Biochemical Pharmacology, Vol. 46, No. 11, pp. 1945–1951, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

ACETONE-DEPENDENT REGULATION OF CYTOCHROMES P4502E1 AND P4502B1 IN RAT NASAL MUCOSA

VINCENZO LONGO* and MAGNUS INGELMAN-SUNDBERG†
Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-17177 Stockholm, Sweden

(*Received* 30 *April* 1993; *accepted* 13 *August* 1993)

Abstract—The inducibility and molecular regulation of cytochrome P4502E1 (CYP2E1) has been examined in nasal mucosa of rats after acetone treatment and compared to that of cytochrome P4502B1 (CYP2B1). Twenty-four hours following treatment with acetone (5 mL/kg) for 2 days, the amount of CYP2E1 as well as the rate of microsomal 4-nitrophenol hydroxylase activity had increased by a factor of 2–3, in microsomes isolated from nasal mucosa. The increase in CYP2E1 was accompanied by a corresponding increase of CYP2E1 mRNA, as determined by northern and slot blot analyses. In contrast, hepatic and renal CYP2E1 mRNA, studied in the same rats, did not increase, despite the fact that the amount of CYP2E1 was increased 3- and 5-fold, respectively. The amount of CYP2B1, an isozyme known as acetone-inducible in other tissues, decreased significantly by acetone, as detected by immunoblot analysis. After 48 hr, the amount of CYP2E1 enzyme, the level of CYP2E1 mRNA and the rate of 4-nitrophenol hydroxylase activity had returned to normal levels, whereas in liver and kidneys the immunoreactive protein remained 3–4-fold higher than control. The results indicate that acetone does not regulate CYP2E1 in nasal mucosa by post-translational mechanisms, in contrast to the situation observed in liver and kidneys. This indicates a tissue-specific expression of post-translational regulatory systems responsible for P450 stabilization. Furthermore, nasal CYP2B1 also seems to be regulated in a tissue-specific manner by acetone.

Cytochrome P4502E1, (CYP2E1‡ [1]) is constitutively expressed in human and rodent liver [2, 3] and in several extrahepatic tissues including kidneys and nasal mucosa [4, 5]. CYP2E1 is induced by treatment with several xenobiotics and is also induced during certain physiological conditions like fasting and diabetes. CYP2E1 is a very versatile enzyme and presently we know more than 70 different isozyme specific substrates, among them solvents, alcohols and precarcinogens, most of which have the common property of being small and hydrophobic in nature [6].

The regulation of the expression of CYP2E1 is complex and involves both transcriptional and post-transcriptional mechanisms [7–9]. A major mechanism for the induction of CYP2E1 in liver is post-translational stabilization [10]. Isozyme-specific substrates protect the enzyme from a cAMP-dependent phosphorylation on Ser-129 [11], which otherwise causes heme loss and rapid degradation of the isozyme in the endoplasmic reticulum. It appears that this mechanism can elevate the level of CYP2E1 by about 4–5-fold, whereas transcriptional mechanisms are of importance in order to raise the level further [12].

Although several studies have concerned mechanisms for regulation of CYP2E1 in the liver, less is known regarding the molecular mechanisms governing expression in the extrahepatic tissues and particularly in nasal mucosa. The nasal mucosa is constantly exposed to a wide variety of xenobiotics and could be a target tissue for inhaled promutagens and procarcinogens through *in situ* metabolic activation. Several chemicals and carcinogens have been found to be activated to reactive intermediates by nasal cytochrome P450 (P450) [13, 14].

Various forms of P450 have been localized by antibodies to purified hepatic P450 isozymes in nasal mucosa of rabbit [15] and rat [5]. Moreover, specific forms have been found in the olfactory tissue. In this respect, a unique form designated NMb (CYP2G1, [1]), has been purified from microsomes of rabbit nasal mucosa [16] and immunochemical studies demonstrated the presence of this form only in the olfactory epithelium [15]. The P450NMb shows high homology with the olfactory-specific rat P450, called P450olf1 [1].

The nasal mucosa P450 forms are not inducible to the same extent as the hepatic ones, as shown by the lack of induction by classical P450 inducers, including phenobarbital [17], 3-methylcholanthrene, benzo(*a*)pyrene and Aroclor 1254 [17, 18]. However, recently nasal CYP2E1 in rat and rabbit was found to be induced following ethanol or acetone treatment

* Visiting scientist from the Laboratory of Genetics and Biochemical Toxicology, Istituto di Mutagenesi e Differenziamento, CNR, via Svezia 10, 56124-Pisa, Italy.
† Corresponding author: Dr Magnus Ingelman-Sundberg, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-17177 Stockholm, Sweden.

‡ Abbreviations: P450, cytochrome P450; CYP2E1, the major ethanol inducible cytochrome P450 form; CYP2B1, the major phenobarbital inducible cytochrome P450 form, see nomenclature assigned by Nelson *et al.* [1]; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; IgG, immunoglobulin G.

[19, 20]. Elevated levels of CYP2E1 in nasal tissue could imply a higher risk for toxicity or carcinogenicity. Accordingly, a higher incidence of *N*-nitrosonornicotine-induced nasal tumors was observed in rats treated with ethanol as compared to control rats [21].

Knowledge of the regulation of CYP2E1 in nasal mucosa is important for a better understanding of the mechanism of tissue-specific toxicity associated with this P450 form. In this study inducibility and regulation of nasal CYP2E1 and CYP2B1 were monitored in rats after acetone treatment and compared with that of liver and kidneys. The results indicate a tissue-specific regulation of CYP2E1.

MATERIALS AND METHODS

Materials

4-Nitrophenol and acetone were purchased from Merck (Darmstadt, F.R.G.), NADPH from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Cesium trifluoroacetate was obtained from Pharmacia Fine Chemicals Co. (Uppsala, Sweden). Guanidine thiocyanate was from Eastman-Kodak Co. (Rochester, NJ, U.S.A.). Nitrocellulose membranes were obtained from Schleier and Schuell (Keene, NH, U.S.A.). Nylon membranes were purchased from Sartorius GmbH (Göttingen F.R.G.). Alkaline-phosphate-linked goat anti-rabbit immunoglobulin G (IgG), 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate-toluidine salt color developing reagents for western blot analysis and nitrocellulose membrane were from Bio-Rad (Richmond, CA, U.S.A.).

Anti-(P450 IgG) against rat CYP2E1 was prepared in rabbits as described by Johansson *et al.* [22]. Rabbit anti-rat IgG against CYP2B1 was kindly donated by Dr Anders Åström, (University of Stockholm, Sweden) and has previously been characterized [23]. All other chemicals and solvents were of the highest grade available and obtained from common commercial sources.

Methods

Animal treatment and preparation of microsomes. The study was performed with male Sprague–Dawley rats (180–200 g). The animals were treated with acetone (5 mL/kg of body weight) intragastrically once daily for 2 days. These rats received food and water *ad libitum* until they were killed, which took place 24 or 48 hr after the last injection. Microsomes were prepared from rat liver, kidney and nasal mucosa as described previously [24].

Western blot analysis. Microsomes were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) using the method of Laemmli [25] in a Bio-Rad Protean Mini II apparatus. Western blot analysis was performed according to Towbin *et al.* [26] using 5% (w/v) milk as a blocking agent and an alkaline-phosphatase-linked goat anti-rabbit IgG as the secondary antibody. Color development using 4-nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate-toluidine salt reagents was as per manufacturers instructions. Immunoquantification was carried out with a densitometer (Image Quant Software on the Personal Densitometer of Molecular Dynamics).

Northern blot and slot blot analysis. Total RNA was isolated from the frozen rat nasal mucosa, liver and kidney by the guanidinium/cesium trifluoroacetate method essentially as described by Okayama *et al.* [27]. The RNA was size fractionated by electrophoresis in 1.25% agarose-formaldehyde gels and then transferred to nylon membrane as described by Sambrook *et al.* [28] or directly applied to the nitrocellulose membrane using a slot blot apparatus (Minifold II, Schleicher and Schuell). The membranes were hybridized with a nick translated [^{32}P]dCTP (Amersham Corp., Arlington Heights, IL, U.S.A.) labeled CYP2E1 cDNA probe, kindly donated by Dr Frank Gonzalez (NIH, Bethesda, MA, U.S.A.) or with a ^{32}P-labeled β-actin probe (donated by the late Prof. Håkan Persson, Karolinska Institutet, Stockholm, Sweden), as described by Sambrook *et al.* [29]. After washing, the membranes were exposed at −70° onto Kodak XAR-5 film with a Lightning Plus intensifying screen. The bands or slots on the autoradiographs were quantified by densitometry. Procedures for other assays have been described before [22]. Statistical analysis was carried out using Student's *t*-test; $P < 0.05$ was considered significant.

RESULTS

Effect of acetone-treatment on CYP2E1 and CYP2B1 in nasal mucosa after 24 hours

As shown in Fig. 1A, CYP2E1 in nasal mucosa was faintly detectable in control microsomes. However, pretreatment of rats with acetone resulted in a 2.3-fold increase in the amount of nasal microsomal CYP2E1 protein, accompanied by a similar increase in the rate of microsomal hydroxylation of 4-nitrophenol (Fig. 1A, Table 1). By contrast, this treatment did cause a 50% decrease in the amount of CYP2B1 in the mucosal microsomes (Fig. 1B, Table 1). The net effect of acetone treatment on the P450-level was a 50% increase. The effect of acetone treatment was less and different in nasal mucosa as compared to liver and kidney, where CYP2E1, CYP2B1 and 4-nitrophenol hydroxylase increased 4–8-fold.

Analysis of CYP2E1 mRNA following acetone treatment

In order to explore the mechanism of CYP2E1 induction after acetone treatment, the level of CYP2E1 mRNA from nasal mucosa was measured using ^{32}P-labeled CYP2E1 cDNA (Fig. 2). Northern blot analysis revealed the recognition of a single mRNA species in both control and acetone rats with the same molecular size as that of liver and kidney. Quantification of the mRNA transcripts revealed a significant 2.3-fold increase by the acetone treatment (Fig. 2), similar in magnitude to the increase in CYP2E1 protein. By contrast, no significant changes in the CYP2E1 mRNA were seen in liver or kidney.

Effects of acetone 48 hr after treatment

As shown in Table 2, 48 hr after the last intragastric dose of acetone, immunoreactive CYP2E1, CYP2E1

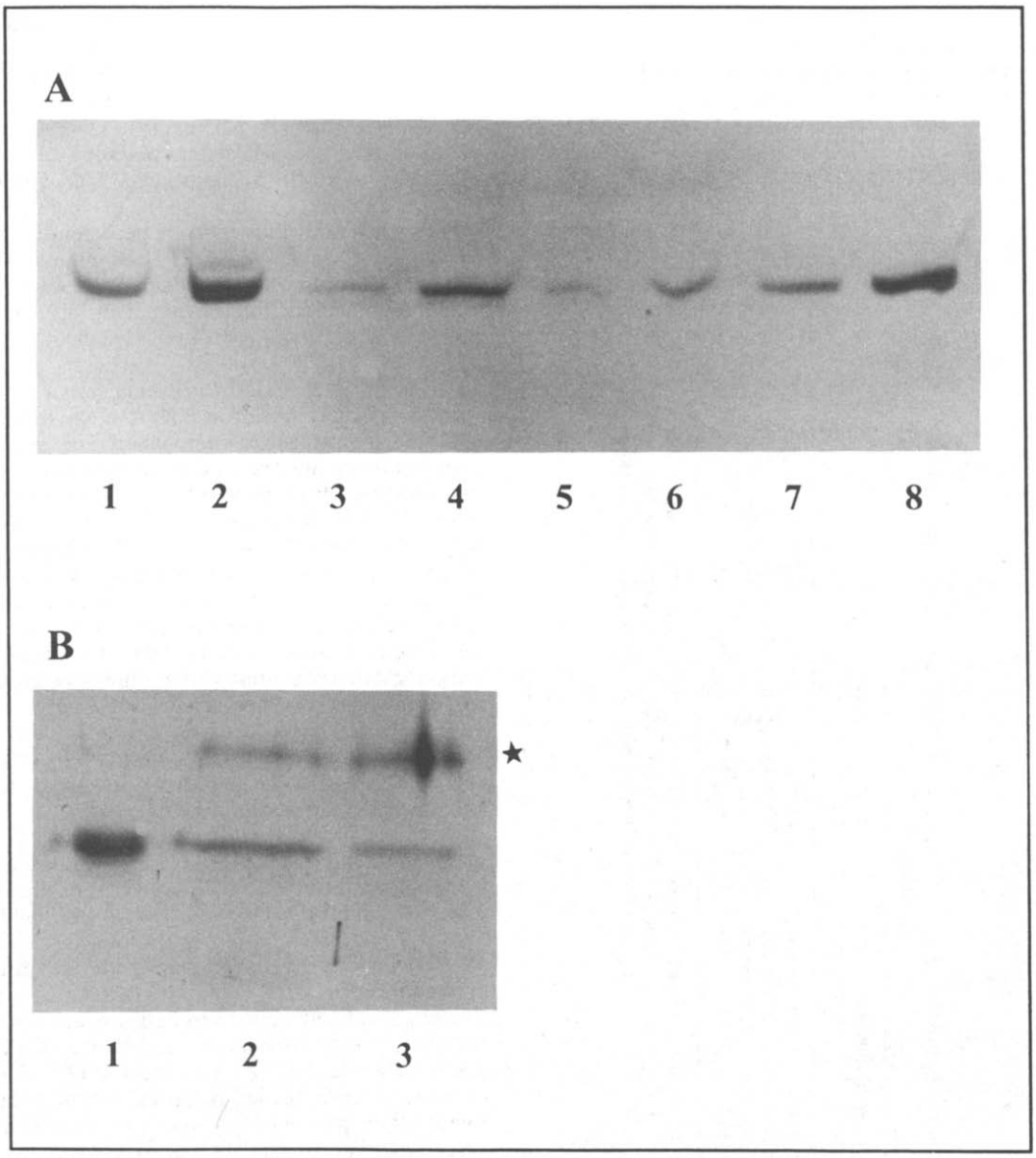

Fig. 1. Immunoblot analyses of CYP2E1 (A) and CYP2B1 (B) in rat microsomes. (A) Pooled microsomes from nasal mucosa, liver and kidney were subjected to SDS–PAGE on 8.7% acrylamide gels, transferred to nitrocellulose and probed with anti CYP2E1 IgG. Lane 1, control liver microsomes (2.5 μg); lane 2, liver microsomes from acetone-treated rats (2.5 μg), lane 3, microsomes from control nasal mucosa (70 μg); lane 4, nasal mucosal microsomes from acetone-treated rats (70 μg); lane 5, microsomes from control nasal mucosa (30 μg); lane 6, nasal microsomes from acetone-treated rats (30 μg); lane 7, microsomes from control kidney (22 μg); lane 8, kidney microsomes from acetone-treated rats (22 μg). (B) Analysis of CYP2B1. Lane 1, purified CYP2B1; lane 2, nasal microsomes from control rats (70 μg); lane 3, nasal microsomes from acetone-treated rats (70 μg). The band indicated with an asterisk has previously been shown to be an artefact [23].

mRNA and 4-nitrophenol hydroxylase activity in nasal mucosa had returned to constitutive levels. By contrast, kidney CYP2E1 protein remained elevated (about 3-fold) 48 hr after acetone treatment, whereas the 4-nitrophenol hydroxylase activity had declined to control levels. Similar differences between immunoreactive protein and 4-nitrophenol hydroxylase activity were seen in liver microsomes (data not shown) and are consistent with previously described results [30].

DISCUSSION

The present study indicates that the mechanisms for acetone-dependent induction of CYP2E1 in nasal mucosa are different from those of liver and kidney.

Table 1. Effect of acetone on total P450, 4-nitrophenol hydroxylase activity, CYP2E1 and CYP2B1 levels 24 hr after treatment in nasal mucosa, liver and kidneys

Parameter	Nasal mucosa		Liver		Kidneys	
	Control	Acetone	Control	Acetone	Control	Acetone
P450 total (nmol/mg protein)	0.11 ± 0.02	0.17 ± 0.02*	0.64 ± 0.06	1.05 ± 0.11*	0.08 ± 0.02	0.14 ± 0.01*
4-NPH (% of control)	100 ± 23	223 ± 35†	100 ± 18	474 ± 72†	100 ± 17	784 ± 93†
CYP2E1 protein (% of control)	100 ± 10	226 ± 15†	100 ± 15	341 ± 35†	100 ± 48	541 ± 90†
CYP2B1 protein (% of control)	100 ± 18	45.5 ± 13†	100 ± 25	470 ± 60†	100 ± 31	436 ± 86†

The data represent mean ± SD of four to five different experiments carried out with microsomes pooled from four to five rats. Statistical analysis was carried out using Student's *t*-test; *$P < 0.05$, †$P < 0.01$ compared to control. The absolute values of 4-nitrophenol hydroxylase (4-NPH) activity were: 0.17 ± 0.04 nmol/mg protein/min (nasal mucosa), 1.04 ± 0.19 nmol/mg protein/min (liver) and 0.12 ± 0.020 nmol/mg/min (kidney).

When studied in the same rats, it was evident that acetone caused a pronounced increase of both CYP2E1 and 4-nitrohydroxylase activity in both liver and kidneys, without any increase of the corresponding mRNA, whereas in nasal mucosa, the elevation of these parameters perfectly correlated with the extent of increase in the CYP2E1 mRNA registered (Table 1). In addition, the induction of CYP2E1 protein was seen to be persistent for 48 hr in kidneys (Table 2). CYP2E1 induction has previously been shown to remain for an equal time period after acetone treatment in liver [30], but had returned to the basal level in nasal mucosa (Table 2).

The turnover of CYP2E1 in liver *in vivo* is biphasic with half lives of 7 and 37 hr [31]. Acetone treatment abolishes the fast phase component. This substrate effect has been shown to be associated with protection of a cAMP-dependent phosphorylation of CYP2E1 on Ser-129 otherwise causing denaturation and rapid degradation of the protein in the endoplasmic reticulum [10, 11]. In the presence of substrate, the protein is broken down according to the autophagosomal-lysosomal pathway only, at a slower rate [30]. The results obtained in nasal mucosa in the present investigation can therefore be interpreted in the manner that the mechanism for substrate dependent CYP2E1 stabilization is not occurring in this tissue. This could have its origin in the absence of expression of critical enzymes involved in the post-translational processing of CYP2E1.

The data obtained cannot discriminate between acetone as a stimulator of CYP2E1 gene transcription or as a factor causing CYP2E1 mRNA stabilization. The rate of CYP2E1 gene transcription has been shown to be increased during prolonged starvation [32] or, as shown recently, during chronic ethanol treatment in the total enteral nutrition model [33]. On the other hand, conditions during streptozotocin-induced diabetes are known to increase CYP2E1 exclusively via mRNA stabilization [7]. Further experiments are needed in order to investigate in detail the mechanisms for acetone-dependent increase of CYP2E1 mRNA in nasal mucosa.

Tissue specificity with regards to action of acetone was also registered in the regulation of CYP2B1. In agreement with previous data, acetone induced CYP2B1 in liver, kidney and nasal mucosa has been shown to involve an increased rate of gene transcription [22]. CYP2B1 is constitutively expressed in nasal mucosa at higher levels than in liver of control rats [34]. However, in contrast to the well established inducibility of CYP2B1 in liver and kidney, we found a significant decrease to 50% of the original level of CYP2B1 in nasal mucosa after acetone treatment (Table 1).

We have reported previously that CYP2E1-dependent activities decline much faster than the immunoreactive CYP2E1 in rat liver after acetone treatment [30]. This was interpreted as an inactivation of CYP2E1 prior to degradation and indicated two phases of post-translational regulation of CYP2E1, an acute inactivation in the endoplasmic reticulum before of the degradation by lysosomal route. Our results shown a similar course in kidney, where 4-nitrophenol hydroxylase activity returned to the

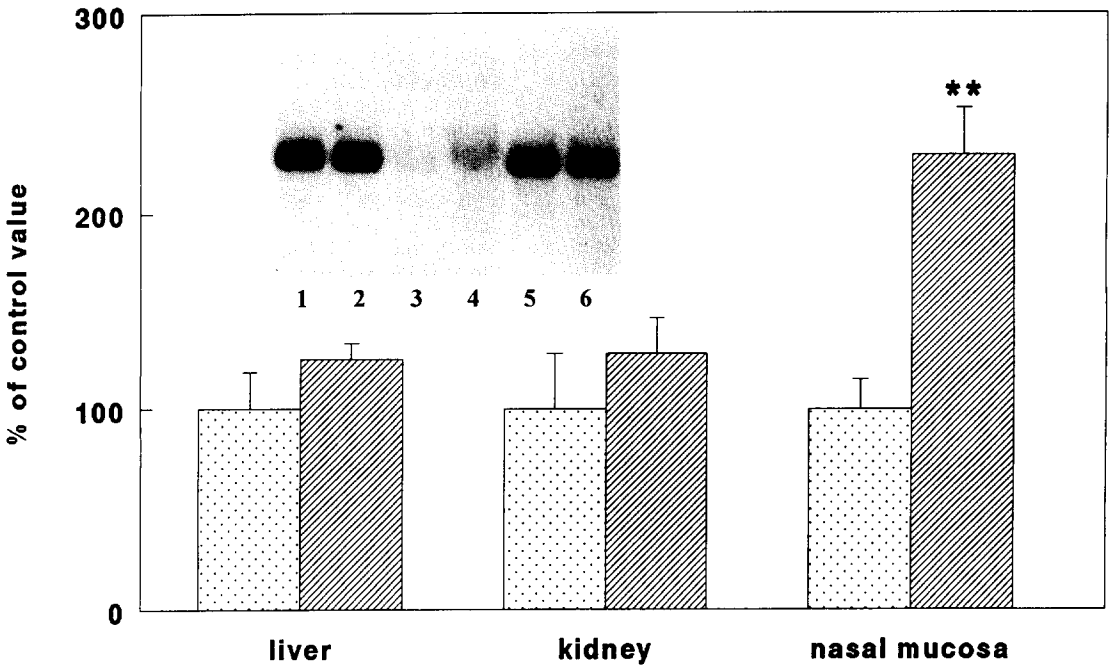

Fig. 2. Analysis of the amount of nasal, hepatic and renal CYP2E1 mRNA from control (▨) and acetone-treated (▨) rats. Total RNA was isolated from control and acetone-treated rats (□) and subjected to electrophoresis, blotting and hybridization as described in Materials and Methods. The relative signals were normalized with respect to the intensities obtained using β-actin cDNA. Values are mean ± SD of four determinations on pooled samples of total RNA (three to four rats for each determination). **Value significantly different from control ($P < 0.01$). Insert, northern blot analysis. Lane 1, control liver (3 μg); lane 2, acetone liver (3 μg); lane 3, control nasal mucosa (45 μg); lane 4, nasal mucosa from acetone-treated rats (45 μg); lane 5, control kidney (10 μg); lane 6, kidney from acetone-treated rats (10 μg).

Table 2. Effect of acetone, 48 hr after treatment, on CYP2E1 protein, CYP2E1 mRNA and 4-nitrophenol hydroxylase activity in rat nasal mucosa and kidneys

Parameter	Nasal mucosa		Kidneys	
	Control	48 hr	Control	48 hr
CYP2E1 protein	100 ± 15	115 ± 17	100 ± 35	290 ± 30*
CYP2E1 mRNA	100 ± 20	110 ± 18	100 ± 15	90 ± 10
4-NPH	100 ± 25	95 ± 18	100 ± 15	105 ± 20

The results are expressed as per cent of control. Each experiment used pooled fractions from four rats. The values represent mean ± SD of four experiments. * Results significantly different from control ($P < 0.01$). 4-NPH, 4-nitrophenol hydroxylase.

control level in spite of the amount of CYP2E1 protein expressed after 48 hr acetone treatment.

In conclusion, the results of the present study indicate that regulatory mechanisms governing CYP2E1 and CYP2B1 expression after acetone are different for nasal tissue compared to hepatic and renal tissues. In addition, nasal specific P450 isozymes exist which do differ from those in the liver with regard to their substrate specificities and mechanism of action [1, 16]. This makes this tissue different with respect to its mechanisms of interactions with xenobiotics.

Acknowledgements—Dr V. Longo was supported by a grant from the European Science Foundation (RF/92/31). This work was supported by grants from The Swedish Alcohol Research Fund, Lundströms Stiftelse and from the Swedish Medical Research Council.

REFERENCES

1. Nelson DR, Kamataki T, Waxman DJ, Guengerich FP, Estabrook RW, Feyereisen R, Gonzalez FJ, Coon MJ, Gunsalus IC, Gotoh O, Okuda K and Nebert DW, The P450 superfamily: update on new sequences, gene mapping, accession numbers, early trivial names

of enzymes, and nomenclature. *DNA Cell Biol* **12**: 1–51, 1993.
2. Wrighton SA, Thomas PE, Molowa DT, Haniu M, Shively JE, Maines SL, Watkins PB, Parker G, Mendez-Picon G, Levin W and Guzelian PS, Characterization of ethanol-inducible human liver *N*-nitrosodimethylamine demethylase. *Biochemistry* **25**: 6731–6735, 1986.
3. Puccini P, Menicagli S, Longo V, Santucci A and Gervasi PG, Purification and characterization of an acetone-inducible cytochrome P450 from hamster liver microsomes. *Biochem J* **287**: 863–870, 1992.
4. Ding X, Koop DR, Crump BL and Coon MJ, Immunochemical identification of cytochrome P450 isozyme 3a ($P450_{ALC}$) in rabbit nasal and kidney microsomes and evidence for differential induction by alcohol. *Mol Pharmacol* **30**: 370–378, 1986.
5. Jayyosi Z and Thomas PE, Identification of cytochrome P450 isozyme and characterization of testosterone oxidation pathways in rat nasal epithelium. *FASEB J* **4**: A 1971, 1990.
6. Terelius Y, Lindros KO, Albano E and Ingelman-Sundberg M, Isozyme specificity of cytochrome P450-mediated hepatotoxicity. In: *Frontiers of Biotransformation* (Eds. Ruckpaul K and Rein H), Vol 8, 187–232. Akademie Verlag, Berlin, 1992.
7. Song BJ, Matsunaga T, Hardwick JP, Park SS, Veech RL, Yang CS, Gelboin HV and Gonzalez FJ, Stabilization of cytochrome P450j messenger ribonucleic acid in the diabetic rat. *Mol Endocrinol* **1**: 542–547, 1987.
8. Koop DR and Tierney DJ, Multiple mechanisms in the regulation of ethanol-inducible cytochrome P4502E1. *BioEssay* **12**: 429–435, 1990.
9. Ingelman-Sundberg M, Ethanol-inducible cytochrome P4502E1. Regulation, radical formation and toxicological importance. In: *Oxy Radicals* (Eds. Poli G, Albano E and Dianzani M), pp. 287–301. Birkenhauser, Basel, 1993.
10. Eliasson E, Johansson I and Ingelman-Sundberg M, Ligand-dependent maintenance of ethanol-inducible cytochrome P450 in primary rat heptocyte cell cultures. *Biochem Biophys Res Commun* **150**: 436–443, 1988.
11. Eliasson E, Johansson I and Ingelman-Sundberg M, Substrate-, hormone-, and cAMP-regulated cytochrome P450 degradation. *Proc Natl Acad Sci USA* **87**: 3225–3229, 1990.
12. Ronis MJJ, Crough J, Mercado C, Irby D, Valentine C, Lumpkin CK, Ingelman-Sundberg M and Badger TM, Cytochrome P450 CYP2E1 induction during alcohol exposure occurs by a two step mechanism associated with blood alcohol concentrations in rats. *J Pharmacol Exp Ther* **264**: 944–950, 1993.
13. Hong JY, Ding X, Smith TJ, Coon MJ and Yang CS, Metabolism of (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific carcinogen, by rabbit nasal microsomes and cytochrome P450s NMa and NMb. *Carcinogenesis* **13**: 2141–2144, 1992.
14. Longo V, Pacifici GM, Panattoni G, Ursino F and Gervasi PG, Metabolism of diethylnitrosamine by microsomes of human respiratory nasal mucosa and liver. *Biochem Pharmacol* **38**: 1867–1869, 1989.
15. Ding X and Coon MJ, Immunochemical characterization of multiple forms of cytochrome P450 in rabbit nasal microsomes and evidence for tissue-specific expression of P450s NMa and NMb. *Mol Pharmacol* **37**: 489–496, 1990.
16. Ding X and Coon MJ, Purification and characterization of two unique forms of cytochrome P450 from rabbit nasal microsomes. *Biochemistry* **27**: 8330–8337, 1988.
17. Hadley WM, Dahl AR, Benson JM, Hahn FF and McClellan RO, Cytochrome P450 dependent monooxygenases in nasal epithelial membranes effect of phenobarbital and benzo(a)pyrene. *Proc West Pharmacol Soc* **25**: 197–199, 1982.
18. Bond JA, Some biotransformation enzymes responsible for polycyclic aromatic hydrocarbon metabolism in rat nasal turbinates: effects on enzyme activities of *in vitro* modifiers and intraperitoneal and inhalation exposure of rats to inducing agents. *Cancer Res* **43**: 4805–4811, 1983.
19. Hong JY, Smith T, Lee MJ, LI W, Ma BL, Ning SM, Brady JF, Thomas PE and Yang CS. Metabolism of carcinogenic nitrosamines by rat nasal mucosa and the effect of diallyl sulfide. *Cancer Res* **51**: 1509–1514, 1991.
20. Ding X and Coon MJ, Induction of cytochrome P450 isozyme 3a (P450IIEI) in rabbit olfactory mucosa by ethanol and acetone. *Drug Metab Dispos* **18**: 742–745, 1990.
21. Castonguay A, Rivenson A, Trushin N, Reinhardt J, Spathopoulos S, Weiss CJ, Reiss B and Hecht SS, Effect of chronic ethanol consumption on the metabolism and carcinogenicity of *N'*-nitrosonornicotine in F344 rats. *Cancer Res* **44**: 2285–2290, 1984.
22. Johansson I, Ekström G, Scholte B, Purycki D, Jörnvall H and Ingelman-Sundberg M, Ethanol-, fasting-, and acetone-inducible cytochromes P450 in rat liver: regulation and characteristics of enzymes belonging to the IIB and IIE gene subfamilies. *Biochemistry* **27**: 1925–1934, 1988.
23. Bühler R, Lindros KO, Nordling Å, Johansson I and Ingelman-Sundberg M, Zonation of cytochrome P450 isozyme expression and induction in rat liver. *Eur J Biochem* **204**: 407–412, 1992.
24. Longo V, Citti L and Gervasi PG, Biotransformation enzymes in nasal mucosa and liver of Sprague–Dawley rats. *Toxicol Lett* **44**: 289–297, 1988.
25. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685, 1970.
26. Towbin H, Staehlin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76**: 4350–4355, 1979.
27. Okayama H, Kawaichi M, Brownstein M, Lee F, Yokota T and Arai K, High-efficiency cloning of full-length cDNA; construction and screening of cDNA expression libraries for mammalian cells. In: *Methods in Enzymology* (Eds. Wu R and Grossman L), Vol. 154, pp. 3–28. Academic Press, New York, 1987.
28. Sambrook J, Fritsch EF and Maniatis T, Electrophoresis of RNA through gels containing formaldehyde. In: *Molecular Cloning a Laboratory Manual* (Eds. Ford N, Nolan C and Ferguson M), Vol. 1, pp. 7.43–7.48. Cold Spring Harbor Laboratory Press, New York, 1989.
29. Sambrook J, Fritsch EF and Maniatis T, Hybridization of radiolabeled probes to immobilized nucleic acids. In: *Molecular Cloning a Laboratory Manual* (Eds. Ford N, Nolan C and Ferguson M), Vol. 1, pp. 9.47–9.55. Cold Spring Harbor Laboratory Press, New York, 1989.
30. Ronis MJJ, Johansson I, Hultenby K, Lagercrantz J, Glaumann H and Ingelman-Sundberg M, Acetone-regulated synthesis and degradation of cytochrome P4502E1 and cytochrome P4502B1 in rat liver. *Eur J Biochem* **198**: 383–389, 1991.
31. Song BJ, Veech RL, Park SS, Gelboin HV and Gonzalez FJ, Induction of rat hepatic *N*-nitrosodimethylamine demethylase by acetone is due to protein stabilization. *J Biol Chem* **264**: 3568–3572, 1989.
32. Johansson I, Lindros KO, Eriksson H and Ingelman-Sundberg M, Transcriptional control of CYP2E1 in

perivenous liver region and during starvation. *Biochem Biophys Res Commun* **173**: 331–338, 1990.

33. Badger TM, Huang J, Ronis MJJ and Lumpkin CK, Induction of cytochrome P450 2E1 during chronic ethanol exposure occurs via transcription of the CYP2E1 gene when blood alcohol concentrations are high. *Biochem Biophys Res Commun* **190**: 780–785, 1993.
34. Nef P, Heldman J, Lazard D, Margalit T, Jaye M, Hanukoglu I and Lancet D, Olfactory-specific cytochrome P450. *J Biol Chem* **264**: 6780–6785, 1989.