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COMPARISON OF HAMSTER AND MOUSE REVEALS INTERSPECIES DIFFERENCES IN THE REGULATION OF HEPATIC CYP2A ISOZYMES

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Abstract—Three CYP2A-related activities [coumarin 7-hydroxylase (COH), testosterone 7α- (test7α) and 15α-hydroxylases (test15α)], identified in hamster liver and analysed by immunoinhibition, and western and northern blotting, were found to be similar to mouse and human CYP2As. In the microsomal fractions, anti-mouse CYP2A5 antibody recognised three bands of about 48, 49 and 52 kDa, suggesting the presence of at least three proteins immunologically similar to mouse CYP2A5. The 49 kDa band migrated close to mouse CYP2A5 and changes in its expression followed COH and test15α activities. Test7α activity did not associate with any of the individual bands detected on western blots despite its strong inhibition by the antibody. Despite the immunological and catalytic similarities between mouse and hamster CYP2A enzymes, their regulation is different. In mice, the enzyme activities are higher in females than males, are induced by pyrazole (PY) and phenobarbital (Pb), and are not affected by 3-methylcholanthrene (MC). In hamsters, activities are not higher in females, induced by MC and reduced by PY. MC and PY appear to regulate expression at the mRNA level, while Pb seems to act post-transcriptionally by increasing either the synthesis or the stability of the protein. Our data indicate that the modes of expression and regulation of CYP2A-related enzymes make the hamster different from mice and humans with respect to the mechanism of metabolism of certain drugs and carcinogens.

The cytochrome P450 (CYP‡) system, the principal catalyst for xenobiotic oxidations, has been quite well characterized in rats, mice and humans. However, the enzymatic basis of drug and carcinogen activation in hamsters is less well understood although this species is widely used, particularly in studies on carcinogens [1–3]. Understanding the enzymatic basis of drug oxidation is important for studies on drug and carcinogen metabolism, and for interspecies comparisons, which are often required in toxicity testing and in the extrapolation of animal data to humans.

Recently, several reports have suggested the importance of enzymes of the CYP2A subfamily in xenobiotic metabolism [4–8]. It appears that certain members of this sub-family have an exceptional mode of regulation compared to several other CYPs and show important interspecies variation in their expression. For example, the expression of Cyp2a-4 and 2A-5 is strongly stimulated by certain hepatotoxins such as pyrazole (PY) and cobalt in mice [9, 10]. Furthermore, CYP2A6/2A-5 is highly expressed in humans and mice with wide inter-individual variation, but is absent in rat liver [11].

Since CYP2A5 and possibly other members of this subfamily activate well-known carcinogens such as nitrosamines and aflatoxin B_1 [7, 8], the high degree of variation in their expression may contribute to interspecies and interindividual differences in the toxic response to these chemicals.

Very little is known about CYP2As in hamsters, apart from one report by Fukuhara *et al.* [12] suggesting that this species has a CYP isozyme 73% homologous to mouse CYP2A-4. It is not known how the catalytic properties of this enzyme relate to mouse or human CYP2As. Furthermore, the hamster enzyme is inducible by 3-methylcholanthrene (MC), while mouse CYP2A-4 is not, which suggests differences in regulation between mice and hamsters.

We now report on hamster liver enzymes orthologous to mouse and human CYP2As, indicating that hamster does indeed have enzymes similar to CYP2As. The unique profile of the enzymes and their regulation make the hamster different from the two other species.

MATERIALS AND METHODS

Chemicals. These were obtained from following sources: coumarin, MC, metyrapone, testosterone, bicinchonic acid, anti-rabbit IgG conjugated with alkaline phosphatase and 5-bromo-4-chloro-3-indolyl phosphate (Sigma Chemical Co., St Louis, MO, U.S.A.); phenobarbital (Pb) and corn oil (Yliopiston Apteekki, Helsinki, Finland); pyrazole (PY) (E.

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‡ Abbreviations: CYP, cytochrome P450; CYP2A, cytochromes P450 of the 2A family; COH, coumarin 7-hydroxylase, a CYP2A isozyme highly active in the 7-hydroxylation of coumarin (CYP2A6 in humans; CYP2A-5 in mice); test15α, testosterone 15α-hydroxylase, a CYP2A isozyme highly active in the 15α-hydroxylation of testosterone (CYP2A? in humans; CYP2A-4 in mice); test7α, testosterone 7α-hydroxylase, a CYP2A isozyme highly active in the 7α-hydroxylation of testosterone; Pb, phenobarbital; MC, 3-methylcholanthrene; PY, pyrazole.

Merck Ag, Darmstadt, Germany); NADPH (Boehringer Mannheim GMBH, Germany); [4-^{14}C]-testosterone (54.5 mCi/mmol; Amersham, U.K.); nitrocellulose BA 83 sheets (Schleider & Schnell, Feldbach, Switzerland). All other chemicals were of the highest grade commercially available.

Production of a polyclonal antibody against CYP2A-5 and validation of its specificity by immunoinhibition and the immunoblotting technique have been described before [5,10]. Cloning of cDNA for mouse CYP2A-5 and its use in northern blot analysis for CYP2As have been described previously [13].

Treatment of animals and preparation of microsomes. Male and female (8–10 weeks old) Syrian golden hamsters were obtained from Charles River Laboratories (Boston, MA, U.S.A.). Hamsters were given PY in saline (100 mg/kg), MC in corn oil (25 mg/kg) or Pb in saline (50 mg/kg) as single i.p. injections for three consecutive days. Control animals received only vehicle. During the treatment the animals received water and pelleted food (Hankkija Ltd, Finland) *ad lib.* and were killed 24 hr after the last injection by decapitation. Livers were quickly removed and divided into two parts. The first part was frozen quickly in liquid nitrogen and stored in −80° for RNA preparation. The microsomal fraction from the second part was isolated according to Lang and Nebert [14].

Mono-oxygenase assays. Microsomal protein and CYP contents were determined by the methods of Smith *et al.* [15] and Omura and Sato [16], respectively. Coumarin 7-hydroxylase (COH) activity was measured according to Juvonen *et al.* [4] and testosterone hydroxylase activities as described by Waxman *et al.* [17].

Immunoinhibition of mono-oxygenase activities. After preliminary experiments to find conditions for maximal inhibition, immunoinhibition of COH and testosterone 15α- and 7α-hydroxlase (test15α and test7α) activities were performed by adding anti-CYP2A-5 antibody (in a ratio of 5 mg IgG to 1 mg microsomal protein) to the reaction mixtures 10 min before the start of the reaction by NADPH. Otherwise the assays were carried out as described above. Protein concentration was held constant by adding preimmune IgG.

Other inhibition studies. COH (100 μM) and test15α and -7α (100 μM) were inhibited with the respective substrates and metyrapone (5–1000 μM). The compounds were added to the reaction mixtures in incubation buffers immediately before starting the reactions.

SDS–PAGE and western blot analysis. Electrophoresis of the microsomes was run in 9% acrylamide gels (30 mA/gel) according to Laemmli [18] and the proteins were transferred onto nitrocellulose sheets according to Towbin *et al.* [19]. A 1:300 dilution of anti-CYP2A5 antibody was used to detect the immunorecognisable proteins.

Preparation of RNA and northern blots. Pieces of livers were pooled for each group and subsequently weighed and homogenized in guanidine thiocyanate. RNA was prepared by Chomczynski and Sacchi [20]. Northern blots were performed by standard procedures [21], except that 2% agarose gel was used. The filter was hybridized with a cDNA probe of mouse CYP2A-5 as described previously [22] with the following minor modifications. After electrophoresis RNA was blotted on to a Hybond-C Extra membrane (Amersham). The RNA was fixed by baking at 80° for 2 hr. The filters were prehybridized for at least 2 hr at 45° 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° (3 × 15 min). The filters were exposed to Hyperfilm-MP (Amersham) in Cronex cassettes with an intensifier screen at −70°.

RESULTS

Liver microsomal enzyme activities related to the CYP2A subfamily

From previous studies, it was known that COH, test15α and test7α activities are catalysed by different CYP2A isozymes, at least in mice and humans [22, 23]. Therefore, these activities were chosen as indicators for CYP2As in hamster liver. Table 1 shows the three activities in females and males and their response to three inducers. The basal activity of COH seems to be somewhat higher in males than females and is increased by MC and Pb. PY, in contrast, decreases the activity.

The COHs of the hamster and DBA/2 mouse, differ in at least three respects (Table 1). First, the female predominance of COH expression in mice is reversed in hamsters; second, COH is MC-inducible only in the hamster; third, PY strongly induces mouse COH but inhibits it in hamsters to 10–15% of control.

In the hamster, test15α activity was higher than that of COH, but regulation of these two activities, in relation to both sex and inducers, seems to be similar, although changes in test15α were not as great as in COH. Furthermore, sex differences were not as obvious for test15α as for COH. On the other hand, test7α activity, which in uninduced animals is much higher than the other two activities, seems to be differently regulated since the three "inducers" decrease it to various extents (Table 1).

Kinetic analysis (data not shown) of the microsomal activities gave one apparent K_m value for each in about the same concentration range (Table 1). Within the range given the K_m values were the same for all microsomal preparations.

Western blot analysis

Western blot analysis of hamster liver microsomes by anti-mouse CYP2A5 antibody revealed three

Table 1. COH, test15α and test7α activities, and K_m values of hamster liver microsomes of untreated males and females, and after MC, Pb and PY treatments

Activity	Sex	Control	MC	Pb	PY	K_m (μM)
COH	F	42 ± 6.5	158 ± 9.8*	180 ± 15.5*	5.5 ± 1.5*	2.5–6.0
	M	68 ± 7.3	141 ± 20.5*	245 ± 30.8*	7.8 ± 2.3*	3.0–10
Test15α	F	406 ± 85	1080 ± 140*	550 ± 110	200 ± 50	5–8
	M	508 ± 82	1010 ± 200†	930 ± 250†	340 ± 60	7–20
Test7α	F	3080 ± 610	1210 ± 140*	1110 ± 200*	1420 ± 230*	5–7
	M	3360 ± 220	660 ± 90*	1840 ± 80*	1590 ± 220*	5–10
COH of DBA2 liver	F	400 ± 45	350 ± 20	1050 ± 80	1230 ± 170	2–5
	M	170 ± 15	120 ± 10	1230 ± 150	950 ± 130	2–5

Activities are means ± SD of six individuals as pmol/mg protein × min. For comparison, coresponding activities of COH in DBA2 mouse liver are shown.

K_m values were obtained by using pools of microsomes of each group.

Scheffe's F-test was used to analyse the statistical significance of the results. Controls vs treated: *$P \leq 0.001$, †$P \leq 0.01$.

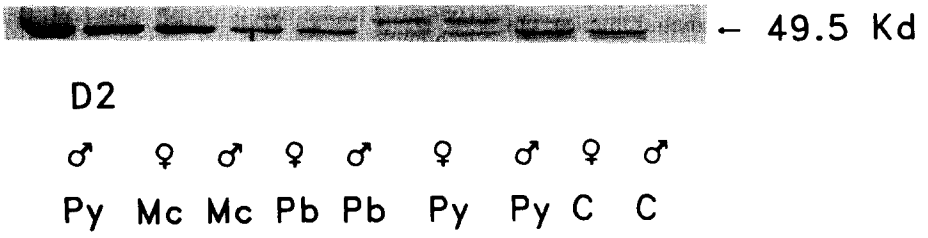

Fig. 1. Western blot analysis of female and male hamster liver microsomes from control (C) animals and after treatment with MC, Pb or PY. Twenty micrograms of microsomal protein was applied to the wells and probed with anti-CYP2A5 antibody as described in Materials and Methods. For comparison, microsomes from PY-treated D2 mouse livers were included. Pools from six animals were used.

bands: one minor with an apparent molecular weight of 48 kDa, one sharp major band of approximately 49 kDa and one of about 52 kDa (Fig. 1). This suggests that immunologically similar proteins to those of mouse CYP2A5 are present in hamster liver. The intensity of the 49 kDa band is increased slightly by Pb, strongly by MC and reduced by PY; in contrast, the 52 kDa band is increased by PY and reduced by MC (Fig. 1), suggesting different regulation of the two proteins. When changes in the intensities of the bands are compared to the enzyme activities, it appears that COH and test15α associate with the major 49 kDa band in that all are increased by MC and reduced by PY. On the other hand, changes in test7α activity do not seem to associate with any band.

Immunoinhibition of COH, test15α and test7α activities by anti-mouse CYP2A5 antibody

A strong inhibition of COH activity was found in all groups, suggesting immunological similarities between the mouse and hamster enzymes. Test15α was also inhibited, particularly after induction by MC or Pb. A strong inhibition of test7α was seen in all groups, which was somewhat unexpected since test7α activity was not associated with any band on the western blots (Table 2).

Table 2. Immunoinhibition of hamster liver COH, test15α and test7α activities

Activity	Sex	Control	MC	Pb	PY
COH	F	1.4 (46)	4.3 (173)	1.3 (165)	4.8 (5.2)
	M	1.9 (77)	5.2 (157)	1.0 (235)	15.0 (8.2)
Test15α	F	32 (380)	30 (981)	19 (490)	49 (190)
	M	28 (520)	10 (1200)	11 (870)	41 (330)
Test7α	F	11 (2800)	4 (1540)	16 (1050)	15 (1290)
	M	7 (3100)	10 (620)	15 (1690)	9 (1430)

Remaining activities as percentage of control value (= 100) are shown.

Values in brackets are the non-inhibited enzyme activities as pmol/mg protein × min when determined in the presence of the corresponding amount of preimmune serum. Preliminary experiments were carried out to find out the amount of antibody giving maximal inhibition.

Pools of microsomes from six individuals were used and the values are means of three experiments.

Inhibition of COH, test15α and test7α by metyrapone, and test15α and test7α by coumarin

The IC_{50} values of metyrapone for COH, test15α and test7α were 5, 30 and >150 μM, respectively. The values were essentially the same in control and treated hamsters and for males and females. A strong inhibition ($IC_{50} \sim 10$ μM) of test15α by coumarin was found in all groups, except after PY treatment. By comparison, test7α was inhibited only weakly or not at all, with the notable exception of male hamsters after MC treatment, in which the inhibition was almost as strong as in the case of test15α ($IC_{50} = 15$ μM) (data not shown).

Despite the strong inhibition of test15α by coumarin, testosterone had no effect on COH. This is in accordance with our previous observations with mice [22].

Taken together, our data suggest that hamster liver contains enzymes immunochemically and catalytically similar to the CYP2As of mice and men. The 49 kDa band on western blots seems to associate with hamster liver test15α and COH activities, and has a molecular weight almost identical to those of mouse CYP2A4 and CYP2A5, which are responsible for the corresponding enzyme activities in mice. On the other hand, test7α was not associated with any of the bands recognised on western blot, and changes in the 52 kDa band did not follow any of the three enzyme activities. It is possible therefore that testosterone 7α-hydroxylation is catalysed by one or more enzymes which may not be revealed on western blotting, and that the 51 kDa protein, despite its immunological similarity to mouse CYP2A5, does not catalyse any of these reactions. Alternatively test7α is catalysed by two or more of the immunoreactive bands and therefore will not associate to any individual band.

Despite the similarities, there are profound differences in the regulation of hamster and mouse enzymes. Test15α and COH are pronounced in male hamsters and in female mice; by PY they are induced in mice and reduced in hamster, while by MC they are induced hamsters and (slightly) reduced in mice.

Northern blot analysis

A full-length mouse CYP2A-5 cDNA hybridizes with hamster liver mRNA(s) which comigrates with the mouse mRNA (Fig. 2). Weaker signals are obtained with hamster preparations compared to mouse, which suggests that either there is less hybridizable mRNA or the homology between the hamster mRNA and cDNA is less than 100% (or both). As could be expected from the enzyme activities and western blot analysis ("49 kDa band"), the amount of hybridizable mRNA is increased after MC and decreased after PY treatment compared to controls. Interestingly, the level of mRNA in Pb-treated hamsters was about the same as in the controls, even though the enzyme activities were increased. This suggests that the mechanism of regulation of CYP2A by Pb, MC and PY is not the same. While MC and PY seem to affect either the transcription rate or half life of the mRNA, Pb affects either the rate of protein synthesis or stability. It is also possible that for example two highly similar mRNAs are recognised by the probe, one of which is increased and the other one decreased following Pb treatment, which would give the appearance of no change on the northern blot.

DISCUSSION

Until now, no data on hamster liver test7α, test15α or COH and their relation to CYP2As have been reported. However, it appears from our results that enzymes similar to mouse CYP2A5, capable of catalysing the three oxidations, are present in hamster liver microsomes.

Previously, we have shown that test15α and COH activities are catalysed in the mouse by CYP2A4 and 2A5, respectively [22]. These two enzymes are highly homologous (98.3%), and their immunological similarities and same molecular weight make them indistinguishable on western blots, although their catalytic properties are clearly distinct and their regulation, although similar, is not identical [22]. The mouse test7α is also a member of the CYP2A subfamily but is only 75% homologous to CYP2A-4 and 2A-5, and is clearly different in its regulation compared to the two other proteins [23]. Based on its homology, however, it is possible that this protein is also recognised by the CYP2A5 antibody.

While the anti-CYP2A5 antibody recognises one band on western blots from mouse liver microsomes, as many as three can be found for hamster, suggesting the presence of more than one protein immunologically similar to mouse CYP2As. The 49 kDa band has a molecular weight closest to that of mouse CYP2A4/2A5 and shows the best association with test15α and COH activities. This protein could therefore be comparable to mouse CYP2A4/2A5. At present, we do not know whether hamster test15α and COH are two different enzymes, as in the mouse, but the somewhat different response to inducers, differences in immunoinhibition and the fact that coumarin is able to inhibit test15α but not *vice versa* (as seen in the mouse) suggest that this is the case [22].

The other two bands do not seem to relate to any of the three activities. It is possible, however, that more than one enzyme contributes to microsomal oxidation. This could be the case for test7α, which is strongly inhibited by the anti-CYP2A5-antibody, yet does not associate with any of the bands on the western blot, and is strongly inhibited by coumarin only after MC treatment which selectively increases only one band. It could also be that while proteins in the 49 kDa band play a major role in COH and test15α metabolism, other bands may have minor activity.

In untreated animals, COH activity is higher in the mouse while test15α and in particular test7α activities are higher in the hamster [22, 23]. This suggests species differences in the expression of CYP2As that become evident in their response to inducers.

Interestingly, test15α and COH are MC-inducible only in hamsters; the corresponding mouse enzymes are PY-inducible although this treatment inhibits them in hamsters. The mechanism of induction of hamster test15α/COH by MC is not known, except

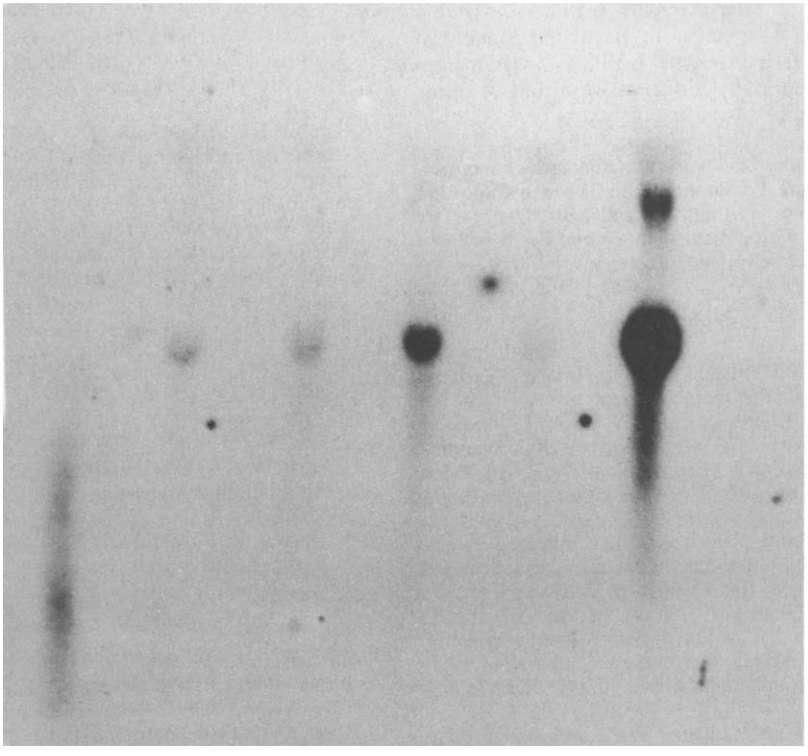

Fig. 2. Northern blots of mRNAs from livers of control, Pb-, PY- and MC-treated male hamsters and PY-treated DBA2 mice. Twenty micrograms of total mRNA from each sample was electrophoresed and probed with a full-length cDNA of mouse CYP2A-5. Pools of six animals were used.

that it seems to take place pretranslationally through either stimulated transcription or mRNA stabilization. Whether or not the induction is mediated by the Ah-receptor or another mechanism remains to be seen.

In mouse, PY seems to increase CYP2A5 by mRNA stabilization rather than increased transcription [9]. Interestingly, the 3′ end of mouse CYP2A5 mRNA has a sequence necessary for stabilization [13], but hamster mRNA, which is 73% homologous to mouse CYP2A-5 (and MC-inducible), does not [12]. The structure of hamster COH is not known and it remains to be seen whether differences in the 3′-end of COH mRNAs can explain the opposite responses of the two species to PY. On the other hand, anti-CYP2A5 antibody recognises a 52 kDa band on western blots which is increased by PY. It is therefore possible that certain hamster CYP2As are regulated similarly to mouse CYP2A-5. Thus, it seems that while mice and hamsters may have catalytically and immunologically similar enzymes of the CYP2A family, there are profound differences in regulation between the two species.

Interspecies comparisons have shown that mouse and human COH are similar (CYP2A-5 in mouse and 2A6 in humans) [11, 24], whereas test15α (CYP2A-4) is practically non-existent in humans [25]. Furthermore, rat livers do not have an enzyme similar to mouse CYP2A-5 or human 2A6 and their COH is extremely low [11]. Until the reasons for these species differences are elucidated, extrapolations between species for reactions catalysed by the CYP2A enzymes will be difficult.

Only a few studies on hamster CYP2As have been published. Fukuhara *et al.* [12] and Lai and Chiang [26] have independently isolated and cloned an MC-inducible CYP isozyme from hamster liver (P450 AFB and P450 MC1, respectively) which according to their amino acid sequences is the same enzyme. This protein has a 73% homology and the same number of amino acids (494) as CYP2A-5 [13]. Whether or not one of the proteins (the 49 kDa) recognised in the present study by anti-mouse CYP2A-5 antibody is P450 AFB/P450 MC1 is not known at present.

Several reports suggest that certain members of the CYP2A family oxidize important drugs and carcinogens such as aflatoxin B_1 and nitrosamines, at least in mice and humans [6–8]. Our preliminary experiments have shown that this also seems to be the case in hamsters, in accordance with the observations by Fukuhara *et al.* [12, 27]. Since hamsters are used as an animal model in carcinogenicity studies [28, 29], it is important to

understand the enzymatic basis of carcinogen activation in this species, in particular since the regulation of at least some CYP2As in hamsters seems to be essentially different from that of other species.

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REFERENCES

1. Yang CS, Smith T, Ishizaki H and Hong JY, Enzyme mechanisms in the metabolism of nitrosamines. In: *Relevance to Human Cancer of N-Nitroso Compounds Tobacco Smoke and Mycotoxins*. IARC Scientific Publication No. 105 (Eds. O'Neill IK, Chen J and Bartsch H), pp. 265–274. International Agency for Research on Cancer, Lyon, 1991.
2. Lijinsky W and Kovatch RM, Comparative carcinogenesis by nitrosomethyl-alkylamines in Syrian hamsters. *Cancer Res* **48**: 6648–6652, 1988.
3. Yoo J-SH, Ning SM, Patten CJ and Yang CS, Metabolism and activation of *N*-nitrosodimethylamine by hamster and rat microsomes: comparative study with weanling and adult animals. *Cancer Res* **47**: 992–998, 1987.
4. Juvonen RO, Shkumatov VM and Lang MA, 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.
5. Lang MA, Juvonen R, Järvinen P, Honkakoski P and Raunio H, Mouse liver P450Coh: genetic regulation of the pyrazole-inducible enzyme and comparison with other P450 isoenzymes. *Arch Biochem Biophys* **271**: 139–148, 1989.
6. Crespi CL, Penman BW, Leakey JAE, Arlotto MP, Stark A, Parkinson A, Turner T, Steimel DT, Rudo K, Davies RL and Langenbach R, Human cytochrome P450IIA3: cDNA sequence, role of the enzyme in the metabolic activation of promutagens, comparison to nitrosamine activation by human cytochrome P450IIE1. *Carcinogenesis* **11**: 1293–1300, 1990.
7. Forrester LM, Neal GE, Judah DJ, Glancey MJ and Wolf CR, Evidence for involvement for multiple forms of cytochrome P-450 in aflatoxin B_1 metabolism in human liver. *Proc Natl Acad Sci USA* **87**: 8306–8310, 1990.
8. Camus A-M, Geneste O, Honkakoski P, Béréziat J-C, Henderson CJ, Wolf CR, Bartsch H and Lang MA, Individual nitrosamine metabolism is highly variable: role of cytochromes P-4502A6 and 2E1 in the dealkylation on *N*-nitroso-dimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) in mice and men. *Mol Carcinogen* **7**: 268–275, 1993.
9. Hahnemann B, Salonpää P, Pasanen M, Mäenpää J, Honkakoski P, Juvonen R, Lang MA, Pelkonen O and Raunio H, Effect of pyrazole, cobalt and phenobarbital on mouse liver cytochrome P-450 2a-4/5 (CYP2A-4/5) expression. *Biochem J* **286**: 289–294, 1992.
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. Raunio H, Syngelmä T, Pasanen M, Juvonen R, Honkakoski P, Kairaluoma MA, Sotaniemi E, Lang MA and Pelkonen O, Immunochemical and catalytical studies on hepatic coumarin 7-hydroxylase in man, rat and mouse. *Biochem Pharmacol* **37**: 3889–3895, 1988.
12. Fukuhara M, Nagata K, Mizokami K, Yamazoe Y, Takanaka A and Kato R, Complete cDNA sequence of a major 3-methylcholanthrene-inducible cytochrome P-450 isozyme (P-450AFB) of Syrian hamsters with high activity toward aflatoxin B_1. *Biochem Biophys Res Commun* **162**: 265–272, 1989.
13. Squires EJ and Negishi M, Reciprocal regulation of sex-dependent expression of testosterone 15α-hydroxylase (P-450$_{15\alpha}$) in liver and kidney of male mice by androgen. *J Biol Chem* **263**: 4166–4171, 1988.
14. Lang MA and Nebert DW, Structural gene products of *Ah* locus. Evidence for many unique P-450-mediated monooxygenase activities reconstituted from 3-methylcholanthrene-treated C57BL/6N mouse liver microsomes. *J Biol Chem* **265**: 12058–12067, 1981.
15. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC, Measurement of protein using bicinchonic acid. *Anal Biochem* **150**: 76–85, 1985.
16. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J Biol Chem* **239**: 2379–2385, 1964.
17. Waxman DJ, Ko A and Walsh C, 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.
18. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685, 1970.
19. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4354, 1979.
20. Chomczynski P and Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**: 156–159, 1987.
21. Davis LG, Dibner MD and Battey JF, *Basic Methods in Molecular Biology*, pp. 143–146. Elsevier, Amsterdam, 1986.
22. Negishi M, Lindberg R, Burkhart B, Ichikawa T, Honkakoski P and Lang M, Mouse steroid 15α-hydroxylase gene family: identification of type II P-45015α as coumarin 7-hydroxylase. *Biochemistry* **28**: 4169–4172, 1989.
23. Lindberg RLP and Negishi M, Mouse testosterone 7α-hydroxylase (P450$_{7\alpha}$): isolation, sequencing and expression of P450$_{7\alpha}$ cDNA and regulation by sex and dexamethasone in mouse liver. In: *Drug Metabolizing Enzymes: Genetics, Regulation and Toxicology. Proceedings of the VIIIth Jutland Symposium on Microsomes and Drug Oxidations, Stockholm, Sweden, 1990* (Eds. Ingelman-Sundberg M, Gustafsson, J-Å and Orrenius S), p. 135.
24. Miles JS, McLaren AW, Forrester LM, Glancey MJ, Lang MA and Wolf CR, Identification of the human liver cytochrome P-450 responsible for coumarin 7-hydroxylase activity. *Biochem J* **267**: 365–371, 1990.
25. Mäenpää J, Syngelmä T, Honkakoski P, Lang MA and Pelkonen O, Comparative studies on coumarin and testosterone metabolism in mouse and human livers. *Biochem Pharmacol* **42**: 1229–1235, 1991.
26. Lai TS and Chiang JYL, Cloning and characterization of two major 3-methylcholanthrene inducible hamster liver cytochrome P450s. *Arch Biochem Biophys* **283**: 429–439, 1990.
27. Fukuhara M, Mizokami K, Sakaguchi M, Niimura Y, Kato K, Inouye S and Takanaka A, Aflatoxin B_1 specific cytochrome P-450 isozyme (P-450-AFB) inducible by 3-methylcholanthrene in Golden hamsters. *Biochem Pharmacol* **39**: 463–469, 1990.
28. Flavell DJ and Lucas SB, Potentiation by the human

liver fluke *Opisthorchis viverrini* of the carcinogenic action of *N*-nitrosodimethyl-amine upon the biliary epithelium of the hamster. *Br J Cancer* **46**: 985–989, 1982.

29. Flavell DJ and Lucas SB, Promotion of *N*-nitrosodimethylamine-initiated bile duct carcinogenesis in the hamster by the human liver fluke, *Opisthorchis viverrini*. *Carcinogenesis* **4**: 927–930, 1983.