et al.*, 1989), and some metals such as cobalt and indium (Legrum *et al.*, 1979; Legrum & Netter, 1980; Mangoura *et al.*, 1989). This last finding is in apparent contrast with early reports showing that heavy metals usually suppress the ability of the *P*-450 system to metabolize xenobiotics by decreasing the amount of *P*-450, probably by interfering with haem metabolism (Maines, 1984). Moreover, pyrazole and several other nitrogen-containing heterocyclic compounds often decrease *P*-450-associated activities other than COH in the mouse (Juvonen *et al.*, 1987).

In a recent study (Kozer *et al.*, 1991), we showed that COH activity was increased and the amount of steady-state hepatic *P*-450Coh (isoenzyme catalysing coumarin 7-hydroxylation; Cyp2a-5) mRNA was increased following cobalt and pyrazole treatment in DBA/2 and C57BL/6 mice. In the present paper, we have studied the molecular mechanisms involved in the observed selective induction of COH activity.

MATERIALS AND METHODS

Chemicals and reagents

Coumarin, 7-hydroxycoumarin, ethoxyresorufin and pyrazole were from Sigma (St. Louis, MO, U.S.A.). Phenobarbital sodium was purchased from the University Apothecary (Helsinki, Finland). Nucleic-acid-grade agarose, DNA and RNA molecular size markers were from Pharmacia (Uppsala, Sweden). Guanidine thiocyanate was from Fluka (Buchs, Switzerland). ^{32}P-labelled nucleotides were obtained from NEN–Du Pont (Wilmington, DE, U.S.A.). [4-^{14}C]Testosterone was obtained from Amersham. Restriction enzymes were purchased from Boehringer Mannheim. All reagents used were of the highest quality available from the sources cited.

Treatment of animals

Adult male C57BL/6N and DBA/2N mice (body weight about 25 g) were used throughout the study. We chose to experiment with two mouse strains genetically differing at the *Coh* locus governing the constitutive level of COH activity. The DBA/2 strain has been shown to have a high COH activity, whereas the C57BL/6 strain exhibits low constitutive activity (Wood, 1979; Juvonen *et al.*, 1987; Lang *et al.*, 1989). In dose

Abbreviations used: COH, coumarin 7-hydroxylase; EROD, ethoxyresorufin *O*-de-ethylase; DMNAD, dimethylnitrosamine *N*-demethylase; P-450$_{15\alpha}$, mouse cytochrome *P*-450 isoenzyme catalysing testosterone 15α-hydroxylation; *P*-450Coh, mouse *P*-450 isoenzyme catalysing coumarin 7-hydroxylation; DTT, dithiothreitol; TCPOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene. In the current *P*-450 gene nomenclature (Nebert *et al.*, 1991), mouse Cyp2a-4 encodes for a *P*-450 isoform catalysing testosterone 15α-hydroxylation and the Cyp2a-5 product catalyses coumarin 7-hydroxylation. p15α-15 is the trivial name for Cyp2a-5 cDNA (Squires & Negishi, 1988).

‡ Permanent address: Department of Pharmacology and Toxicology, University of Marburg, Marburg, Germany.

§ Current address: IARC, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France.

‖ To whom correspondence and requests for reprints should be addressed.

range experiments, $CoCl_2$ (50, 125 and 250 μmol/kg, subcutaneous) and pyrazole (50, 100 and 200 mg/kg, intraperitoneal) were given as single injections. As a reference inducer, phenobarbital was injected intraperitoneally at a dose of 80 mg/kg. The mice were fasted overnight before killing.

Preparation of microsomes and mono-oxygenase assays

Livers from mice were either used individually or pooled. The microsomal fraction was prepared by differential centrifugation. Microsomal protein content was determined by the method of Bradford (1976). Cytochrome *P*-450 content was determined as originally described by Omura & Sato (1964). COH activity was measured by the method of Aitio (1978), using 100 μM-coumarin as substrate. Ethoxyresorufin *O*-de-ethylase (EROD) activity was assayed using the end-point method of Burke *et al.* (1977), with 1 μM-ethoxyresorufin as a substrate. Dimethylnitrosamine *N*-demethylase (DMNAD) activity was assayed according to Anderson & Angel (1980). Testosterone 15α-hydroxylase activity was determined according to Waxman *et al.* (1983).

Western immunoblotting

P-450Coh was purified as described (Juvonen *et al.*, 1988). The preparation and validation of the anti-*P*-450Coh antibody has been reported earlier (Lang *et al.*, 1989). SDS/PAGE and immunoblotting were performed using the semi-dry method of Kyhse-Andersen (1984). After resolving microsomal proteins in a 10% gel and transferring on to nitrocellulose filters, the antibody–protein complex was visualized by the peroxidase reaction according to Hawes *et al.* (1982).

Preparation of cDNA probes

The cloning of Cyp2a-5 cDNA (trivial name p15α-15) has been described previously (Burkhart *et al.*, 1985; Squires & Negishi, 1988). Because of the high degree of sequence similarity between Cyp2a-4 and Cyp2a-5 DNAs (98.3%), Cyp2a-5 cDNA does not discriminate between them (Squires & Negishi, 1988). The cloning of CYP2E1 cDNA has been described (Song *et al.* 1986). As a control probe, an 18 S RNA oligonucleotide (Albretsen *et al.*, 1988) was used. The full-length cDNAs were labelled with [α-^{32}P] dCTP using the Pharmacia oligolabelling kit to a specific radioactivity of about 10^9 c.p.m./μg of DNA. The 18 S oligomeric probe was end-labelled with [γ-^{32}P]ATP.

Preparation of RNA

Livers from 3–5 mice were divided into two parts. One part was used to isolate microsomes. The other part of each liver was quickly immersed in liquid nitrogen, weighed, and pooled livers were homogenized with an Ultra-Turrax homogenizer (IKA–Laborteknik, Staufen, Germany) in guanidine thiocyanate. RNA was prepared by the CsCl centrifugation method of Chirgwin *et al.* (1979). Northern blots of total cellular RNA were prepared after electrophoresis in 1% agarose gels by blotting on to a Hybond-C Extra membrane (Amersham). The RNA was fixed by baking at 80 °C for 2 h. The filters were prehybridized for at least 2 h at 45 °C in prehybridization buffer consisting of 5 × SSPE (1 × SSPE is 0.15 M-NaCl/10 mM-NaH_2PO_4/1 mM-EDTA, pH 7.4), 5 × Denhardt's solution (1 × Denhardt's is 0.02% Ficoll/0.02% polyvinylpyrrolidine/0.002% BSA), 50% formamide, 0.1% SDS and sonicated salmon sperm DNA (100 μg/ml). The hybridization was done overnight in 5–20 ml of prehybridization buffer supplemented with 10% dextran sulphate, 5 × SSPE, 1 × Denhardt's solution, 50% formamide, 0.1% SDS and labelled Cyp2a-5 cDNA. After hybridization, the filters were washed first at room temperature (3 × 15 min) in 2 × SSC/0.1% SDS (1 × SSC is 0.15 M-NaCl/0.015 M-sodium citrate) and subsequently in 0.1 × SSC/0.1% SDS at 55 °C (3 × 15 min). The filters were exposed for various times to Hyperfilm-MP (Amersham) in Cronex cassettes with an intensifier screen at −70 °C. A Shimadzu CS-9000 dual-wavelength flying spot scanner was used for densitometric determinations.

Differentiation between Cyp2a-4 and Cyp2a-5 mRNAs

A PCR-based analysis method was used to differentiate between the highly similar Cyp2a-4 and Cyp2a-5 mRNAs. In brief, total cellular RNA was used as template for single-stranded cDNA synthesis, and 12-mer primers (5′ primer GAGCGCATCCAA; 3′ primer CACGAGTCTAGG), constructed to bind to areas just outside the *Cla*I sites in Cyp2a-4, were added to the cDNA reaction tubes with Taq polymerase. Amplification was done in 25 cycles in a Perkin–Elmer Cetus thermocycler. The amplification product (1 kb) was digested with *Pst*I, which cuts only in Cyp2a-5 cDNA, generating 710 bp and 290 bp fragments. The digested material was electrophoresed in a 1% agarose gel and blotted on to a filter, which was hydridized with a ^{32}P-labelled *Cla*I fragment of Cyp2a-4 cDNA. The films were developed after exposure of between 60 min and overnight at room temperature. The details of the method will be described elsewhere (P. Salonpää, O. Pelkonen & H. Raunio, unpublished work) and are available on application to the authors. The basis of the experimental strategy is described by Squires & Negishi (1988).

Nuclear run-off analysis

Nuclei were isolated from pooled livers according to Lakso & Negishi (1992) and stored frozen before used. A 400 μg portion of nuclei was incubated for 60 min at 28 °C in 100 μl of reaction buffer [50 mM-Hepes, pH 8.0, 16% (w/v) glycerol, 5 mM-$MgCl_2$, 150 mM-KCl, 2 mM-dithiothreitol, (DTT), 0.5 mM-ATP, -CTP and -GTP and 250 μCi of [^{32}P]UTP (3000 Ci/mmol)]. The reaction was terminated by DNAase treatment (50 μg/ml for 5 min at 30 °C). The nuclei were pelleted by brief centrifugation (10000 *g*) and lysed in TE buffer (10 mM-Tris, pH 7.4, 1 mM-EDTA) containing 0.3% SDS and 25 μg of yeast tRNA for 15 min. After extraction with phenol/chloroform (1:1, v/v), RNAs were precipitated with ethanol. The pellets were suspended in TE buffer containing 1 mM-DTT, 3.5 mM-$MgCl_2$ and 100 mM-$CaCl_2$, digested with 10 μg of DNAase/ml at 30 °C for 30 min and precipitated with ethanol. p15α-15 cDNA was immobilized on to a Hybond-C Extra membrane in a Schleicher & Schuell slot-blot apparatus. After hybridization with RNA produced by the nuclei, the filters were washed and visualized as described above.

Statistical analysis

The Kruskall–Wallis analysis of variance method (Theodorson-Norheim, 1986) was used for statistical calculations.

RESULTS

Dose–response experiments

Fig. 1 shows the dose-dependence of the effects of cobalt and pyrazole on COH activity and p15α-15 (Cyp2a-5)-hybridizable mRNA. Although the basal COH activities were lower in the B6 mice, the extent of increase by both compounds was greater in these animals. The most efficient inducing dose of cobalt was 250 μmol/kg in the D2 strain and 125 μmol/kg in the B6 strain. The decrease in COH activity in the B6 strain at the highest dose may have been due to liver injury, which was detected visually for B6 but not for D2 mice. Pyrazole gave the most efficient response at the highest dose (200 mg/kg) in both mouse strains.

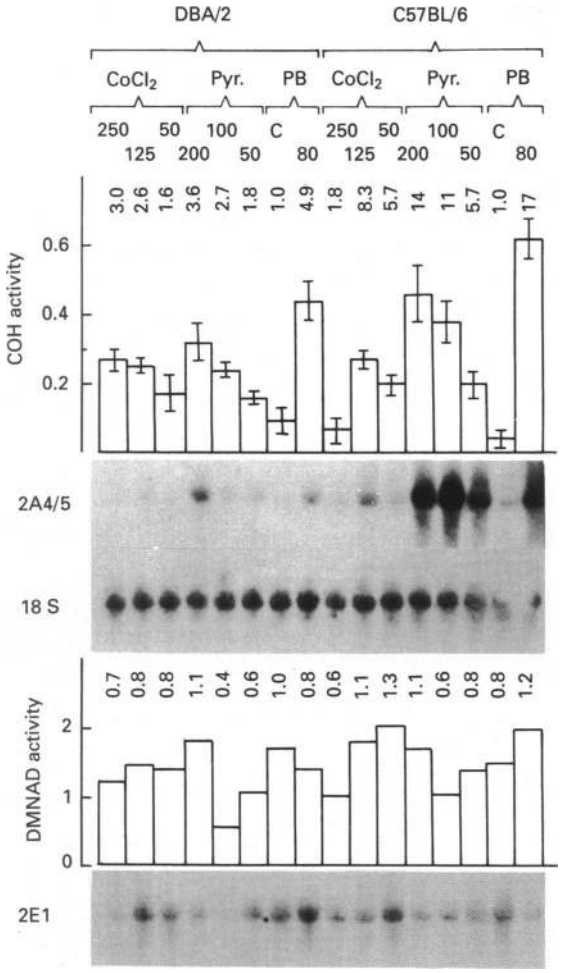

Fig. 1. Dose–response experiments

$CoCl_2$ (50, 125 and 250 μmol/kg) and pyrazole (Pyr) (50, 100 and 200 mg/kg) were administered as single injections. Phenobarbital (PB, 80 mg/kg) was used as a reference inducer. The mice were killed 24 h after the treatment. COH and DMNAD activities are in pmol of product formed/min per mg of microsomal protein. Each value represents the mean of 3–5 mice. The numbers above activity bars are fold increases relative to control. The Northern blots were hybridized with Cyp2a-4/5, CYP2E1 and 18 S oligomeric probes.

In Northern blots the elevation in the levels of Cyp2a-4/5 mRNA was more pronounced in the B6 mice than in the D2 mice (Fig. 1). Densitometric analysis showed 3.5-, 9.5- and 6.5-fold increases (relative to 18 S RNA) in the Cyp2a-4/5 mRNA level in the B6 mice after treatment with cobalt, pyrazole and phenobarbital respectively. The fold increases were greater in the D2 mice due to the very low constitutive level of the mRNA.

There was some variation in the CYP2E1 hybridization signal, with no clear dose–response relationship. No treatment increased the activity of DMNAD (Fig. 1).

Time course experiments

Single doses of the inducers were selected and the responses were monitored as a function of time. Fig. 2 shows the time courses of COH, EROD and DMNAD activities in the D2 mice. Only COH activity was appreciably induced by either pyrazole or cobalt. Fig. 3 shows the changes in the amount of Cyp2a-4/5 mRNA in the same experiment. Fig. 4 shows a similar experiment in the B6 mice. Again, the response to pyrazole was more pronounced than that to cobalt. After pyrazole treatment, the maximal mRNA level occurred at 12 h in D2 mice and 24 h in B6 mice, which explains the lower level of mRNA in the D2 RNA samples in the dose–response experiment (see Fig. 1). With cobalt the extent of increase was much smaller, with a peak at 6 h in both strains (Figs. 3 and 4). Despite these inducer and strain-dependent differences in Cyp2a-4/5 mRNA levels, the behaviour of the enzyme activities was rather similar in both strains and after both inducers.

EROD activity (Cyp1a-1-mediated) and some parameters related to Cyp2e-1 expression were also measured. No relationship with Cyp2a-4/5 expression seemed to exist (Figs. 2–4). In contrast, both pyrazole and cobalt tended to slightly decrease EROD and DMNAD activities.

Immunoblotting experiments

In immunoblots, anti-*P*-450Coh antibody revealed a protein species with a minimum molecular mass of 50 kDa (Fig. 5). The 50 kDa band co-migrated with purified *P*-450Coh and is known from previous studies to represent both $P\text{-}450_{15\alpha}$ (*P*-450 isoenzyme catalysing testosterone 15α-hydroxylation) and *P*-450Coh (Raunio *et al.*, 1988*b*; Negishi *et al.*, 1989). Densitometric analysis showed that the amount of this protein was increased 2–7-fold by the inducers.

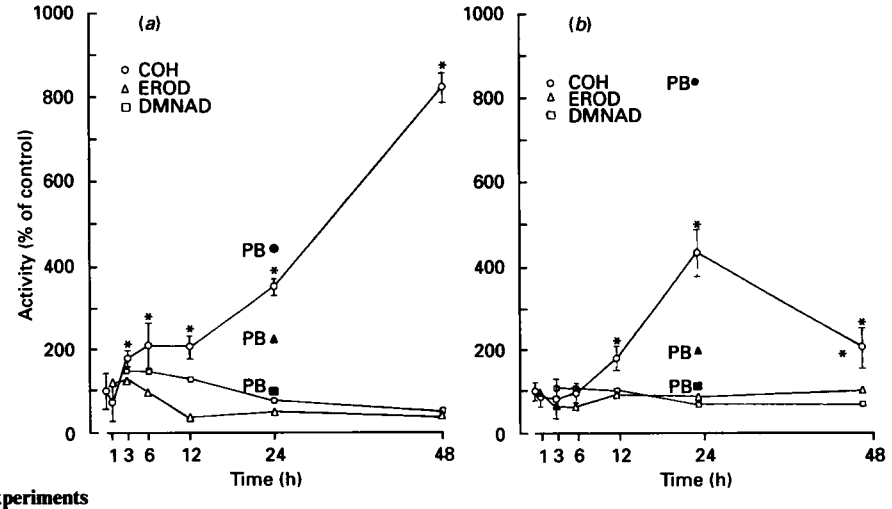

Fig. 2. Time course experiments

COH, EROD and DMNAD activities were determined at different time points after the administration of (*a*) pyrazole (200 mg/kg) or (*b*) cobalt (125 μmol/kg) to D2 mice. Phenobarbital (PB) was given at a dose of 80 mg/kg. The COH value at each time point represents the mean from at least three animals; the bars represent S.E.M. A significant difference versus the control sample ($P < 0.05$) is denoted by *. EROD and DMNAD activities were assayed from pooled samples.

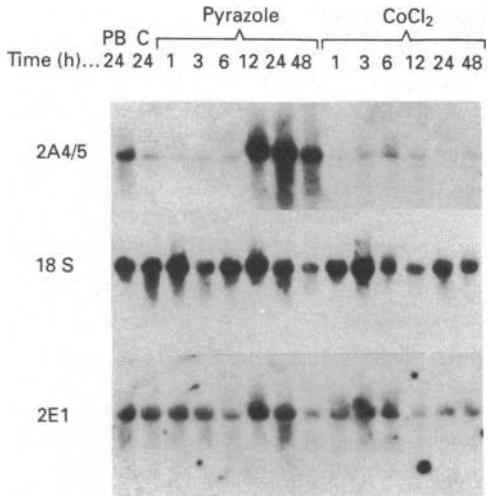

Fig. 3. Northern blot analysis in D2 mice

Total RNA was isolated from D2 mouse liver at different time points after the administration of single doses of cobalt (125 μmol/kg) or pyrazole (200 mg/kg). The blots were hybridized with the indicated probes. PB, phenobarbital-treated; C, control.

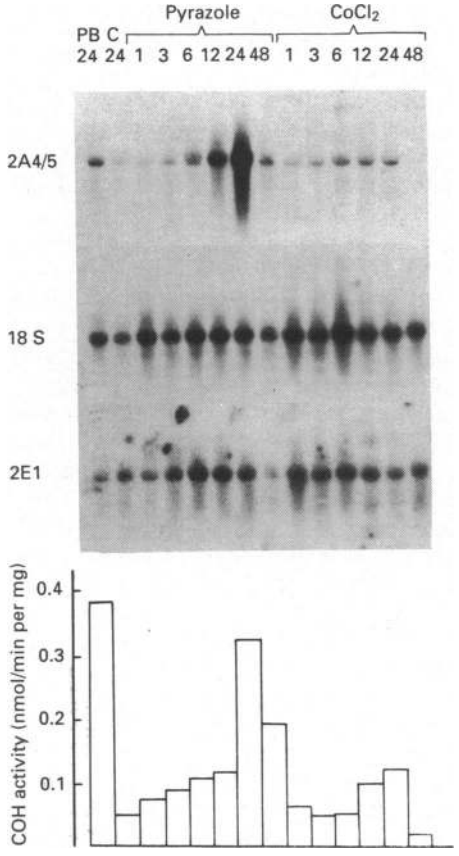

Fig. 4. Northern blot analysis in B6 mice

Total RNA was isolated from B6 mouse liver at different time points after the administration of single doses of cobalt (125 μmol/kg) or pyrazole (200 mg/kg). The corresponding COH activity is shown in the bar graph. PB, phenobarbital-treated; C, control.

Table 1 gives a summary of the changes in COH catalytic activity and the respective mRNA and protein contents at optimal inducing doses. There was generally good agreement between changes in these parameters, especially in the D2 mice.

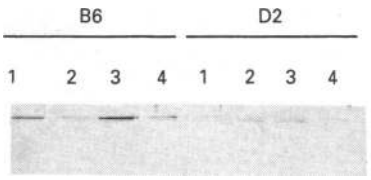

Fig. 5. Western blot analysis

P-450Coh/*P*-450$_{15\alpha}$ proteins were detected in mouse liver microsomes after treatment with pyrazole (lane 1), cobalt (2), phenobarbital (3) and vehicle only (4). A 15 μg sample of microsomal protein was applied to each lane. After visualization of the antibody–antigen complex by the peroxidase reaction, relative concentrations of the proteins were estimated by densitometry.

Table 1. Summary of inducer-produced changes in COH activity and mRNA and protein content

The agents were administered as single injections of optimal inducing doses. The numbers denote fold increases relative to the values in control mice. The mRNA values are related to the 18 S RNA loading controls. The values are from mice killed 24 h after treatment, except the mRNA values of cobalt-treated mice, which were obtained at the peaks *6 h and †12 h after the treatment. PB, phenobarbital.

	Increase (fold)					
	DBA/2			C57BL/6		
Parameter	Cobalt	Pyrazole	PB	Cobalt	Pyrazole	PB
COH activity	3.0	3.6	4.9	8.3	14	17
mRNA content	1.8*	4.3	3.3	4.0†	6.2	2.8
Protein content	3.0	4.0	7.4	0.7	1.3	2.3

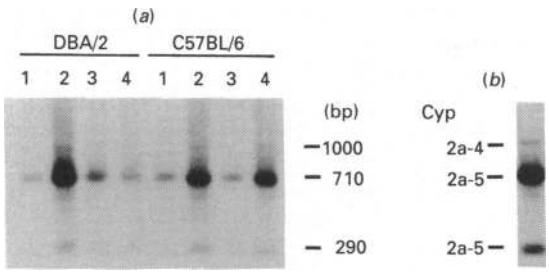

Fig. 6. Effect of the inducers on the relative contribution of Cyp2a-4 and Cyp2a-5

Single-stranded cDNAs were prepared from total RNA isolated from the livers of D2 and B6 mice. A 1000 bp fragment comprising the *Cla*I–*Cla*I region of Cyp2a-4/5 was amplified by PCR. The amplified product was cut with *Pst*I, which is unique for Cyp2a-5. The digested DNA was electrophoresed and blotted on to a nylon membrane which was hybridized with ^{32}P-labelled *Cla*I fragment of Cyp2a-5 (p15α-15) cDNA. Uncut Cyp2a-4 appears as a 1000 bp band and *Pst*I-cut Cyp2a-5 as 710 bp plus 290 bp bands. Panel (*b*) is an overnight exposure illustrating the relative intensities of the bands in a control animal (DBA/2 strain). Panel (*a*) shows the effect of treatments on the relative proportion of Cyp2a-4 and 4a-5: lane 1, control; 2, pyrazole treatment; 3, cobalt treatment; 4, phenobarbital treatment.

In the B6 mice the large increases in COH activity were not entirely reflected at the mRNA and protein levels, which may be due to the low constitutive COH activity in this strain.

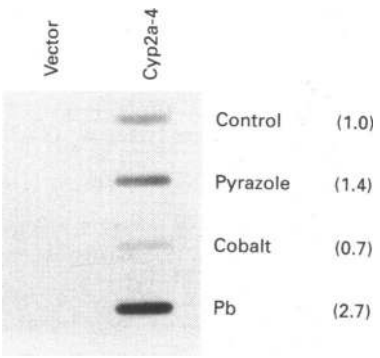

Fig. 7. Nuclear run-off analysis

B6 mouse liver nuclei were isolated 6 h after single injections of cobalt chloride (125 μmol), pyrazole (200 mg/kg) or phenobarbital (PB; 80 mg/kg) and processed as described. Hybridization of the transcripts with vector and Cyp2a-5 cDNA are shown. The relative densitometric values are shown in parentheses.

Table 2. Effects of cobalt, pyrazole and phenobarbital on testosterone 15α-hydroxylase activity

Testosterone 15α-hydroxylase assays were done with microsomal fractions prepared from pooled livers (3–5 mice) treated for 24 h with vehicle, cobalt, pyrazole or phenobarbital. The fold inductions are shown in parentheses.

	Activity (pmol/min per mg of protein)			
Strain	Control	Cobalt	Pyrazole	Phenobarbital
DBA/2	177 (1.0)	166 (0.9)	140 (0.8)	214 (1.2)
C57BL/6	85 (1.0)	133 (1.6)	130 (1.5)	269 (3.2)

Measurement of relative Cyp2a-4 and Cyp2a-5 mRNA levels

To study the relative contributions of Cyp2a-4 and Cyp2a-5 to the total Cyp2a-5-hybridizable mRNA, a PCR-enhanced diagnostic analysis based on the method of Squires & Negishi (1988) was used. In this method the two isoforms are visualized as a 1 kb band (Cyp2a-4) or as a 710 bp plus a 290 bp band (Cyp2a-5). As shown in Fig. 6(*b*), Cyp2a-5 was the predominant component in untreated control mice, representing about 90% of the total Cyp2a-4/5 mRNA. Treatment with the inducers affected mainly Cyp2a-5 (Fig. 6*a*). Although the PCR method used cannot be considered to be strictly quantitative, the fold increases between samples generally agreed well with those obtained in Northern blots.

Nuclear run-off analysis

Fig. 7 shows the changes in the transcription of Cyp2a-4/5 after treatment of B6 mice for 6 h with optimal doses of the inducers. Pyrazole and cobalt did not appreciably affect transcription, but phenobarbital increased the rate 2–3-fold. The experiment was repeated several times using actin cDNA as a reference, with similar results. Phenobarbital decreased the actin transcription rate by up to 50%. It is of interest that TCPOBOP, a potent phenobarbital-type *P*-450 inducer in mice, also suppressed actin transcription (results not shown).

Effects on testosterone 15α-hydroxylase activity

Although *P*-4502a-4 and 2a-5 are very similar (98.3% identity at the amino acid level), they have distinct substrate specificities with *P*-4502a-4 catalysing testosterone 15α-hydroxylation and *P*-4502a-5 catalysing coumarin 7-hydroxylation (Negishi *et al.*, 1989; Lindberg *et al.*, 1989). To elucidate whether cobalt and pyrazole affect also *P*-4502a-4, testosterone 15α-hydroxylase activity was assayed (Table 2). Relatively small changes were observed in this activity after cobalt or pyrazole pretreatments, and even in B6 animals the increase in testosterone 15α-hydroxylase activity was much smaller than the increase in COH activity. Phenobarbital seemed to be a more potent inducer of this activity than either cobalt or pyrazole.

DISCUSSION

The present data show that cobalt chloride, pyrazole and phenobarbital cause an elevation in the amount of hepatic Cyp2a-5 mRNA, an increase in the amount of *P*-450Coh protein, and an increase in *P*-450Coh-mediated COH catalytic activity in D2 and B6 mice, with strain- and dose-dependent differences in the inducibility.

The cDNA probe (p15α-15) and antibody (anti-*P*-450Coh) used in this study do not distinguish between the very similar (98.3% identity) Cyp2a-4 and Cyp2a-5 mRNAs and proteins. Cyp2a-5 is, however, the protein that is primarily affected by cobalt and pyrazole. First, using a diagnostic restriction endonuclease digestion analysis (Squires & Negishi, 1988), it has been shown previously that pyrazole causes only a modest increase in Cyp2a-4 mRNA in male mice, while potently increasing Cyp2a-5 mRNA expression (Negishi *et al.*, 1989). This is supported by the PCR-enhanced diagnostic analysis used in the present study: in both D2 and B6 mice the constitutive level of Cyp2a-5 greatly exceeded that of Cyp2a-4. Treatment with the inducers preferentially increased Cyp2a-5. Secondly, testosterone 15α-hydroxylase, the activity catalysed by *P*-4502a-4, is increased only marginally by cobalt or pyrazole. Therefore only a minor part of the increased expression of Cyp2a-4/5 by cobalt and pyrazole can be ascribed to Cyp2a-4.

Earlier studies suggested that the inductive properties of cobalt resembled those of pyrazole (Legrum *et al.*, 1979; Legrum & Netter, 1980; Juvonen *et al.*, 1985, 1987). Based on the data in this paper there are also some important differences in the effects of these two compounds. First, pyrazole treatment led to a much more pronounced increase in mRNA levels than did cobalt pretreatment. This increase did not correlate fully with the increases in the amount of the *P*-450Coh protein or in the COH activity. Secondly, the effects of pyrazole and cobalt seemed to be somewhat different in the two mouse strains studied.

This and previous studies have revealed many differences in the regulation of Cyp2a-4/5 expression among different inbred mouse strains. The present nuclear run-off data indicate that, in B6 male mice, phenobarbital increases the transcription rate of Cyp2a-4/5, whereas pyrazole and cobalt act by some post-transcriptional mechanism. Aida & Negishi (1991) showed recently that neither pyrazole nor phenobarbital affect the transcription of Cyp2a-4/5 in D2 male mice. They suggested that the levels of Cyp2a-5 mRNA and COH activity are increased by mechanisms involving message stabilization after pyrazole treatment and increased translational efficiency or stability of *P*-450Coh protein after phenobarbital treatment (Aida & Negishi, 1991).

It is of interest to compare the effects of cobalt and pyrazole with those of two other inducers of COH in the mouse, i.e. phenobarbital and TCPOBOP. Although both of these compounds increase COH activity in the same way as cobalt or pyrazole, they also markedly induce the activity of pentoxyresorufin *O*-de-ethylase (Raunio *et al.*, 1988*a*), an activity that is

considered to be diagnostic for phenobarbital-inducible *P*-450 isoenzymes (Burke *et al.*, 1985). This property does not appear to be characteristic of cobalt or pyrazole, which in fact decrease pentoxyresorufin *O*-de-ethylase activity. It is worth noting that all of these structurally widely varying compounds share COH induction as a part of their variable spectrum of effects. The mechanistic elucidation of this phenomenon should also illuminate the induction mechanism of phenobarbital, a problem that remains unsolved after more than 20 years of research.

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