Biochemical Pharmacology, Vol. 51, pp. 403–411, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02184-1

Expression of Xenobiotic-Metabolizing Cytochrome P450 Forms in Human Full-Term Placenta

Jukka Hakkola,[1] *Markku Pasanen,*[2] *Janne Hukkanen,*[1] *Olavi Pelkonen,*[1] *Jukka Mäenpää,*[1] *Robert J. Edwards,*[3] *Alan R. Boobis*[3] *and Hannu Raunio*[1]

[1]Department of Pharmacology and Toxicology, University of Oulu, 90220 Oulu, Finland,
[2]Department of Pharmacology and Toxicology, University of Kuopio, 90721 Kuopio, Finland, and
[3]Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 0NN, U.K.

ABSTRACT. The expression of individual xenobiotic-metabolizing cytochrome P450 (CYP) genes in human placenta was studied at the mRNA level by reverse transcriptase-polymerase chain reaction (RT-PCR). mRNAs of CYP1A1, CYP2E1, CYP2F1, CYP3A3/4, CYP3A5, and CYP4B1 were detected by RT-PCR, and CYP1A2, CYP2A6/7, CYP2B6/7, CYP2C8-19, CYP2D6, and CYP3A7 were not detected. Several enzyme activity assays and immunoblots were used to further characterize expression of forms producing detectable mRNA transcripts. The catalytic activities of 7-ethoxycoumarin O-deethylase (ECOD), 7-ethoxyresorufin O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) were substantially increased in response to maternal cigarette smoking, and paralleled the amount of CYP1A1 mRNA and protein. Aromatase activities were slightly lower in placentas exposed to cigarette smoke compared with nonexposed placentas. These data show that several xenobiotic-metabolizing CYP genes are expressed in human placenta at a low level. The significance of such low-level expression is unknown, but it may have local physiological or toxic consequences. BIOCHEM PHARMACOL 51;4:403–411, 1996.

KEY WORDS. cytochrome P450; maternal-fetal exchange; smoking; polymerase chain reaction

The placenta plays a vital role in maintenance of pregnancy by delivering oxygen and nutrients from the maternal circulation to the fetus and by returning fetal metabolites to the mother. The placenta also has many crucial endocrine and metabolic functions [1, 2]. Numerous foreign compounds reach the placenta through the maternal circulation and human placental tissue is capable of oxidizing several of them, although the substrate profile compared with liver appears to be more restricted [3].

CYP‡§ enzymes constitute an important part of the complex system that metabolizes a number of endogenous and exogenous substrates [4]. The CYP superfamily consists of families and subfamilies defined on the basis of their amino acid sequence similarities. The CYP forms can be functionally divided into 2 groups: families CYP5–27 are involved with synthesis of important endogenous molecules, and the xenobiotics are metabolized mainly by families CYP1–4 [4, 5].

In humans, 19 xenobiotic-metabolizing P450s are currently known [5]. The majority of these genes are expressed most abundantly in liver, but the CYP1A1, CYP2F1, and CYP4B1 forms have been detected mainly in extrahepatic tissues [5]. The P450 enzymes usually catalyze oxidative metabolism of xenobiotics as a part of the process of inactivation and elimination from the body [6]. The activity of CYP enzymes may sometimes lead to the formation of reactive metabolites with toxic or carcinogenic consequences [6, 7]. It has become evident that xenobiotic-metabolizing CYP enzymes may also be involved in vital cell functions, affecting growth, differentiation, and homeostasis [8, 9].

Relatively little is known about the individual CYP forms present in human placenta. *CYP1A1* is expressed in placentas of those women who were exposed to polychlorinated biphenyls or smoked cigarettes [3, 10–13], but the significance of this induction to fetal toxicity is unknown [8, 14]. In addition, mRNA and protein of CYP3A7, the prominent form in fetal liver, have been detected in early-term human placenta [15]. *CYP4B1* cDNA has been cloned from a human placenta cDNA library [16]. To understand more thoroughly the function of CYP system in placenta and its significance in the metabolism of xenobiotics and endogenous compounds, infor-

* Corresponding author: J. Hakkola, Department of Pharmacology and Toxicology, University of Oulu, Kajaanintie 52 D, 90220 Oulu, Finland. Tel. 358-81-537 5242; FAX. 358-81-330 687.

† A preliminary report of this study was presented at the 10th International Symposium on Microsomes & Drug Oxidations, Toronto, Canada, July 18–21, 1994.

‡ *Abbreviations:* CYP, cytochrome P450; RT-PCR, reverse transcriptase-polymerase chain reaction; AHH, aryl hydrocarbon hydroxylase; ECOD, 7-ethoxycoumarin O-deethylase; and EROD, 7-ethoxyresorufin O-deethylase.

§ Under the recommended P450 nomenclature (4) used in this study, italicized form names refer to the gene or cDNA and nonitalicized names to the mRNA or protein.

Received 7 June 1995; accepted 4 October 1995.

mation on the expression of individual members of the CYP superfamily in placental tissue is required.

The study of CYP gene expression in placenta has been hindered by the low levels of the CYP forms present. The RT-PCR method enables the sensitive and specific detection of low quantities of mRNA [17]. In this study we used the RT-PCR method to examine the expression of all known xenobiotic-metabolizing CYP genes in full-term placenta. For CYP1A, CYP2E1, and CYP3A, the content and activity of the expressed proteins were also investigated.

MATERIALS AND METHODS

Human Placental Tissue

Human full-term placentas were obtained from the Department of Obstetrics and Gynaecology, University of Oulu. Clinical data for the donors are summarized in Table 1. Use of the human tissue in this study was approved by the Ethics Committee of the University of Oulu.

Extraction of RNA and cDNA Synthesis

Small pieces of the placenta were cut and frozen in liquid nitrogen immediately after delivery and stored at −70°C for later extraction of RNA. Total RNA was extracted with guanidium thiocyanate followed by centrifugation in cesium chloride. Complementary DNA was synthesized with a First-Strand cDNA Synthesis Kit (Pharmacia P-L Biochemicals, Uppsala, Sweden); 5 μg total RNA was used in each synthesis. The cDNA was stored at −20°C until PCR amplification was performed.

PCR

The PCR reaction contained 1 μL of cDNA (out of 15 μL total), 2.5 U DynaZyme DNA polymerase (Finnzymes, Helsinki, Finland), 5 μL 10x DynaZyme reaction buffer, dNTP reaction mix (Finnzymes) at a final concentration of 200 μM, 20–50 pmol of each primer, and water to a final volume of 50 μL. 35 PCR cycles were performed: 1 min at 94°C, 1 min at 55°C, 2 min at 72°C.

The primers were designed to hybridize only to the desired regions of the cDNA. To exclude the chance of crosshybridization with other sequences, each primer was compared with the EMBL human gene bank using the Genetics Computer Group FASTA program [18]. Because the individual members in the CYP3A subfamily are highly similar to each other, we verified the specificity of each pair of CYP3A primers using full-length cDNAs as amplification templates. The CYP primers, their locations, and the sizes of the PCR products are listed in Table 2. The following primers can detect more than one form: CYP2A6/2A7, CYP2B6/2B7, and CYP3A3/3A4. The CYP2C (8–19) primers were designed to detect all known human *CYP2C* cDNAs (i.e., *2C8*, *2C9*, *2C10*, *2C18*, *2C19*).

In every series of PCR reactions there were 2 negative controls, one containing cDNA synthesis reagents excluding template and the one containing RNA that was not reverse transcribed. Otherwise, these controls were identical to the sample reactions. To control the efficiency of the PCR reaction, a positive control (human liver or lung sample) was used in all PCR series. After the PCR reaction, 8 μL of the reaction mixture was electrophoresed in an agarose gel and stained with ethidium bromide. All PCR reactions were repeated at least twice.

To ensure the identity of the PCR products the DNA was transferred to Qiabrane nylon filter (Qiagen, Chatsworth, CA) and hybridized with specific, ^{32}P-labelled cDNA probes. The *CYP2E1*, *CYP2F1*, *CYP3A4*, and *CYP3A5* full-length cDNAs were generous gifts from Dr. F. J. Gonzalez (Laboratory of Molecular Carcinogenesis, National Cancer Institute, NIH, Bethesda, MD). The *CYP1A1* (800 bp) and *CYP4B1* (600 bp) cDNA probes were prepared by first amplifying the respective sequences and then cloning the amplification products into pCR™II (TA-cloning Kit, Invitrogen, San Diego, CA). Identity of the cloned probes was verified by direct sequencing (Cycle Sequencing Kit, Pharmacia, Uppsala, Sweden).

Semiquantitative PCR Analysis

Quantification of selected CYP cDNAs was achieved by simultaneous amplification with β-actin. The exponentially of

TABLE 1. Clinical data for placenta donors

Placenta	Maternal smoking	Maternal alcohol consumption	Drug treatment during pregnancy	Description of placenta
1	–	–	–	normal
2	–	–	–	normal
3	–	–	–	normal
4	–	–	ferrosulfate 200 mg/day	calcified
5	–	–	paracetamol occasionally	normal
6	20 cigarettes/day, quit a month before delivery	–	–	calcified
7	–	–	pivmecillinam 200 mg/day	normal
8	10 cigarettes/day	?	?	?
10	5–10 cigarettes/day, quit at fifth week of gestation	–	–	thick
11	5 cigarettes/day	–	–	calcified

TABLE 2. Primers used for PCR analysis

CYP	Sense primer location	Antisense primer location	PCR product (bp)	Reference
1A1	TCACAGACAGCCTGATTGAG 928–947	GATGGGTTGACCCATAGCTT 1341–1360	432	19
1A2	TGGCTTCTACATCCCCAAGAAAT 1199–1221	TTCATGGTCAGCCCGTAGAT 1488–1507	308	20
2A6/2A7	GTGTGGACATGATGCCGT 252–269	AGGACTTGAGGCGGAAGT 1385–1402	1151	21
2B6/2B7	CCATACACAGAGGCAGTCAT 1045–1064	GGTGTCAGATCGATGTCTTC 1402–1421	357	22
2C8-19	GCTAAAGTCCAGGAAGAGATTGA	TCCTGCTGAGAAAGGCATGAAGT	332	23, 24
2D6	TGATGAGAACCTGCGCATAG 873–892	ACCGATGACAGGTTGGTGAT 1186–1205	332	25
2E1	AGCACAACTCTGAGATATGG 925–944	ATAGTCACTGTACTTGAACT 1271–1290	365	26
2F1	ATGAACTTGCCGCACCGCGT 1148–1167	AGCGAAAAGCTCTGCAGGAT 1412–1431	283	27
3A3/3A4	CCAAGCTATGCTCTTCACCG 1279–1298	TCAGGCTCCACTTACGGTGC 1583–1602	323	28, 29
3A5	TGTCCAGCAGAAACTGCAAA 1065–1084	TTGAAGAAGTCCTTGCGTGTC 1516–1535	470	30
3A7	CTATGATACTGTGCTACAGT 1041–1060	TCAGGCTCCACTTACGGTCT 1496–1515	474	31
4B1	TGACCATGTGCATCAAAGGAG 1109–1128	AAAGCCATTCTTGGAGCGCA 1487–1506	397	32

amplification was studied as shown in Fig. 1; 25 PCR cycles were used in the actual experiments. Fifty pmol of CYP and 15 pmol of β-actin primers were used. PCR products were electrophoresed in an agarose gel, transferred to a nylon filter and hybridized with cDNA probes. An autoradiography film was exposed to the hybridized filters, and the intensities of the resulting bands were measured by densitometry.

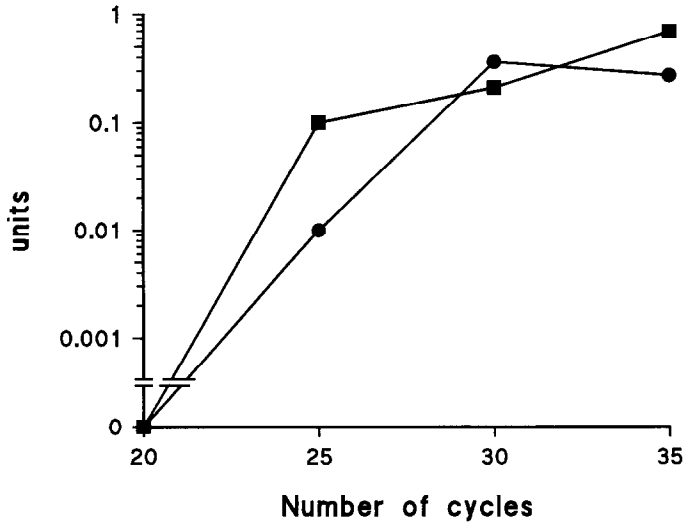

FIG. 1. Exponentiality of CYP1A1 and β-actin co-amplification. ■ CYP1A1, ● β-actin. 5-μL aliquots were removed from the reaction mixture after every 5 cycles (20–35 cycles). These samples were electrophoresed, transferred to nylon filter and hybridized with cDNA probes. Densitometric values were obtained from autoradiographs.

Microsomes and Enzymes Assays

Microsomes were prepared by the standard ultracentrifugation technique as described earlier [33]. Enzyme assays were performed as described: AHH [34], ECOD [35], EROD [36], aromatase [37], androstenedione formation from testosterone [38], cholesterol side-chain cleavage [39], testosterone 6β-hydroxylase [38], benzphetamine N-demethylase [40], erythromycin N-demethylase [41], and chlorozoxazone hydroxylase [42]. All enzyme assays were carried out in duplicate.

Immunoblot Analysis

ANTIBODIES. Antibodies against the human P450 enzymes CYP1A1, CYP3A4/7, and CYP2E1 were produced by targeting antipeptide antibodies against the C-termini of these P450 enzymes. The production of the anti-CYP2E1 has been described previously [43]. Antibodies against CYP1A1 and CYP3A4/7 were produced in a similar manner. The peptides Cys-Met-Gln-Leu-Arg-Ser and Cys-Thr-Val-Ser-Gly-Ala, representing the C-termini of CYP1A1 and CYP3A4/7, respectively (the C-termini of CYP3A4 and CYP3A7 are identical), were synthesized, coupled to keyhole limpet haemocyanin, and used to immunize rabbits as described previously [44]. The production and characterization of the antibody against human CYP1A2 is described elsewhere [45].

SDS-polyacrylamide gel electrophoresis was performed according to Laemmli [46] and protein was transferred to nitrocellulose sheets [47]. Immunoblotting was performed as described previously [48]. Blots of placenta samples developed for CYP1A1 apoprotein were compared with yeast microsomal fraction containing recombinant human CYP1A1 and control

yeast microsomal fraction; these samples were generous gifts from Dr. Michael Ching [49].

Immunoinhibition Studies

Polyclonal antirat CYP2E1 antibody was generously provided by Dr. M. Ingelman-Sundberg (Department of Physiological Chemistry, Karolinska Institute, Stockholm, Sweden). This antibody crossreacts with human CYP2E1 and was used to inhibit ECOD activity in placental samples from one smoking and one nonsmoking mother. Antibody/microsomal protein ratio was 1/1. For the immunoinhibition studies, the ECOD activity was performed according to Greenlee and Poland [50].

RESULTS

PCR

Ten human full-term placentas (6 from nonsmoking and 4 from smoking mothers) were used for detection of CYP mRNAs by RT-PCR. The presence of a band of the correct size in agarose gels was regarded as evidence of gene expression. To ensure the quality of cDNA and success of PCR, β-actin and 2 abundantly expressed CYP forms, CYP11A1 (cholesterol side-chain cleavage) and CYP19 (aromatase), were amplified. These forms were readily detected in all of the placenta samples by RT-PCR. Ethidium bromide-stained gels of β-actin, CYP11A1, and CYP19 amplification products are shown in Fig. 2.

Expression of forms in families *CYP1–CYP4* was then studied. The PCR products were transferred to nylon filters and hybridized with specific cDNA probes. The autoradiographs from these filters are shown in Fig. 3. The results indicate that *CYP1A1*, *CYP2E1*, *CYP2F1*, *CYP3A3/4*, *CYP3A5*, and *CYP4B1* were expressed in at least some of the samples, and all other known xenobiotic-metabolizing forms (CYP1A2, CYP2A6/7, CYP2B6/7, CYP2C8–19, CYP2D6, CYP3A7) were absent.

Several studies have suggested that maternal smoking induces CYP1A1 in placenta. Therefore, a semiquantitative RT-PCR assay based on co-amplification of β-actin and CYP1A1 messages was performed. The results showed an association between the level of CYP1A1 expression and maternal smoking (Fig. 4). CYP1A1 mRNA could also be detected in placentas of nonsmoking mothers (Fig. 4), particularly upon longer exposure of the autoradiography film (data not shown). Although the qualitative amplification of CYP4B1 suggested that its mRNA might be modulated by maternal cigarette smoking (Fig. 3), experiments in which CYP4B1 and β-actin were co-amplified failed to corroborate this (data not shown).

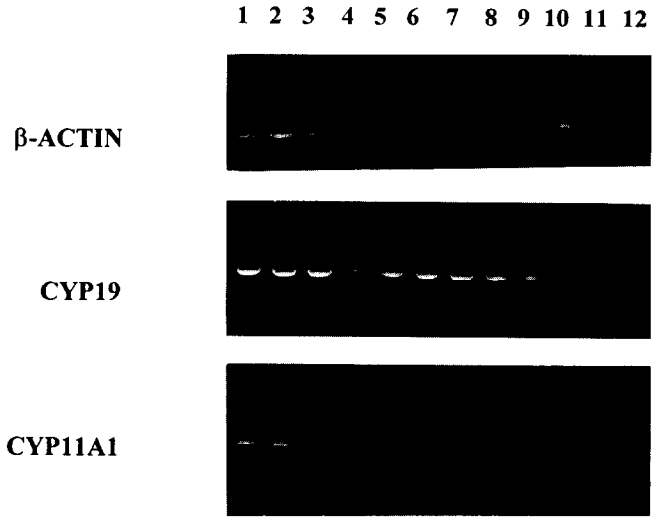

FIG. 2. Amplification of β-actin, CYP19, and CYP11A1 in placentas. Lane 1, placenta 1; lane 2, placenta 2; lane 3, placenta 3; lane 4, placenta 4; lane 5, placenta 5; lane 6, placenta 6; lane 7, placenta 7; lane 8, placenta 8; lane 9, placenta 10; lane 10, placenta 11; lanes 11 and 12, negative controls. Primers: β-actin: sense TGACGGGGTCACCCACACTGTGCCCATCTA, antisense CTAGAAGCATTGCGGTGGACGATGGAGGG; CYP 19: sense ATGCTGATCGCAGCTCCTGA, antisense GCAAGGACAAGTCGTGTATC; CYP11A1: sense TGAGTCCATCACTAACGTCA, antisense GTGCCATCTCATACAAGTGC. CYP19 is present, but poorly visible in placenta 11 (lane 10).

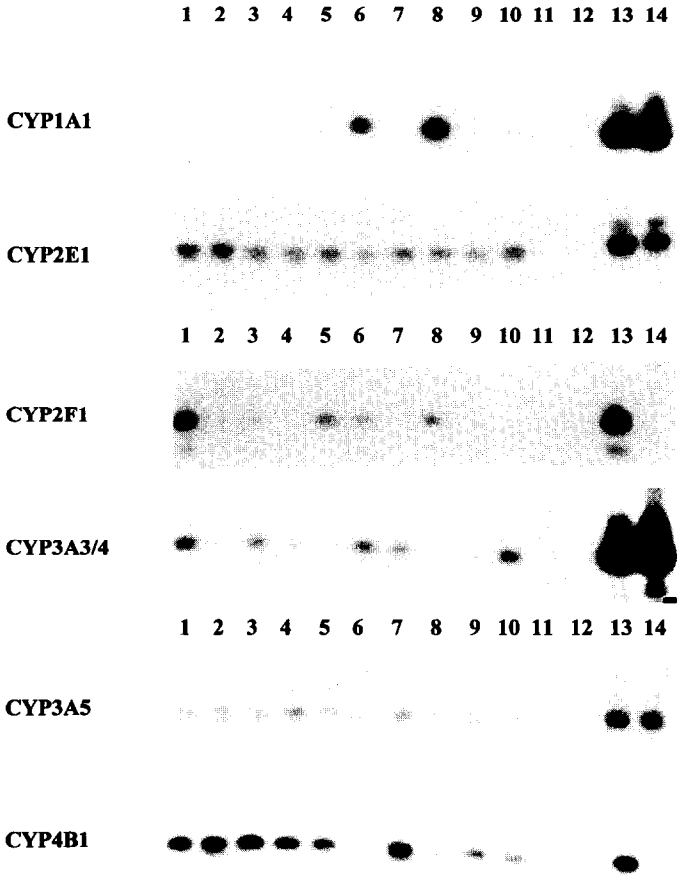

FIG. 3. Autoradiographs of the hybridized PCR products of CYP genes. Lane 1, placenta 1; lane 2, placenta 2; lane 3, placenta 3; lane 4, placenta 4; lane 5, placenta 5; lane 6, placenta 6; lane 7, placenta 7; lane 8, placenta 8; lane 9, placenta 10; lane 10, placenta 11; lanes 11 and 12, negative controls; lanes 13 and 14, adult liver, except CYP2F1 and CYP4B1 where lane 13, adult lung and lane 14, empty.

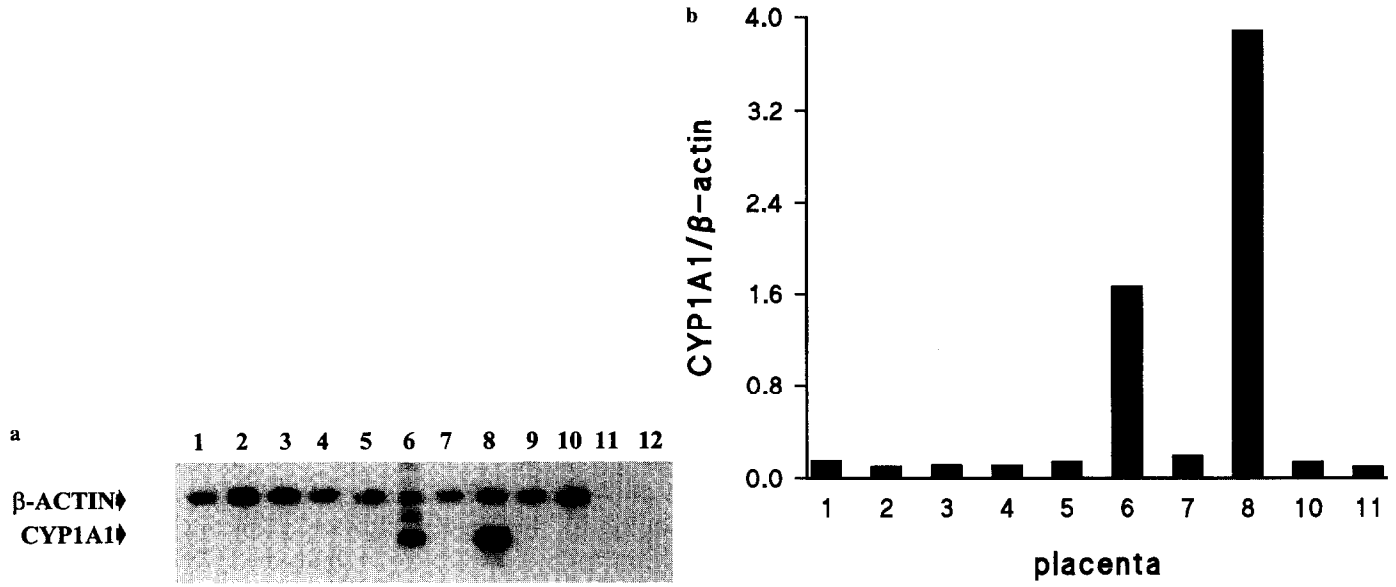

FIG. 4. (a) Co-amplification of CYP1A1 and β-actin. PCR products were electrophoresed in agarose gel, transferred to nylon filter and hybridized with CYP1A1 and β-actin cDNAs. The autoradiography exposure time was 1 hr. Lane 1, placenta 1; lane 2, placenta 2; lane 3, placenta 3; lane 4, placenta 4; lane 5, placenta 5; lane 6, placenta 6; lane 7, placenta 7; lane 8, placenta 8; lane 9, placenta 10; lane 10, placenta 11; lanes 11 and 12, negative controls. (b) Relative CYP1A1 mRNA contents in placentas. Densitometric values of CYP1A1 and β-actin bands were obtained from the autoradiograph in Fig. 4a, and the CYP1A1 values were corrected against those of β-actin.

Immunoblot Analysis and Enzyme Assays

Enzyme assays and immunoblot analysis were performed, in an attempt to detect expressed proteins corresponding to positive PCR results. The catalytic activities of aromatase (CYP19), cholesterol side-chain cleavage (CYP11A1), androstendione formation, AHH, ECOD, and EROD were readily detected. (Table 3). In placenta number 8, AHH, ECOD, and EROD activities were increased 19-fold, 9.5-fold, and 634-fold, respectively, in comparison with the mean activities in the placentas from nonsmoking mothers. There was a tendency to a lower aromatase activity in placentas from smoking mothers (Table 3), which is in agreement with Kitawaki *et al.* [51]. ECOD is among the only monooxygenase activities consistently found in all placentas regardless of exposure to cigarette smoke [3]. Human CYP2E1 has been shown to possess high ECOD activity in the vaccinia virus expression system [52]. Anti-CYP2E1 antibody, however, did not affect ECOD activity (data not shown), suggesting that this form does not mediate the constitutive activity of ECOD in placenta. The

TABLE 3. Enzyme activities of placental microsomes

Placenta	AHH	ECOD	EROD	AROM	ANDR	SCC
Placentas from nonsmokers						
1	110	17.1	0.07	42	270	nd
2	100	21.0	0.04	52	340	609
3	106	22.0	0.12	48	290	781
4	92	3.6	0.18	11	270	nd
5	96	15.5	0.07	46	370	843
7	104	11.0	0.34	27	230	423
mean	101	15.0	0.14	38	295	664
SD	6.7	6.9	0.11	15.6	51.2	188.7
Placentas from smokers or ex-smokers						
6	416	35.5	35.2	28	250	1115
8	1914	142.0	89.2	28	340	504
10	94	5.2	0.47	21	170	930
11	100	10.6	1.05	20	250	1130
mean	631	48.3	31.5	24	253	919
SD	868	63.8	41.8	4	69	292

AHH (fmol/mg protein/min), ECOD (pmol/mg protein/min), EROD (pmol/mg protein/min), aromatase (AROM) (pmol/mg protein/min), androstendione formation from testosterone (ANDR) (pmol/mg protein/min), cholesterol side-chain cleavage (SCC) (fmol/mg protein/min), nd (not determined).

activities of testosterone 6β-hydroxylase (CYP3A), benzphetamine N-demethylase (CYP3A?), erythromycin N-demethylase (CYP3A), and chlorozoxazone hydroxylase (CYP2E1) were below the level of detection in all placentas.

Antibodies against human CYP1A1, CYP1A2, CYP2E1, and CYP3A4/7 were used in immunoblotting experiments. Among the 10 placenta samples, immunoreactive CYP1A1 was detected only in sample number 8 (Fig. 5). Immunoreactive CYP1A2, CYP2E1, and CYP3A4/7 were not detected in any of the placenta samples (results not shown).

DISCUSSION

The main finding of this study was that mRNA of several individual xenobiotic-metabolizing CYP forms can be detected in full-term placentas by the RT-PCR method. Members in each of the families CYP1–4 were found. All of these forms, however, appear to be expressed at a very low level compared with liver. Apart from the cigarette smoke-induced CYP1A1, these forms could not be detected at protein level. A clear merit of the RT-PCR method is that it gives an overall picture of the possible CYP forms present in a given tissue. In other words, a form not detectable by RT-PCR is highly unlikely to be present, and a detectable form may be present at meaningful levels. Detection of mRNAs, thus, guides the direction of further studies.

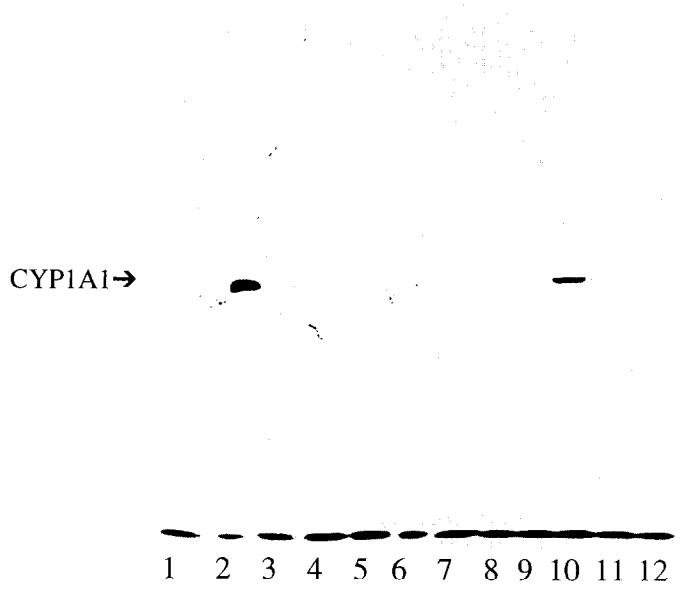

FIG. 5. Immunoblot of human placenta microsomal fractions developed with an antihuman CYP1A1 antibody. Either 10 μg yeast microsomal fraction containing recombinant P450 or 50 μg of placenta microsomal fraction was applied to each lane. The immunoblot was developed with an antipeptide antibody directed against human CYP1A1 as described in the text. Lane 1, recombinant control; lane 2, recombinant human CYP1A1; lane 3, placenta 1; lane 4, placenta 2; lane 5, placenta 3; lane 6, placenta 4; lane 7, placenta 5; lane 8, placenta 6; lane 9, placenta 7; lane 10, placenta 8; lane 11, placenta 10; lane 12, placenta 11.

There is considerable evidence that a member of the CYP1A subfamily is present in placentas from cigarette-smoking women [3]. Enzymatic data suggest that the form is CYP1A1 rather than CYP1A2 [53]. This study unequivocally confirms that CYP1A1, but not CYP1A2, is inducible in full-term placenta at the mRNA and protein level. In placenta samples 6 and 8, induction of CYP1A1 was demonstrated by semiquantitative PCR, the increase in AHH, EROD, and ECOD activities, and the detection of immunoreactive CYP1A1 apoprotein (only sample 8). The donor of placenta number 10 quit smoking at the fifth week of gestation and, thus, no induction of CYP1A1 could be expected. Induction of CYP1A1 also could not be seen in placenta number 11. The donor of placenta 11 smoked slightly less than the other smokers. This, however, is unlikely to be the reason for the total lack of induction. The cause for this phenomenon is unknown, but genetic differences in the factors affecting CYP1A1 induction may be the explanation. Both the PCR method and immunoblotting showed that CYP1A2 was absent. It is of interest that expression of CYP1A1 mRNA could also be detected in placentas from nonsmoking mothers. Whether this is true constitutive expression, or due to slight induction by environmental compounds, is unclear.

The Ah receptor, mediating CYP1A1 induction, has been shown to be abundantly expressed in human placenta [54, 55]. Presumably, mechanisms similar to the well-characterized murine CYP1A1 regulation system are behind the strong induction of this form in placenta. Interestingly, the murine *Ah receptor* gene promotor region has been shown to contain 2 DNA elements which, in other genes (human glycoprotein hormone α-subunit and placental lactogen II), confer placenta-specific expression [56]. Together, these findings suggest that CYP1A1 could be constitutively expressed in human placenta and that the induction of this form may be regulated in a tissue-specific fashion. It would be of considerable interest to elucidate the significance of CYP1A1 induction in cigarette smoke-caused fetal toxicity, such as lower birth weight and fetal anomalies [8].

CYP2E1 has been detected in several human extrahepatic tissues including lung [57], leukocytes from diabetics [58], and umbilical vein endothelial cells [59]. We were able to detect CYP2E1 mRNA from placenta by RT-PCR and have, thus, demonstrated yet another example of extrahepatic expression of this gene. However, CYP2E1 protein could not be detected immunochemically and there was no measurable chlorozoxazone 6-hydroxylase activity in placenta microsomes. This may be due to the lower sensitivity of the enzyme assay and immunoblotting methods used in this study relative to RT-PCR. Although it is well established that extremely low levels of mRNA can be detected by PCR, the correspondingly minute amounts of protein cannot be detected by current methods. On the other hand, there is evidence that posttranscriptional and posttranslational mechanisms play a major role in the regulation of CYP2E1 [60] and the absence of active protein could be due to lack of stabilizers of substrates. Indeed, CYP2E1 mRNA may not even be translated into protein in human placenta [61].

The first indication of the presence of the CYP3A subfamily in human placenta was demonstrated by Schuetz *et al.* [15], who were able to detect CYP3A7 with RNA- and immunoblotting techniques from first and second trimester placental samples. In this study we detected CYP3A3/4 and CYP3A5 but not CYP3A7 from full-term placentas with RT-PCR. The antipeptide antibody targeted against the C-terminus of CYP3A4/7 bound strongly to recombinant CYP3A4 (result not shown). Because the C-termini of CYP3A4 and CYP3A7 are identical, it is highly likely that the antibody will bind to both forms. However, no immunoreactive protein was found in any of placenta samples. The PCR method also failed to detect CYP3A7 and, although CYP3A4 was detected, the level of expression appeared to be extremely low. Consistent with this, no activity of testosterone 6β-hydroxylase activity, a functional marker of CYP3A, could be detected in any of the placentas studied. Interestingly, although the level of CYP3A7 increases from the first to second trimester of pregnancy [15], this enzyme is not detectable by either PCR or immunoblotting toward the end of pregnancy. CYP3A7 is also the most abundant P450 enzyme in fetal liver [62], and only small amounts can be detected in adult liver [63, 64]. It is tempting to speculate from these results that CYP3A7 has a specialized function during early pregnancy.

CYP2F1 [27] and *CYP4B1* [32] were originally cloned from human lung cDNA libraries. CYP2F1 has not been detected in any other tissue. However, in this study, we show that CYP2F1 mRNA is expressed at a low level in human placenta. In addition to lung, *CYP4B1* has been cloned from a placental cDNA library [16] and, in the present study, we found that placental expression of this gene is readily detectable by RT-PCR. Neither CYP2F1 nor CYP4B1 could be detected in human liver by RT-PCR [64]. Thus, these forms appear to be expressed only in extrahepatic tissues. CYP4B1 may participate in fatty acid hydroxylations as does rat CYP4A1 [65]. The function of CYP2F1 is unclear.

The observed lack of expression of CYP2A6, CYP2B6, CYP2C, and CYP2D6 mRNAs in placentas agrees well with the known lack of oxidative activity towards some marker substrates (e.g, S-mephenytoin and debrisoquine [3].

This study demonstrates the presence of mRNA of several CYP forms in families CYP1–4 in human placenta. Only limited detection of the respective proteins was achieved, which could be due to the relative insensitivity of the current protein detection methods. The significance of this low level expression is unclear. However, even very low activities may have local toxic consequences, especially in the activation of procarcinogens. There have also been suggestions that the main function of drug-metabolizing enzymes in extrahepatic tissues could be physiological, perhaps the metabolism of endogenous effector ligands [9]. This hypothesis could explain the low level of expression of several CYP forms in placenta.

We thank Dr. Päivi Pienimäki and Kaarina Pitkänen for assistance. The antirat CYP2E1 antibody was kindly provided by Dr. M. Ingelman-Sundberg and CYP2E1, CYP2F1, CYP3A4, *and* CYP3A5 *cDNAs were gifts from Dr. F. J. Gonzalez. This study was supported by the Academy of Finland (Medical Research Council, contract No. 1051029 and Environmental Research Council, contract No. 29456) and was carried out within the framework of the COST B1 action.*

References

1. Juchau MR, Drug biotransformation in the placenta. *Pharmacol Ther* **8:** 501–524, 1980.
2. Pasanen M and Pelkonen O, Human placental xenobiotic and steroid biotransformation catalyzed by cytochrome P450 epoxide hydroxylase and glutathione S-transferase activities and their relations to maternal cigarette smoking. *Drug Metab Rev* **21:** 427–446, 1989–1990.
3. Pasanen M and Pelkonen O, The expression and environmental regulation of P450 enzymes in human placenta. *Crit Rev Toxicol* **24:** 211–229, 1994.
4. 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.
5. Gonzalez FJ, Human cytochromes P450: problems and prospects. *Trends Pharm Sci* **13:** 346–352, 1992.
6. Wrighton SW and Stevens JC, The human hepatic cytochromes P450 involved in the drug metabolism. *Crit Rev Toxicol* **22:** 1–21, 1992.
7. Miles JS and Wolf CR, Developments and perspectives on the role of cytochrome P450s in chemical carcinogenesis. *Carcinogenesis* **12:** 2195–2199, 1991.
8. Beresford AP, CYP1A1: friend or foe. *Drug Metab Rev* **25:** 503–517, 1993.
9. Nebert DW, Drug-metabolizing enzymes in ligand-modulated transcription. Biochem Pharmacol **47:** 25–37, 1994.
10. Welch RM, Harrison YE, Gommi BW, Poppers PJ, Finster M and Conney AH, Stimulatory effect of cigarette smoking on the hydroxylation of 3 4-benzpyrene and the N-demethylation of 3-methyl-4-monothylaminoazobenzene by enzymes in the human placenta. *Clin Pharmacol Ther* **10:** 100–109, 1969.
11. Wong TK, Everson RB and Hsu S-T, Potent induction of human placental monooxyganase activity by previous dietary exposure to polyclorinated biphenyls and their thermal degradation products. *Lancet* **I:** 721–724, 1985.
12. Pasanen M, Stenbäck F, Park SS, Gelboin HV and Pelkonen O, Immunohistochemical detection of human placental cytochrome P-450-associated mono-oxygenase system induciple by maternal cigarette smoking. *Placenta* **9:** 267–275, 1988.
13. Pasanen M, Haaparanta T, Sundin M, Sivonen P, Vähakangas K, Raunio H, Hines R, Gustafsson J-Å and Pelkonen O, Immunochemical and molecular biological studies on human placental cigarette smoke-induciple cytochrome P-450-dependent monooxygenase activities. *Toxicology* **62:** 175–187, 1990.
14. Pelkonen O, Fetoplacental biochemistry—xenobiotic metabolism and pharmacokinetics. In: Occupational Hazards and Reproduction (Eds. Hemminki K, Sorsa M and Vainio H), pp. 113–126. Hemisphere, Washington, DC, 1985.
15. Schuetz JD, Kauma S and Guzelian PS, Identification of the fetal liver cytochrome CYP3A7 in human endometrium and placenta. *J Clin Invest* **92:** 1018–1024, 1993.
16. Yokotani N, Sogawa K, Matsubara S, Gotoh O, Kusunose M and Fujii-Kuriyama Y, cDNA cloning of cytochrome P-450 related to $P\text{-}450_{p\text{-}2}$ from the cDNA library of human placenta, genestructure and expression. *Eur J Biochem* **187:** 23–29, 1990.
17. Foley KP, Leonard MW and Engel JD, Quantitation of RNA using the polymerase chain reaction. *Trends Genet* **9:** 380–385, 1993.
18. Devereux J, Haeberli P and Smithies O, A comprehensive set of sequence analysis preograms for VAX and convex systems. *Nucleic Acids Res* **12:** 387–395, 1984.

19. Jaiswal AK, Gonzalez FJ and Nebert DW, Human dioxin-inducible cytochrome P_1-450: complementary DNA and amino acid sequence. *Science* **228:** 80–83, 1985.
20. Jaiswal AK, Nebert DW and Gonzalez FJ, Human $P_3$450 cDNA and complete amino acid sequence. *Nucleic Acids Res* **14:** 6773–6774, 1986.
21. Yamano S, Tatsuno J and Gonzalez FJ, The CYP2A3 gene product catalyzes coumarin 7-hydroxylation in human liver microsomes. *Biochemistry* **29:** 1322–1329, 1990.
22. Yamano S, Nhamburo PT, Aoyama T, Meyer UA, Inaba T, Kalow W, Gelboin HV, McBride OW and Gonzalez FJ, cDNA cloning and sequence and cDNA-directed expression of human P450 IIB1: identification of a normal and two variant cDNAs derivated from the CYP2B locus on chromosome 19 and differential expression of the IIB mRNAs in human liver. *Biochemistry* **28:** 7340–7348, 1989.
23. Kimura S, Pastewka J, Gelboin HV and Gonzalez FJ, cDNA and amino acid sequences of two members of human P450IIC gene subfamily. *Nucleic Acids Res* **15:** 10053–10054, 1987.
24. Romkes M, Faletto MB, Blaisdell JA, Raycy JL and Goldstein JA, Cloning and expression of complementary DNAs for multiple members of the human cytochrome P450IIC subfamily. *Biochemistry* **30:** 3247–3255, 1991.
25. Kimura S, Umeno M, Skoda RC, Meyer UA and Gonzalez FJ, The human debrisoquine 4-hydroxylase (CYP2D) locus: sequence and identification of the polymorphic CYP2D6 gene a related gene and a pseudogene. *Am J Hum Genet* **45:** 889–904, 1989.
26. Song B-J, Gelboin HV, Park SS, Yang CS and Gonzalez FJ, Complementary DNA and Protein sequences of ethanol-inducible rat and human cytochrome P-450s. *J Biol Chem* **35:** 16689–16697, 1986.
27. Nhamburo PT, Kimura S, McBride OW, Kozak CA, Gelboin HV and Gonzalez FJ, The human CYP2F gene subfamily: identification of a cDNA encoding a new cytochrome P450 cDNA-directed expression and chromosome mapping. *Biochemistry* **29:** 5491–5499, 1990.
28. Molowa DT, Schuetz EG, Wrighton SA, Watkins PB, Kremers P, Mendez-Picon G, Parker GA and Guzelian PS, Complete cDNA sequence of a cytochrome P-450 induciple by glucocorticoids in human liver. *Proc Natl Acad Sci USA* **83:** 5311–5315, 1986.
29. Gonzalez FJ, Schmid BJ, Umeno M, McBride OW, Hardwick JP, Meyer UA, Gelboin HV and Idle JR, Human P450PCN1: sequence chromosome localization and direct evidence through cDNA expression that P450PCN1 is nifedipine oxidase. *DNA* **7:** 79–86, 1988.
30. Aoyama T, Yamano S, Waxman DJ, Lapenson DP, Meyer UA, Fischer V, Tyndale R, Inaba T, Kalow W, Gelboin HV and Gonzalez FJ, Cytochrome P-450 hPCN3 a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver. *J Biol Chem* **264:** 10388–10395, 1989.
31. Komori M, Nishio K, Ohi H, Kitada M and Katamaki T, Molecular cloning and sequence analysis of cDNA containing the entire coding region for human fetal liver cytochrome P-450. *J Biochem* **105:** 161–163, 1989.
32. Nhamburo PT, Gonzalez FJ, McBride OW, Gelboin HV and Kimura S, Identification of a new P450 expressed in human lung: complete cDNA sequence cDNA-directed expression and chromosome mapping. *Biochemistry* **28:** 8060–8066, 1989.
33. Pelkonen O and Pasanen M, Effect of heparin on the subcellular distribution of human placental 7-ethoxycoumarin O-deethylase activity. *Biochem Pharmacol* **30:** 3254–3256, 1981.
34. Nebert DW and Gelboin HV, Substrate-indicible microsomal aryl hydroxylase in mammalian cell culture. I. Assay and properties of induced enzyme. *J Biol Chem* **243:** 6242–6249, 1968.
35. Aitio A, A simple and sensitive assay of 7-ethoxycoumarin decthylation. *Anal Biochem* **85:** 488–491, 1978.
36. Burke MD, Prough RA and Mayer RT, Characteristics of a microsomal cytochrome P-448-mediated reaction: ethoxyresorufin O-deethylation. *Drug Metab Disp* **5:** 1–8, 1977.
37. Pasanen M, Human placental aromatase activity: use of a C_{18} reverse-phased cartridge for separation of tritiated water or steroid metabolites in placentas from both smoking and non-smoking mothers in vitro. *Biol Res Pregn* **6:** 94–99, 1985.
38. Waxman DJ, Ko A and Walsh C, Radioselectivity and stereoselectivity of androgen hydroxylations catalysed by cytochrome P-450 isozymes purified from phenobarbital induced rat liver. *J Biol Chem* **258:** 11937–11947, 1983.
39. Pasanen M and Pelkonen O, Cholesterol side-chain cleavage activity in human placenta and bovine adrenals: a one-step method for separation of pregnenolone in vitro. *Steroids* **43:** 517–525, 1984.
40. Cochin J and Axelrod J, Biochemical and pharmacological changes in the rat following chronic administration of morphine nalorphine and normorphine. *J Pharmacol Exp Ther* **125:** 105–110, 1959.
41. Nash T, The calorimetric estimation of formaldehyde by means of the Hantzoch reaction. *Biochem J* **55:** 416–421, 1953.
42. Peter R, Böcker R, Beaune BH, Iwasaki M, Guengerich FP and Yang CS, Hydroxylation and chlorozoxazone as a specific probe for human liver cytochrome P-450IIE1. *Chem Res Toxicol* **3:** 566–573, 1990.
43. Edwards RJ, Singleton AM, Murray BP, Davies DS and Boobis AR, Short synthetic peptides exploited for reliable and spesific targeting of antibodies to the C-termini of cytochrome P450 enzymes. *Biochem Pharmacol* **49:** 39–47, 1995.
44. Edwards RJ, Singleton AM, Sesardic D, Boobis AR and Davies DS, Antibodies to a synthetic peptide that react specifically with a common surface region on two hydrocarbon-inducible isoenzymes of cytochrome P-450 in the rat. *Biochem Pharmacol* **37:** 3735–3741, 1988.
45. Murray BP, Edwards RJ, Murray S, Singleton AM, Davies DS and Boobis AR, Human hepatic CYP1A1 and CYP1A2 content determined with specific anti-peptide antibodies, correlates with the mutagenic activation of PhIP. *Carcinogenesis* **14:** 585–592, 1993.
46. Laemmli UK, Cleavage of structural proteins during the assembly of the head of bacteriophage Y4. *Nature* **227:** 680–685, 1970.
47. Towbin H, Staehelin T and Gordon J, Electroforetic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* **76:** 4350–4354, 1979.
48. Edwards RJ, Murray BP, Murray S, Schulz T, Neubert D, Gant TW, Thorgeirsson SS, Boobis AR and Davies DS, Contribution of CYP1A1 and CYP1A2 to the activation of heterocyclic amines in monkeys and human. *Carcinogenesis* **15:** 829–836, 1994.
49. Ching MS, Lennard MS, Tucker GT, Woods HF, Kelly DE and Kelly SL, The expression of human cytochrome P450IA1 in the *yeast Saccharomyces cerevisiae*. *Biochem Pharmacol* **42:** 753–758, 1991.
50. Greenlee WF and Poland A, An improved assay of 7-ethoxycoumarin O-deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital 3-methylcholantherene and 2378-tetrachloro-p-dioxin. *J Pharmacol Exp Ther* **205:** 596–605, 1978.
51. Kitawaki J, Inoue S, Tamura T, Yamamoto T, Honjo H, Higashiyama T, Osawa Y and Okada H, Cigarette smoking during pregnancy lowers aromatase cytochrome P-450 in the human placenta. *J Steroid Biochem Molec Biol* **45:** 485–491, 1993.
52. Waxman DJ, Lapenson DP, Aoyama T, Gelboin HV, Gonzalez FJ and Korzekwa K, Steroid hormone hydroxylase specifities of eleven cDNA-expressed human cytochrome P450s. *Arch Biochem Biophys* **290:** 160–166, 1991.
53. Sesardic D, Pasanen M, Pelkonen O and Boobis AR, Differential expression and regulation of members of the cytochrome P450IA

gene subfamily in human tissues. *Carcinogenesis* **11:** 1183–1188, 1990.

54. Manchester DK, Gordon SK, Golas CL, Roberts EA and Okey AB, Ah receptor in human placenta: stabilization by molybdate and characterization of binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin 3-methylcholantherene and benzo(a)pyrene. *Cancer Res* **47:** 4861–4868, 1987.
55. Dolwick KM, Schmidt JV, Carver LA, Swanson HI and Bradfield CA, Cloning and expression of a human Ah receptor cDNA. *Molec Pharmacol* **44:** 911–917, 1993.
56. Schmidt JV, Carver LA and Bradfield CA, Molecular characterization of the murine *Ahr* gene. *J Biol Chem* **268:** 22203–22209, 1993.
57. Wheeler CW, Wrighton SA and Guenthner TM, Detection of human lung cytochromes P450 that are immunochemically related to cytochrome P450IIE1 and cytochrome P450IIIA. *Biochem Pharmacol* **44:** 183–186, 1992.
58. Song B-J, Veech RL and Saenger P, Cytochrome P450IIE1 is elevated in lymphocytes from poorly controlled insulin-dependent diabetics. *Clin Endocrinol Metab* **71:** 1036–1040, 1990.
59. Farin FM, Pohlman TH and Omiecinski CJ, Expression of cytochrome P450s and microsomal epoxide hydrolase in primary cultures of human umbilical vein endothelial cells. *Toxicol Appl Pharmacol* **124:** 1–9, 1994.
60. 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.
61. Jones SM, Boobis AR, Moore GE and Stanier PM, Expression of CYP2E1 during human fetal delelopment: methylation of the CYP2E1 gene in human fetal and adult liver samples. *Biochem Pharmacol* **43:** 1876–1879, 1992.
62. Kitada M, Kamataki T, Itahashi K, Rikihisa T, Kato R and Kanakubo Y, Purification and properties of cytochrome P-450 from homogenates of human fetal livers. *Arch Biochem Biophys* **241:** 275–280, 1985.
63. Schuetz JD, Beach DL and Guzelian PS, Selective expression of cytochrome P450 CYP3A mRNAs in embryonic and adult human liver. *Pharmacogenetics* **4:** 11–20, 1994.
64. Hakkola J, Pasanen M, Purkunen R, Saarikoski S, Pelkonen O, Mäenpää J, Rane A and Raunio H, Expression of xenobiotic-metabolizing cytochrome P450 forms in human adult and fetal liver. *Biochem Pharmacol* **48:** 59–64, 1994.
65. Gonzalez FJ, The molecular biology of cytochrome P450s. *Pharmacol Rev* **40:** 243–288, 1989.