0026-895X/92/06958-06$03.00/0

MOLECULAR PHARMACOLOGY, 42:958-963

ACCELERATED COMMUNICATION

Identification of Rabbit Cytochromes P450 2C1 and 2C2 as Arachidonic Acid Epoxygenases

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Received July 17, 1992; Accepted September 22, 1992

SUMMARY

Microsomes prepared from COS-1 cells transiently expressing rabbit cytochromes P450 2C1 and 2C2 catalyzed the metabolism of arachidonic acid to predominantly 11,12- and 14,15-epoxyeicosatrienoic acids (EETs) when microsomal epoxide hydrolase activity was inhibited by 0.2 mM 1,2-epoxy-3,3,3-trichloropropane. P450 2C2 catalyzed the formation of 11,12-EET and 14,15-EET at a ratio of 3.0 and also produced 19-hydroxyeicosatetraenoic acid (19-HETE). The 11,12-EET, 14,15-EET, and 19-HETE represented 48.3, 15.9, and 12.8%, respectively, of the total metabolites formed. P450 2C1 produced a similar but distinct ratio of 11,12-EET to 14,15-EET (2.0) and did not produce any detectable 19-HETE. The 11,12-EET and 14,15-EET represented 63.0 and 31.1%, respectively, of the total metabolites formed. The 8,9- and 5,6-EETs were not detected with either enzyme. The ratio of the 11,12-EET to 14,15-EET was 1.5 with P450 2CAA, a P450 arachidonic acid epoxygenase (P450 2CAA) that had an amino-terminal sequence identical to that of P450 2C2 [*J. Biol. Chem.* **267**:5552–5559 (1992)]. P450 2C1, 2C2, and 2CAA metabolized lauric acid. The ratio of ω−1- to ω-hydroxylated laurate was 3.6, 3.4, and 2.4 for P450 2CAA, P450 2C2, and P450 2C1, respectively. Purified P450 2CAA had a slightly greater apparent molecular weight than expressed P450 2C2 on sodium dodecyl sulfate-polyacrylamide gels. The results clearly establish that rabbit P450 2C1 and 2C2 are arachidonic acid epoxygenases, and they suggest that P450 2CAA and 2C2 are very similar but may not be identical isoforms.

The importance of P450-dependent[1] arachidonic acid metabolism has been well documented. The enzyme system can catalyze the formation of C_{16} to C_{20} HETEs, six regioisomeric *cis*, *trans*-conjugated HETEs, and four regioisomeric EETs. These metabolites, derived from the release of arachidonic acid present in hormone-sensitive lipid pools, have a variety of physiological and pharmacological properties (2–5).

The epoxygenase reaction of P450 has received considerable attention as a result of the potent biological activity of the various EET metabolites (3) and the increase of epoxygenase metabolites during pregnancy-induced hypertension in humans (6). For example, 11,12-DHET inhibits Na^+/K^+-ATPase (7, 8) and 14,15-EET promotes endothelial cell-dependent adhesion of human monocyte tumor U937 cells (9), inhibits the release of renin from rat renal cortical slices (10), amplifies vasopressin-induced calcium flux in rat mesangial cells (11), and activates Na^+/H^+ exchange and was mitogenic in cultured rat glomerular cells (12).

We previously reported the purification of a P450 arachidonic acid epoxygenase from untreated rabbit renal cortex microsomes (13). This isoform, termed P450 2CAA, metabolizes arachidonic acid to two of the four possible regioisomeric EET metabolites and, to a lesser extent, the ω/ω−1-hydroxylated products. The 11,12- and 14,15-EETs represented 45% and 30% of the total metabolites, respectively, whereas 13% of the total metabolites were ω/ω−1-hydroxylated. The amino-terminal amino acid sequence of the first 20 residues was identical to the sequence of rabbit P450 2C2 deduced from the cDNA sequence (14). Based on antibody inhibition studies, Finlayson *et al.* (15) reported that P450 2C2 catalyzed a major portion of the laurate ω−1-hydroxylation in renal tissue. In addition, Imai (16) reported that expressed P450 2C2 was an effective catalyst of lauric and capric acid ω−1-hydroxylation.

The enzymes of the P450 2C gene family have a very high degree of amino acid sequence homology (17–19) while exhibiting different catalytic characteristics (19–21). P450 2C4 and

This work supported by National Institutes of Health Grants PO1 HL41618 and T32 AM07139.

[1] P450 is used as a generic term for one or more forms of P450. Individual forms of P450 are designated according to a proposed uniform system of nomenclature (1).

ABBREVIATIONS: P450, cytochrome P450; HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; DHET, dihydroxyeicosatrienoic acid; HEPES, *N*-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; HBSS, Hanks' balanced salt solution; PMSF, phenylmethanesulfonyl fluoride; HPLC, high pressure liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; 1,2-ETCP, 1,2-epoxy-3,3,3-trichloropropane.

P450 2C5 exhibit about 95% sequence identity (22) yet differ 10-fold in the K_m for progesterone 21-hydroxylation (23). P450 2C1 and P450 2C5 are 70% identical and P450 2C1 exhibits no progesterone 21-hydroxylase activity (23). P450 2C1 and P450 2C2 are 84% identical, but studies comparing fatty acid metabolism between the two enzymes are lacking. In an effort to characterize arachidonic acid metabolism by P450 2C1 and P450 2C2, we have transiently expressed the cDNAs for P450 2C1 and 2C2 in COS-1 cells and compared the catalytic properties with those of P450 2CAA.

Experimental Procedures

Materials. DEAE-Dextran (M_r = 500,000), glucose-6-phosphate, glucose-6-phosphate dehydrogenase (type XII), NADP (grade III), Tris, and EDTA were from Sigma Chemical Co. (St. Louis, MO). Arachidonic and lauric acids were from Nu-Chek Prep, Inc. (Elysian, MN). 1,2-Dilauroyl-*sn*-3-phosphatidylcholine was from Calbiochem (Costa Mesa, CA). Dulbecco's modified Eagle medium, fetal calf serum, trypsin, HBSS, and all other cell culture solutions were from GIBCO (Grand Island, NY). Leupeptin, aprotinin, and PMSF were from Boehringer Mannheim (Indianapolis, IN). Bovine serum albumin (fraction V) was from United States Biochemicals (Cleveland, OH). [1-^{14}C] Arachidonic acid and [1-^{14}C]lauric acid were from American Radiolabeled Chemicals (St. Louis, MO). Ecolite (+) scintillant was from ICN (Costa Mesa, CA). The 1,2-ETCP was provided by Dr. G. D. McCoy (Case Western Reserve University, Cleveland, OH). All organic solvents (each HPLC grade) were from Fisher Scientific (Pittsburgh, PA).

Cell culture. COS-1 cells were obtained from the American Type Culture Collection and were maintained in Dulbecco's modified Eagle medium (no antibiotics) supplemented with 5% (v/v) fetal calf serum at 37° in 5% CO_2. The medium was changed every 48 hr and the cells were passaged approximately once per week as recommended by the American Type Culture Collection.

Plasmid preparation. The full length cDNAs for P450 2C1 and P450 2C2 in pCMV5 were a generous gift from Dr. Byron Kemper, University of Illinois (Urbana, IL), and permission to use the pCMV5 vector was kindly granted by Dr. Mark Stinski, University of Iowa (Iowa City, IA). The *Escherichia coli* strain SCS-1 (Stratagene, La Jolla, CA) was made transformation competent as described by Hanahan (24) and was transformed with the 2C1 and 2C2 plasmids. Plasmids were prepared using alkaline lysis; RNA was then precipitated using 5 M LiCl and the resulting closed circular plasmid was purified by centrifugation through two continuous CsCl gradients, as described by Sambrook *et al.* (25).

Transient expression of P450 2C1 and 2C2 in COS-1 cells. COS-1 cells were transfected with plasmid DNA using the DEAE-dextran procedure described by Clark and Waterman (26), with the plasmids being used at 10 μg/100-mm dish. The medium described for cell culture was substituted into the transfection protocol. Transfection conditions were optimized by expressing the luciferase gene from the pGL2-Control vector (Promega, Madison, WI) and assaying activity with a luciferase assay system (Promega) as recommended by the manufacturer.

Isolation of COS-1 microsomes. Microsomes from COS-1 cells transiently expressing P450 were prepared 96 hr after transfection. The medium was aspirated and the cells on twenty 100-mm plates were incubated for 15 min at room temperature in HBSS with 1 mM EDTA. The cells were scraped and collected by centrifugation at 2000 × *g* for 10 min at 4°. The HBSS was aspirated, and the cells were washed with 10 ml of ice-cold phosphate-buffered saline and centrifuged as described above. The cells were resuspended in 1 ml of homogenization buffer consisting of 0.5 M sucrose, 10 mM Tris-chloride, pH 7.5, 1 mM EDTA, 5 μg/ml leupeptin, 10 μg/ml aprotinin, and 100 μM PMSF and were homogenized with a Kontes (Vineland, NJ) no. 20 glass/Teflon homogenizer for 25 strokes at 1800 rpm. The cell homogenate was centrifuged at 9000 × *g* for 10 min at 4°. The supernatant was added to a 13.7-ml quick-seal centrifuge tube (Beckman, San Ramon, CA). The 9000 × *g* pellet was homogenized with 1 ml of homogenizing buffer and centrifuged two more times as described above, and the supernatants were pooled in the quick-seal tube. Microsomes were pelleted by centrifugation at 216,000 × *g* for 30 min at 4°, resuspended in 100 mM Tris-chloride buffer, pH 7.4, containing 20% glycerol, and stored at −70°. Protein was determined as described by Lowry *et al.* (27).

Isolation of P450 2CAA, cytochrome b_5, and NADPH-P450 oxidoreductase. P450 2CAA was purified as described by Laethem *et al.* (13). NADPH-P450 oxidoreductase was purified by the method of French and Coon (28), modified with the affinity chromatography steps described by Dutton *et al.* (29). Purified P450 oxidoreductase catalyzed the reduction of 45–60 μmol of cytochrome *c*/min/mg of protein at 30° in 300 mM phosphate buffer, pH 7.7 (30). Cytochrome b_5 was prepared as described by Strittmatter *et al.* (31) and had a specific content of 59 nmol of cytochrome b_5/mg of protein.

Fatty acid metabolism. Fatty acid metabolism was carried out as described previously (13). The substrates [7.2 μM [^{14}C]arachidonic acid (0.2 μCi, 55 μCi/μmol) or 6.8 μM [^{14}C]lauric acid (0.2 μCi, 55 μCi/μmol)], dissolved in ethanol, were evaporated to dryness in glass tubes, and either 100 μg of COS-1 microsomes or a reconstituted P450 system consisting of 0.05 nmol of P450 2CAA and 0.15 nmol of NADPH-P450 oxidoreductase was added. This mixture was incubated for 15 min on ice. When the reconstituted system was used, 15 μg of sonicated 1,2-dilauroyl-*sn*-3-phosphatidylcholine were added and the mixture was incubated for an additional 15 min on ice. The remainder of the complete reaction mixture, which contained 50 mM HEPES-chloride buffer, pH 7.6, an NADPH-generating system composed of 0.5 mM NADP and 10 mM glucose-6-phosphate, and in some cases 0.2 nmol of cytochrome b_5, was then added to a final volume of 0.5 ml. Reactions were initiated by the addition of 2.5 units of glucose-6-phosphate dehydrogenase and were incubated at 37° for 15 min (pure enzyme) or 40 min (COS-1 microsomes). Reactions were stopped by acidification to pH 4.5 with 10 μl of 6.7% formic acid and were extracted three times with 2 volumes of ethyl acetate. The organic phases were pooled and dried under reduced pressure. Metabolites were resolved by HPLC as described below. All reactions were conducted under conditions that were linear with respect to time and protein. Recovery of radioactivity was >98%. For some reactions 1 μl of 0.1 M 1,2-ETCP in ethanol was added immediately before the glucose-6-phosphate dehydrogenase. The final concentration of ethanol was 0.2% and had no effect on substrate metabolism.

Reverse phase HPLC. The arachidonic acid extracts were dissolved in 80 μl of 50% acetonitrile containing 0.1% acetic acid, and metabolites were separated on a Beckman model 344 HPLC equipped with a C_{18}-Microsorb column (4.6 × 250 mm, 5 μm; Rainin, Woburn, MA) with a linear gradient (1.25%/min) from 50% acetonitrile in water containing 0.1% acetic acid to 0.1% acetic acid in acetonitrile, at a flow rate of 1.0 ml/min. Radioactivity was monitored with a Radiomatic FLO-ONE A250 detector (Tampa, FL) with Ecolite (+) scintillation cocktail. Lauric acid metabolites were resolved using the same system with a two-part linear gradient. The first part was from 43 to 45% acetonitrile in water containing 0.1% acetic acid (0.22%/min) and the second was from 45% acetonitrile in water containing 0.1% acetic acid to 0.1% acetic acid in acetonitrile (11%/min, held at 0.1% acetic acid in acetonitrile for 3 min).

Immunoblotting. SDS-PAGE was carried out on 8% polyacrylamide separating gels, as described by Laemmli (32). Proteins were transfered to nitrocellulose membranes as described by Schnier *et al.* (33), using the buffer system described by Towbin *et al.* (34). The nitrocellulose sheets were blocked with 1% bovine serum albumin in 10 mM Tris-chloride buffer, pH 7.4, containing 0.9% NaCl (Tris-buffered saline) and were incubated with a 1/500 dilution of mouse ascites fluid containing the 2F5 monoclonal antibody (provided by Dr. Eric Johnson, Research Institute of Scripps Clinic, La Jolla, CA) or 15 μg/ml sheep anti-P450 2C3 (provided by Dr. Patrick Maurel, Institut

National de la Santé et de la Recherche Médicale, Montpellier, France). The blots were washed with Tris-buffered saline containing 0.05% Tween 20 as described previously (33). The secondary antibody was a 1/2000 dilution of rabbit anti-mouse IgG conjugated to horseradish peroxidase (Organon Teknika-Cappel, Durham, NC) or a 1/5000 dilution of rabbit anti-sheep IgG and sheep peroxidase antiperoxidase. The immune complex was detected with chemiluminescence using an ECL detection kit, as described by the manufacturer (Amersham, Arlington Heights, IL), and Kodak X-Omat AR film.

Data analysis. The data were analyzed with Instat statistics software (GraphPAD Software, San Diego, CA), using a one-way analysis of variance followed by the Bonferroni *t* test.

Results

Expression of P450 2C2 in COS-1 cells. Expressed P450 2C2 was detected on immunoblots (Fig. 1) using the 2F5 anti-P450 2C5 monoclonal antibody developed by Reubi *et al.* (35). The antibody does not recognize P450 2C1 (23), and attempts to identify the protein with an anti-P450 2C3 antibody were unsuccessful. Therefore, expression of P450 2C1 was inferred from the enzymatic activity as described below, assuming that the catalytically active enzyme represents full length expression of the protein. No P450 2C2 was detected immunochemically in microsomes from COS-1 cells transiently expressing pCMV5 with no insert cDNA (mock transfections). When the mobility of the expressed P450 2CAA was compared with that of purified P450 2C2, P450 2CAA exhibited an apparent molecular weight slightly greater than that of P450 2C2. The small difference in mobility was not affected by the addition to purified P450 2CAA of microsomal preparations prepared from mock-transfected COS-1 cells (data not shown).

Arachidonic acid metabolism. Arachidonic acid metabolism by microsomes from COS-1 cells transiently expressing P450 2C1 and P450 2C2 was compared with that of purified P450 2CAA (Figs. 2 and 3). No metabolites were observed in reactions using COS-1 microsomes isolated after mock transfections (data not shown). When reactions were run in the absence of an epoxide hydrolase inhibitor, the arachidonic acid metabolites produced by both P450 2C1 and P450 2C2 had retention times corresponding to those of the 11,12- and 14,15-EETs and the 11,12- and 14,15-DHETs (Figs. 2B and 3B). The presence of DHETs was indicative of microsomal epoxide hydrolase. When the epoxide hydrolase inhibitor 1,2-ETCP (36) was included in the incubations, the DHET metabolites were no longer produced and there was a corresponding increase in the EETs (Figs. 2C and 3C). The two EET peaks coeluted with authentic 11,12- and 14,15-EETs, indicating that these are the major arachidonic acid metabolites produced by both enzymes and were identical to the two regioisomeric EETs produced by P450 2CAA (Figs. 2A and 3A). The 11,12- and 14,15-EETs accounted for 63.0 and 31.1%, respectively, of the total metabolites for P450 2C1. For P450 2C2, the 11,12- and 14,15-EETs were 48.3 and 15.9%, respectively, of the total metabolites.

Although the EET metabolites were the same for the three isoforms, the ratio of the two regioisomeric products was distinct for each. In the presence of 0.2 mM 1,2-ETCP, the ratio (average ± standard error of four experiments) of 11,12-EET to 14,15-EET was 1.5 ± 0.1 for P450 2CAA, 3.0 ± 0.25 for P450 2C2, and 2.0 ± 0.1 for P450 2C1. These values were significantly

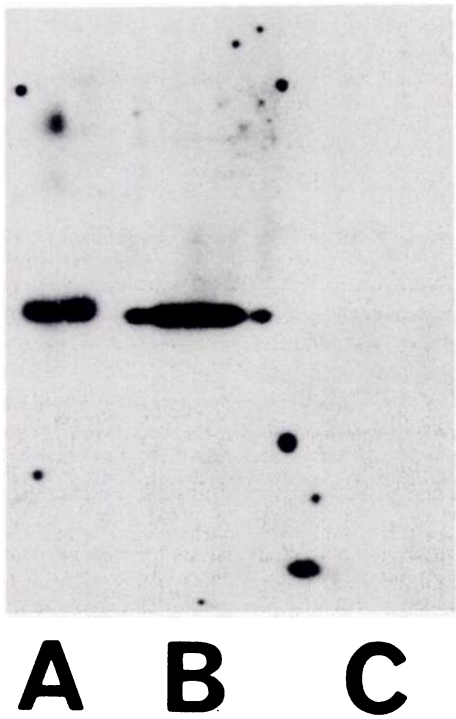

Fig. 1. Chemiluminescent detection of P450 2C2 on immunoblots with 2F5 monoclonal antibody. Proteins were resolved with SDS-PAGE, blotted, and detected as described in Experimental Procedures. *Lane A*, 61 ng of purified P450 2CAA; *lane B*, 260 ng of microsomal protein from COS-1 cells expressing P450 2C2; *lane C*, 260 ng of microsomal protein from mock-transfected COS-1 cells.

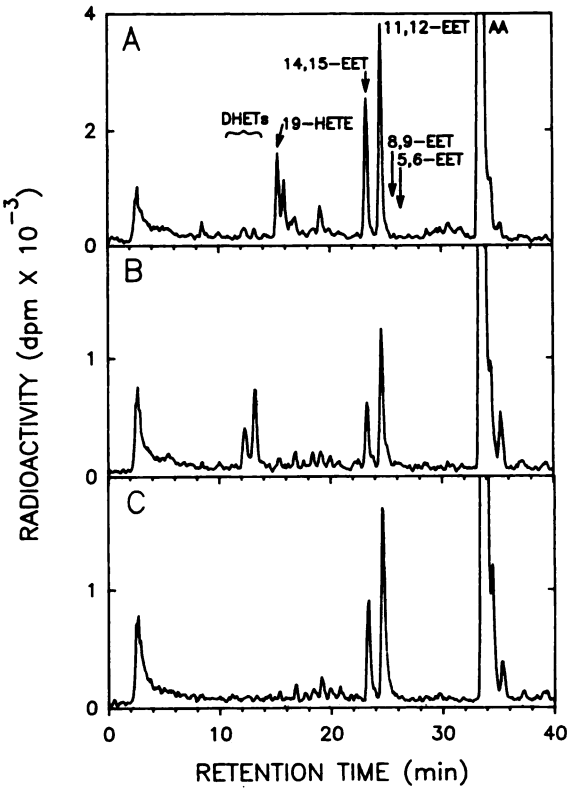

Fig. 2. P450-dependent arachidonic acid (*AA*) metabolism by expressed P450 2C1. The activity of purified P450 2CAA or COS-1 microsomal protein was assayed as described in Experimental Procedures. A, Metabolites from 50 pmol of purified P450 2CAA; B, metabolites from 100 μg of microsomal protein from COS-1 cells expressing P450 2C1; C, same as B except that a 0.2 mM concentration of the epoxide hydrolase inhibitor 1,2-ETCP was included in the reaction. *Arrows* in A, retention times of synthetic 8,9- and 5,6-EET.

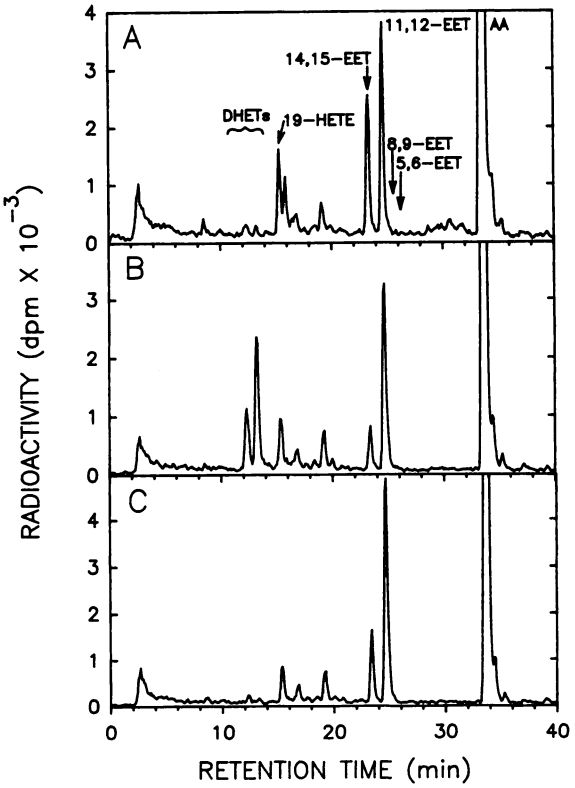

Fig. 3. P450-dependent arachidonic acid (*AA*) metabolism by expressed P450 2C2. The activity of purified P450 2CAA or COS-1 microsomal protein was assayed as described in Experimental Procedures. A, Metabolites from 50 pmol of purified P450 2CAA; B, metabolites from 100 μg of microsomal protein from COS-1 cells expressing P450 2C2; C, same as B except that 0.2 mM 1,2-ETCP was included in the reaction. *Arrows* in A, retention times of synthetic 8,9- and 5,6-EET.

different ($p < 0.05$ between P450 2CAA and 2C1; $p < 0.001$ between P450 2C2 and 2CAA and between P450 2C2 and 2C1). It was possible that the difference in the ratios was due to the incubation conditions, because P450 2C1 and 2C2 were microsomal and P450 2CAA was purified. In control experiments, it was found that the efficiency of extraction of the EETs was not affected by the presence of mock-transfected microsomes or 0.2 mM 1,2-ETCP (data not shown). In addition, the ratio of EET metabolites generated by P450 2CAA was independent of the added cytochrome b_5 and NADPH-P450 oxidoreductase concentrations and was not affected when the reconstituted system was mixed with COS-1 cell microsomes from mock transfections (data not shown). These results suggest that the difference in the ratio of the two EETs for each enzyme reflects the actual metabolism of arachidonic acid to those products and is not an artifact of the methodology or of differences in microsomal preparations.

Both P450 2CAA and P450 2C2 also produced smaller amounts of the ω−1-hydroxylated metabolite 19-HETE. This metabolite was identified by coelution with the metabolite produced by P450 2E1 that was characterized by gas chromatography/mass spectrometry.[2] The 19-HETE metabolite represented 12.8% of the total metabolite for P450 2C2, about the same as that produced by P450 2CAA (13). In contrast, P450 2C1 did not catalyze the formation of any detectable ω−1-hydroxylated metabolite.

Lauric acid metabolism. The results of Finlayson *et al.* (15) and Imai (16) suggest that P450 2C2 is an effective lauric acid ω−1-hydroxylating isoform. We, therefore, also characterized the metabolism of lauric acid with the three enzyme preparations. The lauric acid metabolite profiles for P450 2CAA, 2C2, and 2C1 are shown in Fig. 4. The profiles of all three isoforms are very similar with this fatty acid. The ratio (average ± standard error of three determinations) of ω−1- to ω-hydroxylated metabolite was 3.7 ± 0.7, 3.4 ± 0.1, and 2.4 ± 0.1 for P450 2CAA, P450 2C2, and P450 2C1, respectively. Only the ratios for P450 2CAA and P450 2C1 were significantly different ($p < 0.05$).

Discussion

We demonstrated that transiently expressed P450 2C1 and 2C2 are arachidonic acid epoxygenases. As previously reported for P450 2CAA (13), the major metabolites are the 11,12- and 14,15-EETs, with no 8,9- or 5,6-EET being detectable. The 8,9- and 5,6-EETs are well separated with the chromatographic system described and are detected in reactions using P450 2B4 (37). Both P450 2CAA and 2C2 also metabolize arachidonic acid to the ω−1-hydroxylated product 19-HETE, whereas P450 2C1 failed to produce this metabolite. P450 2C1 and 2C2 can now be grouped among other P450 isoforms known to catalyze the epoxidation of arachidonic acid. This list includes rat P450

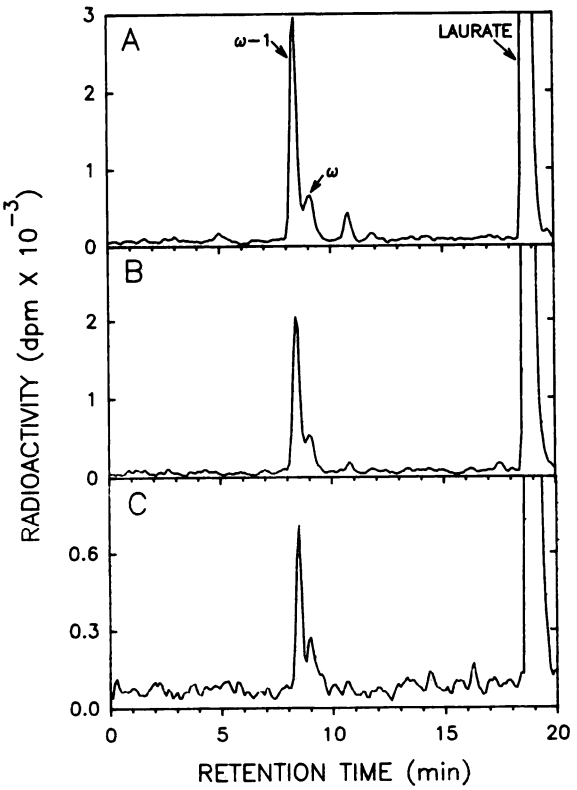

Fig. 4. P450-dependent lauric acid metabolism by expressed P450 2C1 and P450 2C2. The activity of purified P450 2CAA or COS-1 microsomal protein was assayed as described in Experimental Procedures. A, Metabolites from 50 pmol of purified P450 2CAA; B, metabolites from 100 μg of microsomal protein from COS-1 cells expressing P450 2C2; C, metabolites from 100 μg of microsomal protein from COS-1 cells expressing P450 2C1.

[2] R. M. Laethem, M. Balazy, and D. R. Koop. Formation of 19(S)-, 19(R)-, and 18(R)-hydroxyelcosatetraenoic acids by alcohol-inducible cytochrome p450 2E1. Manuscript in preparation.

1A1, 1A2, 2B1, 2B2, and 2C11 (38), rabbit P450 1A1, 1A2, and 2B4 (36), and human P450AA (39). However, P450 2C1, 2C2, and 2CAA are unique in their ability to catalyze specifically the formation of only two of the four possible epoxides, 11,12- and 14,15-EETs. Although P450 2C1 is 84% identical to P450 2C2, it is not able to hydroxylate arachidonic acid at the less reactive C_{19} position, however, the orientation of the substrate must be similar to that in both P450 2CAA and P450 2C2, because only two of the four possible epoxides are produced. Although P450 2C1 cannot hydroxylate arachidonic acid at the penultimate carbon, it is an effective catalyst of this reaction with lauric acid. Thus, it would appear that the orientation of the substrate changes dramatically with the three isoforms as the fatty acid substrate is changed. We are currently evaluating the metabolism of other fatty acids with these three enzymes to determine whether the regioselectivity of metabolism is maintained as the chain length and number of double bonds are varied.

A comparison of the ratio of the 11,12-EET to 14,15-EET produced by P450 2CAA and 2C2 suggests that they are very similar but may not be identical isoforms. There are numerous examples of substrate specificity being influenced by a few critical residues. Kedzie *et al.* (40) reported that an allelic variant of P450 2B1 exhibits regioselective hydroxylation of androstenedione. Although P450 2E1 and P450 2E2 exhibit 97% absolute sequence identity, they also show different rates of product formation and have different affinities for small molecular compounds such as glycerol (41). Kronbach *et al.* (42) reported that the apparent K_m of P450 2C4 for progesterone 21-hydroxylation can be dramatically improved by replacing valine at position 113 with alanine, which is present at this position in P450 2C5. It was also reported that the substrate specificity of $P450_{coh}$ can be changed from coumarin to steroid hydroxylation by changing phenylalanine at position 209 to leucine (43). A single amino acid substitution can also affect the electrophoretic mobility. For example, Kedzie *et al.* (40) reported a difference in the mobility of P450 IIB1-SD and P450 IIB1-WM, proteins that differ by a single amino acid at position 478. Internal amino acid sequence information for P450 2CAA must be obtained to unequivocally identify P450 2CAA as being P450 2C2, a unique P450 2C family member, or an allelic variant of P450 2C2.

The major phenobarbital-inducible P450 in rabbit liver, P450 2B4 (44), is probably the principal hepatic arachidonic acid epoxygenase after phenobarbital treatment. However, this isoform catalyzes the formation of all four regioisomeric EETs (35, 37). P450 2C2 is also induced by phenobarbital treatment in rabbit liver and kidney (45). During phenobarbital treatment, the hepatic arachidonic acid epoxygenase activity of P450 2C2 is masked by the overwhelming amount of P450 2B4. However, P450 2C2 may be the predominant constitutive arachidonic acid epoxygenase in rabbit kidney and liver. It remains to be determined whether an ortholog of P450 2C2 is present in human liver and kidney. Laniado-Schwartzman *et al.* (39) isolated a P450 arachidonic acid epoxygenase from human liver. Based on the amino-terminal sequence this isoform does not appear to be a member of the P450 2C family, and it makes all four of the arachidonic acid EET metabolites. It will be of interest to determine whether any of the human P450 2C isoforms currently characterized are specific for metabolism of arachidonic acid to the 11,12- and 14,15-EETs. It is clear that further studies are necessary to determine what physiological importance the specific production of the 11,12- and 14,15-EETs has in renal and hepatic function.

Acknowledgments

The authors thank Carmen L. Laethem and Eric A. Kienle for excellent technical assistance.

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