Identification and Localization of Five CYP2Cs in Murine Extrahepatic Tissues and Their Metabolism of Arachidonic Acid to Regio- and Stereoselective Products

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ABSTRACT

The CYP2C subfamily has been extensively studied in humans with respect to the metabolism of clinically important drugs, and polymorphisms have been identified in these enzymes. In the present study, a murine model was used to determine the possible physiological functions and extrahepatic distribution of CYP2Cs. Using the reverse transcription-polymerase chain reaction (RT-PCR), Western blotting, and immunohistochemistry, this report demonstrates that the mouse CYP2Cs are extensively distributed in extrahepatic tissues and localized to heart muscle, lung Clara and ciliated cells, kidney collecting ducts, the X-zone of female adrenals, reproductive organs, white blood cells, and eyes (in the optic nerve, rods, and cones). RT-PCR, subcloning, and sequencing of the products indicate that each CYP2C has a unique tissue distribution. Four cDNA fragments representing potentially new CYP2Cs were identified, each with its own organ-specific pattern of expression. Using a bacterial cDNA expression system, we found that recombinant proteins for each of the five full-length murine CYP2Cs metabolize arachidonic acid to different regio- and stereospecific products, including epoxygenes and hydroxyicosatetraenoic acids. Regio- and stereospecific metabolites of arachidonic acid have been reported to affect important physiological functions such as inflammation, neutrophil activation, ion transport, cellular proliferation, and vascular tone. Our results suggest that the presence of CYP2C enzymes in heart muscle, aorta, kidney, lung, adrenals, eyes, and reproductive organs could regulate important physiological and/or pathological processes in these tissues.

The CYP proteins represent a ubiquitous superfamily of monooxygenases that metabolize a vast array of endogenous and exogenous substrates (Guengerich, 1991; Nelson, 1999). Those previously described mammalian CYPs whose function is primarily drug metabolism, are expressed mainly in liver, often present at lower or undetectable levels in extrahepatic tissues (Guengerich, 1992). Other CYPs with endogenous functions such as the CYP2Js are often expressed at high levels in extrahepatic tissues including heart, kidney, and intestine (Wu et al., 1996, 1997; Zhang et al., 1998; Ma et al., 1999). The CYP2Cs have important hepatic functions in metabolizing clinically important drugs in man (Goldstein and de Morais, 1994). However, some CYP2Cs have been reported in human extrahepatic tissues (Klose et al., 1999). CYP2C40 has been identified in murine cecum and colon in an earlier study from our laboratories (Tsao et al., 2000).

Certain CYPs including the CYP2Cs are capable of oxidation of arachidonic acid (AA), and they may potentially play important physiological roles via the generation of bioactive eicosanoids. CYPs metabolize AA to several oxygenated metabolites including the following: 1) four regioisomeric epoxygenes (EETs) (5,6-, 8,9-, 11,12-, and 14,15), which can be further hydrolyzed by epoxide hydrolases to the corresponding dihydroxyeicosatrienoic acids (DHETs); 2) six regioisomeric cis-trans-conjugated monohydroxyicosatetraenoic acids (midchain HETEs); and 3) ω-1 alcohols of arachidonic acid (20- and 19-HETE) (Capdevila et al., 1981, 1992; Oliw et al., 1982). Intestinal microsomal fractions metabolize AA to several regioisomeric EETs and HETEs (Zeldin et al., 1997; Tsao et al., 2000). 20-HETE causes dilation of isolated perfused rabbit mesenteric arteries (Macica et al., 1993), and 11,12-EET causes dose-dependent vasodilation of the rat intestinal microcirculation (Proctor et al., 1987). Many other AA metabolites have been reported to be biologically active. For example, 5,6-EET was found to dilate isolated blood vessels and to inhibit sodium reabsorption and...
potassium secretion in isolated perfused collecting tubules; 19(S)-HETE is a stimulator of renal Na\(^+\)–K\(^+\)-ATPase, and 20-HETE is a potent vasoconstrictor of isolated rat aorta (Schwartzman et al., 1989; Escalante et al., 1990). Interestingly, many of the biological activities of EETs and HETEs are regio- and stereoselective. For example, the (S) enantiomers of 16- and 17-HETEs inhibit proximal tubular ATPase activity, whereas the (R) isomers have negligible effects on ATPase activity (Carroll et al., 1996). Similarly, only 11(R),12(S)-EET, but not its enantiomer 11(S),12(R)-EET, increases the open probability of large-conductance Ca\(^{2+}\)-activated K\(^+\) channels in renal vascular smooth muscle cells (Zou et al., 1996).

In an earlier study, our laboratories cloned five murine CYP2C cDNAs, and preliminary data showed that all five CYP2C recombinant proteins metabolized arachidonic acid with different regiospecific profiles and catalytic rates (Luo et al., 1998). We also identified CYP2C40 as the primary CYP2C isoform in gut (Tsao et al., 2000). In the present study, we examined other extrahepatic organs extensively for expression of the CYP2Cs using Western blotting, RT-PCR cloning methods, and immunohistochemistry. Our previous study showed regiospecificity for the murine CYP2Cs in the production of AA metabolites, and we tentatively identified a midchain HETE peak (Luo et al., 1998). In this study, we further identified the specific HETEs using normal-phase HPLC and determined the stereochemical selectivity of EET production by the CYP2Cs. PCR cloning methods were used to identify the organ-specific expression of the CYP2Cs and potentially new CYP2C fragments in some of these tissues. We propose that the murine CYP2Cs may have important biological functions in numerous extrahepatic tissues.

**Experimental Procedures**

**Materials.** [1-\(1^4\)C]Arachidonic acid was purchased from PerkinElmer Life Sciences (Boston, MA). Midchain HETEs were generous gifts from Dr. J. R. Falck (University of Texas Southwestern Medical School, Dallas, TX). α-Bromo-2,3,4,5,6-pentafluorotoluene, N,N-diisopropylethylamine, dimethylformamide, and Daziald were purchased from Aldrich Chemical (Milwaukee, WI). Rat cytochrome P450 reductase and its antibody were from Calbiochrome (Lafayette, CO). Rat cytochrome P450 reductase, goat anti-rabbit IgG, or rabbit anti-goat IgG were purchased from Jackson ImmunoResearch Laboratories Inc., West Grove, PA. The other antibodies, reagents, and enzymes were purchased from Sigma (St. Louis, MO) unless otherwise specified.

**Isolation of Total RNA and RT-PCR Analysis.** Normal CD-1 female and male mouse extrahepatic tissues were snap-frozen in liquid nitrogen immediately after collection and stored at −80°C until use. Total RNA was extracted using Tri-Reagent (Molecular Research Center Inc., Cincinnati, OH). RT-PCR analysis was performed using a GeneAmp RNA PCR kit (PerkinElmer, Branchburg, NJ). Reverse transcription was performed with 1 μg of total RNA in a buffer containing 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 5 mM MgCl\(_2\), 2.5 mM oligo-dT primer, 1 mM each of dGTP, dATP, dTTP, and dCTP, and 50 units of Moloney murine leukemia virus-reverse transcriptase at 42°C for 1 h. The PCR amplifications were performed in the presence of 2 mM MgCl\(_2\), 0.1 mM forward and reverse primers (Table 1), using 2.5 units of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). The CYP2C primers were designed by aligning the five known murine CYP2C isoforms and choosing the regions of homology that are not shared by other known murine CYPs. After an initial incubation at 95°C for 3 min, samples were subjected to 35 cycles of 30 s at 95°C, 30 s at 55°C, and 90 s at 72°C. The PCR products were electrophoresed on 1.2% agarose gels containing ethidium bromide. PCR products were also cloned into vectors using a TA cloning kit from Invitrogen (Carlsbad, CA) for subsequent identification. DNA was prepared from randomly selected clones and sequenced using an ABI Prism DNA sequencing kit (PerkinElmer).

### TABLE 1

<table>
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<th>Gene</th>
<th>Primer Sequence (5′–3′)</th>
<th>Product Size</th>
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<td>CYP2Cs</td>
<td>Forward: TCCATGCAATGTCACTGCTC</td>
<td>895 bp</td>
</tr>
<tr>
<td>β-Actin</td>
<td>Forward: GAATGAAACAGCTCCATGGC</td>
<td>409 bp</td>
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</table>

The corresponding CYP2C nucleotides for forward and reverse primers are homologous to all five known CYP2Cs at positions 529–549 and 1324–1344, respectively.

**Protein Immunoblotting and Immunohistochemistry.** Microsomal fractions were prepared from frozen normal CD-1 extrahepatic tissues by differential centrifugation at 4°C as previously described (Zeldin et al., 1996). Polyclonal anti-mouse CYP2C38 IgG was raised in New Zealand White rabbits against the partially purified recombinant CYP2C38 protein and purified using a protein A column (Pierce, Rockford, IL) as previously described (Ma et al., 1999; Tsao et al., 2000). For immunoblotting, microsomal fractions and partially purified recombinant proteins were electrophoresed in SDS-10% (w/v) polyacrylamide gels, and the resolved proteins were transferred onto nitrocellulose membranes. Membranes were immunoblotted using rabbit anti-mouse CYP2C38 IgG or goat anti-rabbit IgG, or rabbit anti-goat IgG conjugated to horseradish peroxidase (Amersham Pharmacia Biotech, Buckinghamshire, UK), and visualized using an enhanced chemiluminescence Western blotting detecting system (Amersham Pharmacia Biotech) as previously described (Zeldin et al., 1996).

For immunohistochemistry, specific regions of the mouse liver, lung, kidney, heart, adrenal, eye, and optic nerve were carefully collected and fixed in 10% neutral-buffered formalin overnight (18–24 h), processed routinely, and embedded in paraffin. Localization of CYP2C proteins was determined using anti-CYP2C38 IgG (1:1000 dilution). Slides were deparaffinized in xylene and hydrated through a graded series of ethanol to 1× Automation buffer (Biomed, Foster City, CA) washes. Endogenous peroxidase activity was blocked with 3% (v/v) hydrogen peroxide for 15 min. After rinsing in 1× Automation buffer, the sections were blocked with 5% normal goat serum for 20 min. All antibody incubations were carried out at room temperature in a humidified chamber. The primary antibody, anti-CYP2C38 IgG, was applied and sections were incubated for 1 h. Both preimmune IgG and rabbit nonimmune IgG (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) were used as the negative controls in place of the primary antibody, and mouse liver was used as positive control for immunostaining. The secondary antibody, biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA), was applied at a dilution of 1:600 for 30 min. The bound primary antibody was visualized by avidin-biotin-peroxidase detection using the Vectastain Rabbit Elite kit (Vector Laboratories) according to the manufacturer’s instructions with liquid diaminobenzidine (DAKO Corporation, Carpinteria, CA) as the color-developing reagent. Slides were counterstained with Harris hematoxalin, dehydrated through a graded series of ethanol to xylene washes, and cover-slipped with Permount (Fisher Scientific, Springfield, NJ). Slides were evaluated according to stain distribution, localization, and intensity.

**Regio- and Stereoochemical Analysis of CYP2C AA Metabolites.** The methods for regiochemical analysis of metabolites of AA produced by the reconstituted recombinant murine CYP2Cs were previously described (Luo et al., 1998). For subsequent chiral analysis, the EETs were collected batchwise from HPLC eluents, derivatized to the corresponding EET-pentafluorobenzyl or EET-methyl...
esters, purified by normal phase HPLC, resolved into the corresponding antipodes by chiral-phase HPLC, and quantified by liquid scintillation as previously described (Hammonds et al., 1989; Capdevila et al., 1991). To determine the regiochemical distribution of HETEs, radiolabeled HPLC fractions of unidentified HETEs (Luo et al., 1998) were collected from the reverse-phase HPLC eluent and then chromatographed on a normal-phase HPLC system to resolve individual HETE regioisomers as previously described (Rosolowsky and Campbell, 1996). In all cases, products were identified by comparing their HPLC properties with those of authentic standards.

**Incubations of Mouse Extrahepatic Microsomes with AA.** Microsomal fractions were prepared from frozen mouse kidney, lung, heart, and female adrenals by differential centrifugation at 4°C as described previously (Luo et al., 1998) and resuspended in 50 mM Tris-Cl, pH 7.4, 1 mM dithiothreitol, 1 mM EDTA, and 20% glycerol. Microsomal proteins (2–5 mg/ml) were preincubated by shaking with 50 mM Tris-Cl, pH 7.5, 150 mM KCl, 10 mM MgCl₂, 8 mM sodium isocitrate, 0.5 IU/ml isocitrate dehydrogenase, and [1-14C]AA (25 μCi/μmol) for 10 min at 37°C. Following temperature equilibration, NADPH (1 mM final concentration) was added to initiate the reaction. Aliquots were withdrawn at 20- to 60-min intervals, and the reaction products were extracted into ethyl ether, dried under a steam of nitrogen, analyzed by reverse-phase HPLC, and quantified by on-line liquid scintillation counting using a Radiomatic Flow-One β-detector (Radiomatic Instruments, Tampa, FL) as described previously (Tsao et al., 2000). Metabolites were identified by comparing their reverse-phase HPLC properties with those of authentic standards (Capdevila et al., 1990).

**Results**

**Identification of CYP2Cs in the Murine Extrahepatic Tissues.** Western blotting with an antibody that recognizes all five known murine CYP2C isoforms (Tsao et al., 2000) shows that although liver has the highest quantity of CYP2Cs, these enzymes are widely expressed in extrahepatic tissues, and they are particularly abundant in colon, lung, kidney, heart, and female adrenals (Fig. 1A). Interestingly, multiple bands in the molecular weight range 55 to 58 kDa were found in several organs, suggesting the possibility that more than one CYP2C member exists in these tissues. To demonstrate whether cytochrome P450 reductase 50 reductase distributes in a similar fashion as CYP2Cs, Western blotting with an anti-rat cytochrome P450 reductase was performed. The results showed that murine liver, kidney, and lung have the highest levels of cytochrome P450 reductase (Fig. 1B), and the expression pattern is similar to that of the CYP2Cs. To identify which CYP2Cs are expressed in these tissues, RT-PCR cloning and sequencing of PCR products were performed. PCR products amplified using universal CYP2C primers were cloned into the TA cloning vector and individual clones were selected, DNA extracted, and their sequences were determined. RT-PCR results demonstrate amplification of a 895-bp band from all tissues above, confirm the broad tissue distribution of CYP2C mRNAs and are consistent with the results of Western blotting (Fig. 2). Sequencing demonstrated that different CYP2C isoforms are expressed in different extrahepatic tissues (Table 2). Two CYP2Cs are found to be widely expressed in murine extrahepatic tissues. CYP2C29 mRNAs are expressed in lung, adrenals, heart, aorta, seminal vesicles, testes, and ovary, whereas CYP2C40 mRNAs are present in the intestinal tract, heart, kidney, lung, adrenals, aorta, eye, white blood cells, skin, and ovaries. CYP2C29 is the predominant CYP2C isoform in lung, male adrenals, aorta, and reproductive organs, whereas CYP2C40 is the principal CYP2C isoform in heart, kidney, skin, and intestinal tissues. Correlation of RT-PCR and immunoblotting results suggests that the prominent polypeptide band with the highest molecular weight in Western blots of colon microsomes probably represents CYP2C40, whereas the lower molecular weight band in lung probably represents CYP2C29 (Table 2, Figs. 1 and 2). Other CYP2Cs such as CYP2C37 and CYP2C39 are also expressed in extrahepatic tissues, but their expression pattern is more limited. CYP2C37 is predominant in white blood cells, whereas CYP2C39 is expressed in murine eyes and epididymis. Interestingly, CYP2C37 is the major CYP2C in murine female adrenals, whereas CYP2C29 is the predominant isoform found in male adrenals. In addition to the five known murine CYP2Cs, four potentially new CYP2C fragments were identified in kidney, heart, aorta, and eyes, respectively. The nucleotide sequences for these fragments were 70 to 96% identical to other known CYP2Cs (Table 3). The sequences for these fragments have been submitted to the Committee for Standardized P450 Nomenclature and have been designed CYP2C52p (fragment a), CYP2C52p (fragment b), CYP2C50 (fragment c), and CYP2C51 (fragment d).
Localization of Extrahepatic CYP2C Proteins by Immunohistochemistry. Immunohistochemistry results indicate that the CYP2Cs are expressed in specific cells within these extrahepatic tissues. The distribution of CYP2Cs is summarized in Table 4. In lung, strong immunostaining was not only present in the Clara cells but also in the ciliated epithelial cells in the trachea and bronchi (Fig. 3A). Positive staining was also found in Clara cells in distal airways (Fig. 3C). In the adrenals, the identity and distribution of CYP2Cs differed in males and females. In female adrenals, CYP2Cs were located in medullary cells, inner cortex, and the “X-zone” (Fig. 4A), but only trace amounts of CYP2Cs were found in male adrenals (data not shown). In the heart, strong positive staining was found in the cardiac myocytes (Fig. 4C). In kidney, CYP2Cs were located mainly in distal tubular epithelial cells (Fig. 4E). CYP2C proteins were also found in eyes. Strong positive staining was detected in optic nerves (Fig. 5A), and immunostaining was also detected in specific portions of the retina (rods, cones, inner nuclear layer, and ganglion cells) (Fig. 5C), the periphery of the lens, the epithelium of the cornea, and the ciliary body (Fig. 5E).

Regio- and Stereochemistry of CYP2C AA Metabolites. We have previously shown that 16-HETE is the major AA metabolite of CYP2C40 (Tsao et al., 2000). The present study identifies individual HETEs produced by the other CYP2Cs (Table 5). HETEs were not produced by CYP2C29, whereas several different HETEs were produced by CYP2C37, CYP2C38, and CYP2C39. 16-HETE is the major AA metabolite of CYP2C40; 12-HETE is the major HETE produced by CYP2C37; and 11-HETE is the major HETE produced by CYP2C39 (Table 5).

We have previously shown that the CYP2Cs also produce different EETs (Luo et al., 1998). Stereochemical analyses of CYP2C-derived EETs indicate that they are produced in a highly enantioselective fashion (Table 6). CYP2C29 and
CYP2C40 produce 14,15-EET primarily as 14(R),15(S)-EET, whereas CYP2C39 produces mainly 14(S),15(R)-EET. CYP2C38 and CYP2C39 produce 11,12-EET almost exclusively as the 11(R),12(S)-enantiomer. CYP2C29, CYP2C37, and CYP2C40 produce 8(S),9(R)-EET, but CYP2C38 and CYP2C39 produce mainly 8(R),9(S)-EET.

Characterization of CYP-AA Metabolism of Extrahepatic Tissues. To examine CYP metabolism of AA in extrahepatic tissues, microsomal fractions prepared from murine kidney, lung, heart, and female adrenals were incubated with [1-14C]AA in the presence of NADPH, and the organic soluble metabolites were resolved by reverse-phase HPLC. All of the selected tissues metabolized AA and produced distinct AA metabolite profiles (Fig. 6). Murine kidney exhibited the highest conversion rate (0.055 nmol/mg/min), lung was 0.044 nmol/mg/min, whereas female adrenals and heart had the lowest turnover number (0.025 and 0.022 nmol/mg/min, respectively).

Discussion

The present study demonstrates that murine CYP2Cs are widely expressed in extrahepatic tissues including the heart, lung, kidney, male and female reproductive tissues, endocrine glands, eyes, skin, and brain. Previous studies from our laboratory also found CYP2C40 in high concentrations in murine kidney, testes, adrenal gland, intestinal tract, prostate, ovary, and the epithelium of esophagus, tongue, nasal mucosa, and skin (Murray et al., 1995; Klose et al., 1999; Yokose et al., 1999). CYP2C9 and CYP2C19 proteins were also found in intestine (Klose et al., 1999). In this study, we found extensive expression of murine CYP2C proteins as well as their mRNAs, suggesting the possibility that the mouse could serve as a model for studying the expression and functional significance of the CYP2Cs and that CYP2C expression in human extrahepatic tissues should be investigated more vigorously.

In the present study, CYP2Cs were abundantly found in the murine lung, kidney, heart, and female adrenals. Although the roles of the CYP2Cs in these tissues remain unknown, the fact that the CYP2Cs are differentially expressed in various extrahepatic tissues and metabolize AA to distinct regio- and stereospecific metabolites suggests the possibility that the CYP2Cs may contribute to specific AA metabolite production in some or all of these tissues. The EETs are endogenous constituents of human, rat, rabbit, and guinea pig lung (Knickle and Bend, 1994; Zeldin et al., 1995a, 1996). Results of sequencing selected clones isolated by RT-PCR using universal CYP2C primers from lung RNA indicate that CYP2C29 is the major CYP2C expressed in murine lung. Regiochemical analysis of CYP2C29-derived AA metabolites shows that 14,15-EET, 11,12-EET, and 8,9-EET are the major products. CYP2C29 is stereoselective for production of 14(R),15(S)-EET (83%) and 8(S),9(R)-EET (82%). Interestingly, chiral analysis of the rabbit lung EETs have demonstrated that 14(R),15(S)-EET and 8(S),9(R)-EET are also the predominant enantiomers (Zeldin et al., 1995b). CYP2C proteins are localized to airway epithelial cells including both Clara and ciliated cells. It has been suggested that EETs may contribute to regulation of pulmonary bronchial and vascular tone (Zeldin et al., 1995b; Zhu et al., 2000), modulate airway transepithelial ion transport (Pascual et al., 1998), and may play a role in regulating the volume and composition of the airway surface liquids that affect lung mucociliary clearance (Pascual et al., 1998). The localization of CYP2C29 in the murine lung suggests the possibility that EETs produced by CYP2C29 could be involved in these physiological processes.

Murine CYP2Cs are also expressed in the distal tubular epithelial cells of the kidney. Results of sequencing the RT-PCR clones indicate that CYP2C40 is the major CYP2C in
the murine kidney. We previously reported that CYP2C40 is also the major CYP2C in the intestinal tract and metabolizes AA to a unique metabolite, 16-HETE (Tsao et al., 2000). It has been reported that 16-HETE inhibits kidney tubular ATPase activity and causes vasodilation (Carroll et al., 1996). Recent studies also demonstrated that 16-HETE inhibits adhesion and aggregation of neutrophils, suggesting a possible role of this eicosanoid in resolution of inflammation (Bednar et al., 1997, 2000). Endogenous 16-HETE has been found in the rabbit kidney and is proposed to possess significant biological properties acting either on tubular transport and/or renal vasculature (Carroll et al., 1996). Thus far, CYP2C40 is the only enzyme found to produce 16-HETE (Tsao et al., 2000). CYP2C40-deficient knockout mice could provide the model that provides information concerning the biological functions of 16-HETE in the tissues in which CYP2C40 is expressed.

cDNA fragments of four potentially new members of the murine CYP2C subfamily were also identified in extrahepatic tissues. In heart and aorta, we found two unidentified CYP2C fragments in addition to CYP2C29 and CYP2C40. Recently, CYP-derived EETs have been emphasized to have vasodilatory properties similar to the endothelium-derived hyperpolarizing factor (EDHF) (Fisslthaler et al., 1999). Antisense oligonucleotides to CYP2C8/34 attenuated EDHF-mediated vascular response in native porcine coronary artery endothelial cells (Fisslthaler et al., 1999). Induction of a CYP2C protein with β-naphthoflavone enhanced the formation of 11,12-EET as well as EDHF-mediated hyperpolarization and relaxation. Overexpression of CYP2J2 or addition of physiological concentrations of 11,12-EET have been shown to decrease cytokine-induced endothelial cell adhesion molecule expression in bovine aortic endothelial cells (Node et al., 1999). Taken together, these studies suggest that CYP-derived EETs, especially 11,12-EET, appear to be involved in vascular function. CYP2C29 produces an AA-metabolic profile similar to human CYP2C8 (Zeldin et al., 1995b; Luo et al., 1998). Thus, the mouse could serve as a model for studying the biological functions of CYP2C in human heart.

Surprisingly, CYP2Cs were fairly abundant in murine...
The major cDNA fragment appeared to represent a previously unidentified CYP2C. Other members of CYP2Cs in the murine eyes were identified as CYP2C39 and CYP2C40. CYP2Cs were expressed in different regions of eyes, including corneal and retinal epithelial cells, ganglia, lens, and optic nerve. Other CYPs have been found to be expressed in eyes of various species, including CYP1A1, CYP2E1, CYP3A5, and CYP4B1 (Offord et al., 1999; Mast-yugin et al., 1999), and many of them are located in cornea. Cytochrome P450-derived 12(13)-HETE and 12-hydroxyeicosatrienoic acid have been reported to possess potent inflammatory effects in the eyes (Conners et al., 1995; Mast-yugin et al., 1999). CYP2C40 produces the anti-inflammatory mediator 16-HETE. Future studies will examine the distribution of individual CYP2Cs in the eye and their roles in inflammatory responses in these tissues. Intense staining for the CYP2Cs was also found in nerve cells of the ganglia and optic nerve. Interestingly, EETs have been reported to stimulate the release of neuropeptides (Ojeda et al., 1989), suggesting that AA metabolites of CYP2Cs could have a role in neurotransmission in the eye.

CYP2Cs were highly expressed in female adrenals but only trace amounts were found in male adrenals in the present study. The adrenal gland is one of the major organs involved in the biosynthesis of steroid hormones, and the function and
The morphology of the adrenals are also regulated by these hormones. A distinctive species-specific feature of the mouse adrenals is the X-zone at the junction of the cortex and medulla. In females, the X-zone increases in size with age, reaching a maximum at about 9 weeks and then regresses gradually in virgins and rapidly during the first pregnancy (Greaves, 1990). Interestingly, CYP2Cs are highly expressed in the female adrenals and immunohistochemical analysis shows that CYP2Cs are most abundant in the X-zone. The function of the X-zone is still unclear; however, it is apparent that the X-zone is regulated by hormones and CYP2Cs could conceivably be involved in the biosynthesis of hormones or be regulated by hormones. The major CYP2C in the female adrenals is CYP2C37, but CYP2C29 is the major CYP2C in

**TABLE 6**

Enantioselective composition of EETs produced by recombinant murine CYP2Cs

The regiochemical and stereochemical composition of EETs formed by incubation of recombinant CYP2Cs with [1-14C]AA were quantified by HPLC and liquid scintillation as described under Experimental Procedures. Values shown are averages of at least three different experiments with standard errors less than 5%.

<table>
<thead>
<tr>
<th></th>
<th>8(R),9(S)-EET</th>
<th>8(S),9(R)-EET</th>
<th>11(R),12(S)-EET</th>
<th>11(S),12(R)-EET</th>
<th>14(R),15(S)-EET</th>
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Fig. 6. HPLC profiles for metabolism of arachidonic acid by murine extrahepatic microsomes. Murine kidney (A), lung (B), heart (C), and female adrenal (D) microsomal proteins (2–5 mg/ml each) were used for the reactions. The organic soluble products were extracted immediately into ethyl ether, dried under nitrogen stream, resolved by reverse-phase HPLC, and quantified by on-line liquid scintillation using a Radiomatic Flow-One β-detector. The retention times of authentic standards are indicated by the bars above the respective peaks.
male adrenals, suggesting that CYP2C37 is the CYP2C found in the X-zone of female adrenals. Moreover, different murine CYP2Cs are expressed in the reproductive systems of both sexes. Only CYP2C29 was found in the male reproductive system, but both CYP2C29 and CYP2C40 were found in female reproductive expression, and female CYP2Cs were detected.

In summary, we detected CYP2Cs in murine extrahepatic tissues by immunoblotting and RT-PCR, and their cellular localization was determined by immunohistochemistry. The CYP2Cs are extensively expressed in extrahepatic tissues such as heart, lung, kidney, intestine, adrenals, and eye. Expression is also detected in male and female reproductive organs. The expression of the different CYP2Cs was organ-selective. The CYP2Cs were found to metabolize AA to distinctly different region- and stereospecific products. Recently, extrahepatic CYPs have attracted interest in many fields because of their roles in the metabolic activation of endogenous compounds such as arachidonic acid. The CYP2Cs may have important organ-specific biological functions, and the results of the present study provide preliminary clues to possible functional roles of CYP2Cs in the extrahepatic tissues. We anticipate that the mouse could serve as a useful model system to investigate the possible endogenous biological functions of the human CYP2Cs.

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References


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