Biochemical Pharmacology, Vol. 57, pp. 1407–1413, 1999.

ISSN 0006-2952/99/$–see front matter
PII S0006-2952(99)00015-5

Expression of *CYP2A* Genes in Human Liver and Extrahepatic Tissues

Satu Koskela, Jukka Hakkola,* Janne Hukkanen,* Olavi Pelkonen,* Martti Sorri,† Antti Saranen,* Sisko Anttila,‡ Pedro Fernandez-Salguero,§ Frank Gonzalez§ and Hannu Raunio*‖*

*Department of Pharmacology and Toxicology, and †Department of Otolaryngology, University of Oulu; ‡Finnish Institute of Occupational Health, Helsinki, Finland; and §Laboratory of Molecular Carcinogenesis, National Cancer Institute, Bethesda, MD, U.S.A.

ABSTRACT. Members of the human cytochrome P450 2A (*CYP2A*) subfamily are known to metabolize several promutagens, procarcinogens, and pharmaceuticals. In this study, the expression of the three genes found in the human *CYP2A* gene cluster was investigated in the liver and several extrahepatic tissues by gene-specific reverse transcriptase-polymerase chain reaction (RT-PCR). All three transcripts (CYP2A6, CYP2A7, and CYP2A13) were found to be present in liver. Quantitative RT-PCR analysis showed that CYP2A6 and CYP2A7 mRNAs were present at roughly equal levels in the liver, while CYP2A13 was expressed at very low levels. Two putative splicing variants of CYP2A7 were found in the liver. Nasal mucosa contained a low level of CYP2A6 and a relatively high level of CYP2A13 transcripts. Kidney, duodenum, lung, alveolar macrophages, peripheral lymphocytes, placenta, and uterine endometrium were negative for all transcripts. This survey gives a comprehensive picture of the expression pattern of *CYP2A* genes in liver and extrahepatic tissues and constitutes a basis for a search for functional CYP2A forms and their roles in chemical toxicity in liver and nasal mucosa. BIOCHEM PHARMACOL **57**;12:1407–1413, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. cytochrome P450 gene expression; CYP2A; nasal mucosa; liver; extrahepatic tissues; xenobiotic metabolism

CYP¶ enzymes mediate the oxidative metabolism of numerous exogenous and endogenous compounds [1]. CYP2A is one of the six CYP2 subfamilies known to be present in humans [2]. CYP2A6 is the best-characterized enzyme in this subfamily [3, 4]. It has been shown to catalyze the metabolic activation of several procarcinogens, including several nitrosamines [5–8], aflatoxin B_1 [9–11], and 1,3-butadiene [12]. Clinically used drugs metabolized by CYP2A6 include coumarin [10, 11, 13, 14], methoxyflurane [15], and the novel platelet-activating factor antagonist SM-12502 [16]. CYP2A6 is also a major catalyst in the oxidative metabolism of nicotine [17] and cotinine [18].

Very little is known about the tissue-specific expression of *CYP2A* genes in humans. *CYP2A6* is expressed in the liver at relatively high levels [4, 19]. Indirect evidence suggests that CYP2A protein, participating in nitrosamine metabolism, may be present in lung [20, 21] and kidney [15]. Two recent studies [22, 23] have demonstrated the presence of CYP2A6 transcripts in nasal mucosa.

The organization and structure of the entire human *CYP2A* gene cluster was recently characterized [24, 25]. The cluster consists of three genes [*CYP2A6*, *CYP2A7*, and *CYP2A13*] and two *CYP2A7* pseudogenes localized within a 350-kb region in the long arm of chromosome 19 [24]. The elucidation of the *CYP2A* gene structures made it possible to accurately assess the expression pattern of each of the genes in human tissues. In this study, we have determined, using gene-specific RT-PCR analysis, the levels of expression of each of the *CYP2A* transcripts in liver and several extrahepatic tissues.

MATERIALS AND METHODS

Tissue Samples

Surplus liver tissue was obtained from elective liver surgery at the Department of Surgery, University Hospital of Oulu, Finland. Nasal mucosa samples were obtained during surgery for diseases of nasal cavity or sinuses at the Department of Otolaryngology, University Hospital of Oulu. The nasal mucosa samples were taken from respiratory areas of the nasal cavity. Placental samples were from the repository kept at the Department of Pharmacology and Toxicology, University of Oulu. Uterine endometrial samples were from abrasions done at the Department of Obstetrics and Gynecology, University Hospital of Turku, Finland, and were provided by M. Mäntylä (Department of Anticancer Re-

‖ Corresponding author: Dr. Hannu Raunio, Department of Pharmacology and Toxicology, University of Oulu, FIN-90220 Oulu, Finland. Tel. 358-8-5375235; FAX 358-8-5375247; E-mail: hannu.raunio@oulu.fi

¶ *Abbreviations:* CYP, cytochrome P450; EST, expressed sequence tags; and RT-PCR, reverse transcriptase-polymerase chain reaction.

Received 11 June 1998; accepted 26 October 1998.

TABLE 1. Primers for gene-specific *CYP2A* amplification

Gene	Sense primer	Antisense primer	PCR product [bp]
CYP2A6	TCAAAGGCTATGGCGTGGTA	GCTGACGGTCTCGGTGCCCC	589
CYP2A7	GAACACAGAGCACATATGTG	GCTGACGGTCTCCGTGCCTG	774
CYP2A13	ACAAAGAGTTCCTGTCACTG	CCGCAAAGAAGAGGTTCAGG	320

All primers are shown in 5′–3′ orientation.

search, Orion Corporation, Turku, Finland). Lung samples were from pulmonectomy or lobectomy of individuals operated on for tumorous lung lesions at the Department of Thoracic and Cardiovascular Surgery, University Hospital of Helsinki, Finland. Samples for mRNA extraction were prepared from macroscopically normal peripheral lung tissue. Bronchoalveolar lavage samples consisting mainly of macrophages were obtained from 5 individuals by standard bronchoalveolar lavation. Kidney tissue samples were obtained during surgery to remove kidney tumors. Normal kidney tissue and some kidney tumors were used for analysis. Duodenal tissue was from patients undergoing surgery due to various gastrointestinal diseases. Lymphocytes were isolated from blood samples taken from healthy volunteers. The use of surplus tissue was approved by the Ethics Committees of the participating institutions.

Primers Used for RT-PCR

The primers were designed to distinguish between the highly similar *CYP2A* sequences. To rule out amplification of any transcripts from the two *CYP2A* pseudogenes, all antisense primers were located 3' to the pseudogene truncation point in exon 5. All primer pairs amplify a region including at least one intron, making it possible to monitor for amplification of genomic DNA. The primers used are shown in Table 1. β-Actin primers were purchased from Stratagene.

Preparation of mRNA and cDNA Synthesis

Messenger RNA was extracted from about 20 to 100 mg of tissue with the QuickPrep Micro mRNA Purification and complementary DNA was synthesized with the First-Strand cDNA Synthesis Kit (both from Pharmacia P-L Biochemicals). The cDNA was stored at −20° until the PCR reaction was performed.

Qualitative RT-PCR

PCR reactions contained 1 μL of cDNA (out of 15 μL total), 2.5 U DynaZyme DNA polymerase (Finnzymes), 5 μL 10x DynaZyme reaction buffer, dNTP reaction mix (Finnzymes) at a final concentration of 400 μM, 50 pmol of each primer, and water in a final volume of 50 μL. For extrahepatic tissues 35 PCR cycles were performed using the following conditions: 1 min at 94°, 1 min at 59–62°, and 1 min at 72°. We used 25 cycles to amplify hepatic CYP2A6 and 30 cycles for hepatic CYP2A7. In every series of PCR reactions, there were two negative controls which contained all other reagents except the mRNA samples. After amplification, 8 μL of the reaction mixture was electrophoresed in agarose gel and stained with ethidium bromide. To control for reproducibility, all amplifications were repeated at least twice. The electrophoresed PCR products were transferred to nylon filters and hydridized with the full-length CYP2A6 cDNA [11] labeled with ^{32}P-dCTP (Amersham). After overnight hybridization, the filters were washed three times for 20 min in 0.1X SSC (sodium sodium chloride citrate), 0.1% SDS at 65°. Autoradiography films were exposed to the filters for 16 to 72 hr at room temperature. Amplified products representing CYP2A6, CYP2A7, and CYP2A13 were sequenced to verify their exact identity. Briefly, the amplified DNA was purified with QIAquick PCR Purification columns (Qiagen), boiled at 100° for 3 min, cooled to 42°, and labeled and extended using ^{35}S-dATP (Amersham) and the Pharmacia T7 Sequencing Kit.

Quantitative RT-PCR

Competitive PCR controls for CYP2A6, CYP2A7, and CYP2A13 were prepared as described [26]. In this method, a part of the target sequence is first amplified with the same 3′primer and a recombinant 5′ primer (Table 2) to produce

TABLE 2. Primers for competitive RT-PCR controls

Gene	Recombinant 5′ primer	3′ primer	PCR product [bp]
CYP2A6	TCAAAGGCTATGGCGTGGTAGACGCCCTCCGGGGCACTGG	GCTGACGGTCTCGGTGCCCC	469
CYP2A7	GAACACAGAGCACATATGTGTCAAAGGCTATGGCGTGGCG	GCTGACGGTCTCCGTGCCTG	609
CYP2A13	ACAAAGAGTTCCTGTCACTGCAGCAACAGGCCTTTAAGGA	CCGCAAAGAAGAGGTTCAGG	225

All primers are shown in 5′–3′ orientation.

a shortened template that can be amplified with the original primer pair (Table 1). The obtained PCR products, otherwise identical to the original target templates but containing a 100–150 bp deletion in sequence, were then cloned into the pCRII vector (Invitrogen BV). A serial dilution of this plasmid was then used as a control for the RT-PCR reactions carried out from independent mRNA and cDNA preparations as described above. Every series contained negative controls in which template cDNA was replaced with water. To minimize any differences in efficiency of the reverse transcriptase reaction, all amplifications were repeated from a second independent cDNA synthesis reaction. The same number of cycles was used as in the qualitative RT-PCR. An aliquot of the reaction mixture was electrophoresed in an agarose gel and stained with ethidium bromide. The point where the amount of control molecules is equal to the target molecules determines the amount of the mRNA studied.

RESULTS

Screening of CYP2A Transcripts in Liver and Extrahepatic Tissues

To ensure the quality and integrity of cDNA preparations, tissue samples were first analyzed for the presence of β-actin transcripts, and, where possible, for the presence of mRNA corresponding to CYP forms known to be expressed in each tissue. Only samples with detectable β-actin transcripts and positive CYP controls were subjected to assay with CYP2A-specific primers.

All three CYP2A mRNAs were detected in the liver by qualitative RT-PCR. Analysis of the PCR products in ethidium bromide-stained agarose gels showed bands corresponding to the expected sizes. Additional bands were also visible in the PCR reactions for the *CYP2A7* gene product. CYP2A6 mRNA was readily amplified in 25 cycles, CYP2A7 in 30 cycles, and CYP2A13 in 35 cycles. Sequencing of the PCR products confirmed their identity. The results from 6 to 8 livers after Southern blotting and hybridization are shown in Fig. 1. Single bands representing CYP2A6 product (590 bp) and CYP2A13 product (320 bp) are shown in the top and bottom panels of Fig. 1, respectively. CYP2A7 primers amplified, in addition to the expected 774-bp product, two shorter products of about 600 bp and 450 bp in length (Fig. 1, middle panel).

Nasal mucosa samples were found to contain CYP2A13 mRNA (Fig. 2). The differences in band intensities in Fig. 2 reflect the recovery of mRNA and cDNA from the mucosa samples, since the intensities correlate well with β-actin amplification (data not shown). In some nasal mucosa samples, an amplification product of CYP2A6 was detected, but its abundance was substantially below the level in liver (data not shown). No correct-size products were generated by the CYP2A7 primers in nasal mucosa.

Several other tissues were also examined for the presence of *CYP2A* gene expression. All PCR reactions with the CYP2A primers were done in 35 cycles. All kidney samples, duodenum, lung, alveolar macrophages, peripheral lymphocytes, uterine endometrium, and placental tissue were repeatedly negative even after long-term exposure of the CYP2A6-hybridized filters. In contrast, each tissue examined contained readily detectable levels of various other CYP forms or β-actin transcripts (summarized in Table 3).

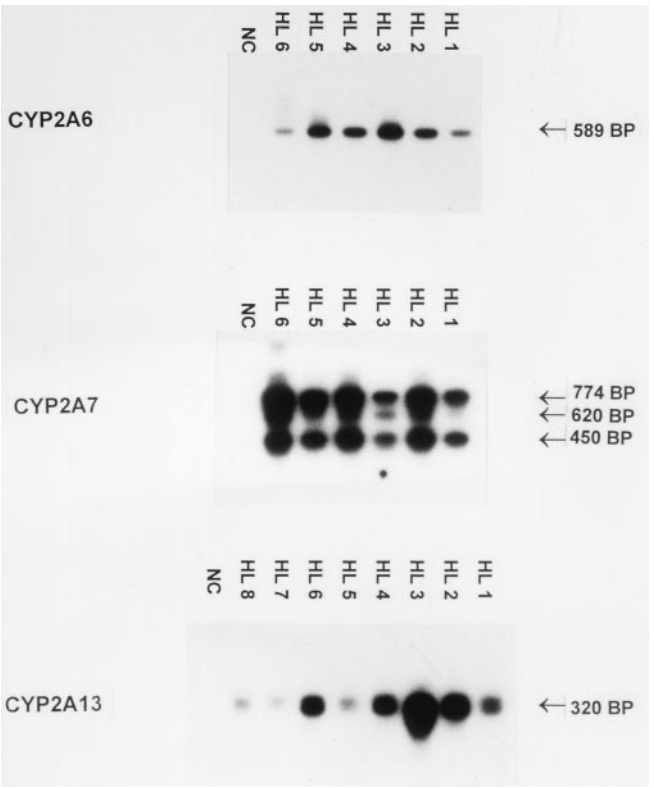

FIG. 1. Amplification of CYP2A6, CYP2A7, and CYP2A13 in liver. mRNA was extracted from human liver samples (HL) and reverse transcribed to cDNA, which was subjected to amplification with *CYP2A* gene-specific primers for 25 cycles (CYP2A6), 30 cycles (CYP2A7), and 35 cycles (CYP2A13). An aliquot of the amplification product from 6–8 samples was electrophoresed in an agarose gel, transferred to nylon membrane and hybridized with ^{32}P-labeled full-length CYP2A6 probe. Autoradiography films were exposed for 16 (CYP2A6) and 72 hr (CYP2A7 and CYP2A13) at room temperature. NC = negative control.

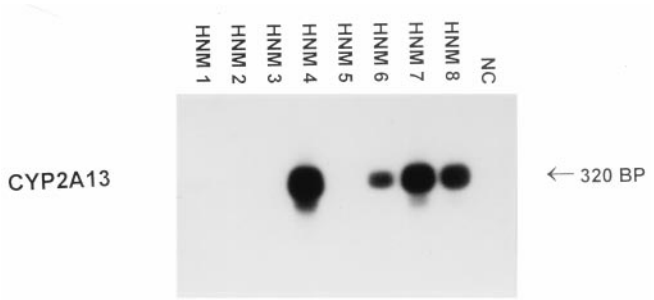

FIG. 2. Amplification of CYP2A13 in nasal mucosa. Eight human nasal mucosal samples (HNM) were treated as the liver samples in Fig. 1. The film was exposed for 72 hr. NC = negative control.

TABLE 3. Summary of detection of CYP2A transcripts indifferent tissues

Tissue	CYP2A6	CYP2A7	CYP2A13	cDNA control*
Liver (N = 8)	+++	++	+	CYP3A4
Nasal mucosa (N = 11)	(+)	−	+	NA
Kidney (N = 10)	−	−	−	CYP3A5
Lung (n = 9)	−	−	−	CYP2F1
Alveolar macrophages (N = 5)	−	−	−	NA
Lymphocytes (N = 8)	−	−	−	NA
Endometrium (N = 4)	−	−	−	CYP11A1
Duodenum (N = 12)	−	−	−	CYP3A4
Placenta (N = 10)	−	−	−	CYP19

*CYP mRNAs that were present in the cDNA preparations. NA = not applicable; all these cDNA preparations contained β-actin transcripts.

These results suggest that physiologically significant amounts of CYP2A mRNAs do not exist in those tissues.

Quantitative RT-PCR

To find out how the expression levels of CYP2A6, CYP2A7, and CYP2A13 compare with each other, quantitative competitive RT-PCR analysis was done in five individual liver samples. There was an approximately 40-fold variation in the content of CYP2A6 mRNA in these livers, while CYP2A7 levels varied only 3-fold (data not shown). The CYP2A6/CYP2A7 ratios ranged from 0.1 to 10.1 (Table 4). Under these assay conditions, the amount of hepatic CYP2A13 was below the limit of detection. Thus, CYP2A13 mRNA constitutes a very minor fraction of total CYP2A expression in the liver. The nasal mucosa samples were too small to yield amounts of mRNA that could be determined spectrophotometrically, hence direct comparison of CYP2A6 and CYP2A13 mRNA contents between nasal mucosa and liver samples was not feasible.

Search for CYP2A Sequences in dbEST DataBank

Since the qualitative RT-PCR results suggested that *CYP2A* genes are expressed almost exclusively in the liver, we searched if sequences related to CYP2A cDNAs are found in the expressed sequence tags (dbEST) database of the National Center for Biotechnology Information (Bethesda, MD). The dbEST release number 052998 (May 1998) was searched using CYP2A as a keyword. Only liver sequences were found among the 1,026,012 entries in the human dbEST database, indicating that extrahepatic expression of *CYP2A* genes in humans indeed occurs rarely.

TABLE 4. Relative levels of CYP2A6 and CYP2A7 in five individual liver samples

Liver sample	Ratio CYP2A6/CYP2A7
HL 1	10.1
HL 2	1.0
HL 4	2.0
HL 6	0.1
HL 7	1.3

The ratio CYP2A6/CYP2A7 was calculated from the amounts of the respective PCR products.

DISCUSSION

This study shows that all three *CYP2A* genes are transcribed in human liver, and that out of several extrahepatic tissues and organs studied, only nasal mucosa appears to contain detectable levels of CYP2A13 and CYP2A6 mRNA. It is well established that the RT-PCR technique can detect extremely small amounts of mRNA, often present at levels that may not be physiologically meaningful. On the other hand, a clear merit of this methodology is its high level of specificity, ensuring that only correct transcripts are being measured. Since the levels of hepatic CYP2A6 mRNAs and proteins are well characterized [4, 19], we used PCR conditions that amplify liver CYP2A cDNAs to clearly visible bands in gel electrophoresis, and applied exactly the same conditions to the other tissues. This allows for a rough comparison of the amount of mRNA expressed between liver and other tissues. Thus, the lack of amplification in the particular tissue being studied was considered to parallel the paucity of significant amounts of mRNA in it.

The present data show that all three *CYP2A* genes are transcribed in human liver. Ding *et al.* used [27] an RT-PCR and diagnostic restriction enzyme-cutting strategy which gave an approximate 1:1 ratio of CYP2A6 and CYP2A7 mRNAs in human liver samples. The present results on the relative amounts of hepatic CYP2A6 and CYP2A7 accord well with those of Ding *et al.* [27]. CYP2A13 mRNA proved to be present at negligible levels in the liver. While CYP2A6 protein efficiently catalyzes coumarin 7-hydroxylation [3, 4], the catalytic activities mediated by CYP2A7 are obscure [13, 27]. Nothing is currently known about CYP2A13 protein properties. A CYP2A7 splicing variant lacking exon 2 has been found [27]. This variant was shown to be present in the liver and to be a major transcript in a human fibroblast cell line [27]. Under our PCR conditions with CYP2A7-specific primers, three amplification products were detected. In addition to the product of the expected size, a fragment of 620 bp that matches in size the splice variant CYP2A7AS [27] was detected in all livers

tested. A strong hybridizing product of about 450 bp was also present in all the livers. These results suggest that several splicing variants of CYP2A7 mRNA exist in the human liver.

Members of the CYP2A subfamily are also expressed in nasal tissue of several animal species. Two cDNAs, CYP2A10 and CYP2A11, have been isolated from a rabbit nasal mucosa cDNA library [28]. These encode a CYP protein [NMa] which is abundantly present in rabbit nasal microsomes [28]. There is also evidence for the presence of protein[s] of the CYP2A subfamily in rat [29–31], mouse [22, 32], and bovine [33, 34] nasal mucosa. Immunohistochemical studies with anti-NMa antibody suggest that CYP2A protein[s] exist in several nasal cell types in humans [35]. Two recent studies [22, 23] show that CYP2A6 mRNA is detectable in human nasal respiratory mucosa by sensitive RT-PCR. Under our PCR conditions, which readily detect CYP2A6 mRNA in the liver, only very faint amplification products were obtained in nasal mucosal samples, while the amount of CYP2A13 mRNA appeared to be higher in the nasal mucosa than in the liver.

The present study demonstrates that CYP2A6 mRNA is present at a very low level in nasal mucosa and that CYP2A13 predominates CYP2A6 in this tissue. This suggests that CYP2A6, if translated to a functional protein, may make a very minor contribution to total P450 content in nasal mucosa. In this study, we did not analyze olfactory epithelia, where CYP2A concentration is highest [29, 30]. On the other hand, CYP2A immunoreactivity also appears to be present in respiratory areas in humans [35], which is in agreement with the data presented here. It is presently unknown whether the *CYP2A13* gene encodes a functional mRNA and protein product. Nevertheless, cross-reactivity of protein(s) in human nasal mucosa with anti-Nma antibody [35] does suggest that a protein belonging to the CYP2A subfamily is present in that organ. This putative protein could mediate the observed relatively high *N*-diethylnitrosamine metabolism in human nasal mucosa [36].

Three *CYP2A* genes also exist in the mouse. In the kidney, CYP2A5 (coumarin 7-hydroxylase) is present at a high level in male mice and cyp2A4 (testosterone 15α-hydroxylase) is predominant in female mice [2]. Both males and females have a similar amount of CYP2A4 and CYP2A5 in the liver. Clear species differences exist, since a CYP2A5 orthologue is not expressed in rat liver or kidney [37]. A recent study shows that heterologously expressed CYP2A6 mediates oxidative defluorination of methoxyflurane, and that coumarin inhibits this reaction in kidney microsomes [15]. In the present study, no transcripts were found of any of the three *CYP2A* genes in human kidney. Tumorous kidney tissue was also negative. It is thus highly unlikely that a CYP2A protein exists in human kidney.

CYP2A is expressed in the lung tissue of rat [37] and mouse [38]. Information concerning the expression of *CYP2A* genes in human lung is important, since CYP2A6 has been shown to be a high-affinity activator of tobacco-specific nitrosamines [5–7]. Recent data obtained with indirect methods suggest that CYP2A6 may catalyze the oxidation of tobacco-specific nitrosamines in human lung microsomes [20]. The results presented here indicate that CYP2A6, CYP2A7, and CYP2A13 mRNAs are not present in whole lung tissue at significant levels. We have also analyzed lung samples with another set of primers detecting CYP2A6 and CYP2A7 simultaneously, with the same result [39]. It is therefore unlikely that CYP2A proteins are present in human lung, and that procarcinogens present in tobacco smoke are metabolized *in situ* in lung cells by CYP2A forms. In agreement with this, a recent study demonstrated that CYP2A6 mRNA can be detected in human bronchial mucosa by RT-PCR, but the corresponding protein was not found [40]. In our own studies, we have found that the CYP2A6 marker activity, coumarin 7-hydroxylation, is absent in human lung microsomes (Raunio et al., unpublished data). In contrast, there is clear evidence for the presence of other CYP proteins in the lung, including members of the CYP1A, CYP2B, and CYP3A subfamilies [41], and these forms may well contribute to metabolic activation of toxic chemicals directly in the lung.

No indications for CYP2A transcripts were found in duodenum, peripheral blood lymphocytes, pulmonary macrophages, uterine endometrium, and placenta. All these tissues contain variable levels of other CYP forms, but in agreement with this study, no evidence exists for the presence of CYP2A members in these tissues in animals [41].

In conclusion, this study shows that *CYP2A* gene transcription occurs in human liver and nasal mucosa and that the transcripts are lacking in a variety of other major organs and tissues. This notion was supported by the lack of extrahepatic ESTs corresponding to CYP2A sequences. These findings establish a basis for further studies on the presence and role of CYP2A proteins in the liver and nasal mucosa.

We thank Risto Tuimala, Sirpa Rintala, and Teuvo Tammela (University of Tampere, Finland) for providing kidney samples, and Gerhard Bookjans and Peter Munzel (Institute of Toxicology, University of Tubingen, Germany) for providing duodenal samples. Pauli Wirta and Markku Pasanen helped in obtaining endometrium and placental samples. Financial support was provided by The Academy of Finland [Contracts 34555 and 29456].

References

1. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC and Nebert DW, P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6:** 1–42, 1996.
2. Chang TKH and Waxman DJ, The CYP2A subfamily. In: *Cytochromes P450. Metabolic and Toxicological Aspects* (Ed. Ioannides C), pp. 99–134. CRC Press, Boca Raton FL, 1996.
3. Pelkonen O and Raunio H, Individual expression of carcinogen-metabolizing enzymes: cytochrome P4502A. *J Occup Environ Med* **37:** 19–24, 1995.

4. Fernandez-Salguero P and Gonzalez FJ, The *CYP2A* gene subfamily: Species differences, regulation, catalytic activities and role in chemical carcinogenesis. *Pharmacogenetics* **5:** S123–S128, 1995.
5. Yamazaki H, Inui Y, Yun C-H, Guengerich FP and Shimada T, Cytochrome P450 2E1 and 2A6 enzymes as major catalysts for metabolic activation of *N*-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. *Carcinogenesis* **13:** 1789–1794, 1992.
6. Tiano HF, Hosokawa M, Clulada PC, Smith PB, Wang R, Gonzalez FJ, Crespi CL and Langenbach R, Retroviral-mediated expression of human cytochrome P450 2A6 in C3H/10T1/2 cells confers transformability by 4-(methylnitrosamino)-1-(3-pyridyl) 1-butanone (NNK). *Carcinogenesis* **14:** 1421–1427, 1993.
7. 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.
8. Camus A-M, Geneste O, Honkakoski P, Bereziat J-C, Henderson CJ, Wolf CR, Bartsch H and Lang MA, High variability of nitrosamine metabolism among individuals: Role of cytochromes P450 2A6 and 2E1 in the dealkylation of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine in mice and humans. *Mol Carcinog* **7:** 268–275, 1993.
9. Aoyama T, Yamano S, Guzelian PS, Gelboin HV and Gonzalez FJ, Five of 12 forms of vaccinia virus-expressed human hepatic cytochrome P450 metabolically activate aflatoxin B_1. *Proc Natl Acad Sci* USA **87:** 4790–4793, 1990.
10. Yun C-H, Shimada T and Guengerich FP, Purification and characterization of human liver microsomal cytochrome P-450 2A6. *Mol Pharmacol* **40:** 679–685, 1991.
11. Salonpää P, Hakkola J, Pasanen M, Pelkonen O, Vähäkangas K, Battula N, Nouso K and Raunio H, Retrovirus-mediated stable expression of human CYP2A6 in mammalian cells. *Eur J Pharmacol Environ Toxicol Pharmacol* **248:** 95–102, 1993.
12. Duescher RJ and Elfarra AA, Human liver microsomes are efficient catalysts of 1,3-butadiene oxidation: Evidence for major roles by cytochromes P450 2A6 and 2E1. *Arch Biochem Biophys* **311:** 342–349, 1994.
13. Yamano S, Tatsuno J and Gonzalez FJ, The CYP2A3 gene product catalyzes coumarin 7-hydroxylation in human liver microsomes. *Biochemistry* **29:** 1322–1329, 1990.
14. 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.
15. Kharasch ED, Hankins DC and Thummel KE, Human kidney methoxyflurane and sevoflurane metabolism. Intrarenal fluoride production as a possible mechanism of methoxyflurane nephrotoxicity. *Anesthesiology* **82:** 689–699, 1995.
16. Nunoya K-I, Yokoi T, Kimura K, Kodama T, Funayama M, Inoue K, Nagashima K, Funae Y, Shimada N, Green C and Kamataki T, (+)-*Cis*-3,5-dimethyl-2-(3-pyridyl) thiazolidin-4-one hydrochloride (SM-12502) as a novel substrate for cytochrome P450 2A6 in human liver microsomes. *J Pharmacol Exp Ther* **277:** 768–774, 1996.
17. Nakajima M, Yamamoto T, Nunoya K-I, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N and Kuroiwa Y, Role of human cytochrome P4502A6 in C-oxidation of nicotine. *Drug Metab Dispos* **24:** 1212–1217, 1996.
18. Nakajima M, Yamamoto T, Nunoya K-I, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N, Kamataki T and Kuroiwa Y, Characterization of CYP2A6 involved in 3′-hydroxylation of cotinine in human liver microsomes. *J Pharmacol Exp Ther* **277:** 1010–1015, 1996.
19. Pelkonen O, Raunio H, Rautio A, Mäenpää J and Lang MA, Coumarin 7-hydroxylase: Characteristics and regulation in mouse and man. *J Irish Coll Phys Surg* **22:** 24–28, 1993.
20. Smith TJ, Stoner GD and Yang CS, Activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in human lung microsomes by cytochromes P450, lipoxygenases and hydroperoxidases. *Cancer Res* **55:** 5566–5573, 1995.
21. Shimada T, Yamazaki H, Mimura M, Wakamiya N, Ueng Y-F, Guengerich FP and Inui Y, Characterization of microsomal cytochrome P450 enzymes involved in the oxidation of xenobiotic chemicals in human fetal livers and adult lungs. *Drug Metab Dispos* **24:** 515–522, 1996.
22. Su T, Sheng JJ, Lipinskas TW and Ding X, Expression of *CYP2A* genes in rodent and human nasal mucosa. *Drug Metab Dispos* **24:** 884–890, 1996.
23. Thornton-Manning JR, Nikula KJ, Hotchkiss JA, Avila KJ, Rohrbacher KD, Ding X and Dahl AR, Nasal cytochrome P450 2A: Identification, regional localization, and metabolic activity toward hexamethylphosphoramide, a known nasal carcinogen. *Toxicol Appl Pharmacol* **142:** 22–30, 1997.
24. Fernandez-Salguero P, Hoffman SMG, Cholerton S, Mohrenweiser H, Raunio H, Rautio A, Pelkonen O, Huang J, Evans WE, Idle JR and Gonzalez FJ, A genetic polymorphism in coumarin 7-hydroxylation: Sequence of the human *CYP2A* genes and identification of variant *CYP2A6* alleles. *Am J Hum Genet* **57:** 651–660, 1995.
25. Hoffman SMG, Fernandez-Salguero P, Gonzalez FJ and Mohrenweiser HW, Organization and evolution of the cytochrome P450 *CYP2A-2B-2F* subfamily gene cluster on human chromosome 19. *J Mol Evol* **41:** 894–900, 1995.
26. Jin C-F, Mata M and Fink DJ, Rapid construction of deleted DNA fragments for use as internal standards in competitive PCR. *PCR Meth Appl* **3:** 252–255, 1994.
27. Ding S, Lake BG, Friedberg T and Wolf CR, Expression and alternative splicing of the cytochrome P-450 CYP2A7. *Biochem J* **306:** 161–166, 1995.
28. Peng H-M, Ding X and Coon MJ, Isolation and heterologous expression of cloned cDNAs for two rabbit nasal microsomal proteins, CYP2A10 and CYP2A11, that are related to nasal microsomal cytochrome P450 form a. *J Biol Chem* **268:** 17253–17260, 1993.
29. Chen Y, Getchell ML, Ding X and Getchell TV, Immunolocalization of two cytochrome P450 isozymes in rat nasal chemosensory tissue. *Neuro Report* **3:** 749–752, 1992.
30. Zupko K, Poria Y and Lancet D, Immunolocalization of cytochromes P-450olf1 and P-450olf2 in rat olfactory mucosa. *Eur J Biochem* **196:** 51–58, 1991.
31. Bereziat J-C, Raffalli F, Schmezer P, Frei E, Geneste O and Lang MA, Cytochrome P450 2A of nasal epithelium: Regulation and role in carcinogen metabolism. *Mol Carcinog* **14:** 130–139, 1995.
32. Walters E, Buchheit K and Maruniak JA, Olfactory cytochrome P-450 immunoreactivity in mice is altered by dichlobenil but preserved by metyrapone. *Toxicology* **81:** 113–122, 1993.
33. Lazard D, Tal N, Rubinstein M, Khen M, Lancet D and Zupko K, Identification and biochemical analysis of novel olfactory-specific cytochrome P-450IIA and UDP-glucuronosyl transferase. *Biochemistry* **29:** 7433–7440, 1990.
34. Longo V, Amato G, Santucci A and Gervasi PG, Purification and characterization of three constitutive cytochrome P-450 isoforms from bovine olfactory epithelium. *Biochem J* **323:** 65–70, 1997.
35. Getchell ML, Chen Y, Sparks DL, Ding X and Getchell TV, Immunohistochemical localization of a cytochrome P-450 isozyme in human nasal mucosa: age-related trends. *Ann Otol Rhinol Laryngol* **102:** 368–374, 1993.

36. Reed CJ, Drug metabolism in the nasal cavity: Relevance to toxicology. *Drug Metab Rev* **25:** 173–205, 1993.
37. Kimura S, Kozak CA and Gonzalez FJ, Identification of a novel P450 expressed in rat lung: cDNA cloning and sequence, chromosome mapping, and induction by 3-methylcholanthrene. *Biochemistry* **28:** 3798–3803, 1989.
38. Honkakoski P, Mäenpää J, Leikola J, Pasanen M, Juvonen R, Lang MA, Pelkonen O and Raunio H, Cytochrome P4502A-mediated coumarin 7-hydroxylation and testosterone hydroxylation in mouse and rat lung. *Pharmacol Toxicol* **72:** 107–112, 1993.
39. Hukkanen J, Hakkola J, Anttila S, Piipari R, Karjalainen A, Pelkonen O and Raunio H, Detection of mRNA encoding xenobiotic-metabolizing cytochrome P450s in human broncho-alveolar macrophages and peripheral blood lymphocytes. *Mol Carcinog* **20:** 224–230, 1997.
40. Mace K, Bowman ED, Vautravers P, Shields PG, Harris CC and Pfeifer AMA, Characterisation of xenobiotic-metabolising enzyme expression in human bronchial mucosa and peripheral lung tissue. *Eur J Cancer* **34:** 914–920, 1998.
41. Raunio H, Pasanen M, Mäenpää J, Hakkola J and Pelkonen O, Expression of extrahepatic cytochrome P450 in humans. In: *Advances in Drug Metabolism in Man* (Eds. Pacifici GM and Fracchia GN), pp. 234–287. European Commission, Office for Official Publications of the European Communities, Luxembourg, 1995.