Molecular Cloning, Expression, and Enzymatic Characterization of the Rat Kidney Cytochrome P-450 Arachidonic Acid Epoxygenase*

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A cDNA containing an open reading frame coding for the rat kidney cytochrome P-450 arachidonic acid epoxygenase was isolated from a male rat kidney cDNA library. Sequence analysis showed that with the exception of 11 nucleotides, this cDNA is identical with the published sequence for rat liver cytochrome 2C23 and encodes a polypeptide of 494 amino acids. Nucleic acid blot hybridization indicated that the levels of expression of the corresponding mRNA are high in rat kidney and liver and are undetectable in brain and heart.

The cDNA coding region was cloned into a pCMV2 vector and expressed in COS-1 cells. The recombinant microsomal protein catalyzed the NADPH-dependent metabolism of arachidonic acid to a mixture of 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acids as the only oxygenation products. The enantiofacial selectivity of the recombinant protein was nearly identical with that reported for the kidney microsomal enzyme and generated 8(R),9(S)-, 11(R),12(S)-, and 14(S),15(R) with optical purities of 95, 85, and 75%, respectively. On the basis of mRNA abundance and the close similarities between the regio- and stereochromical selectivity of the recombinant and kidney microsomal proteins, we concluded that cytochrome P-450 2C23 is the predominant enzyme isoform responsible for arachidonic acid epoxidation in the rat kidney.

The physiological significance of arachidonic acid as the precursor for a variety of oxygenated metabolites is well documented (Ref. 1 and references therein). Thus, it is through the action of regio- and stereospecific oxygenases that cellular mediators such as prostaglandins and leukotrienes are formed (1 and references therein). In 1981, several groups demonstrated a role for microsomal cytochrome P-450 in the in vitro catalysis of arachidonic acid metabolism (2-4). The P-450-arachidonic acid epoxygenase catalyzes the NADPH-dependent formation of 5,6-, 8,9-, 11,12-, and 14,15-EETs.

Studies utilizing purified P-450 isoforms demonstrated that the epoxygenase is highly stereospecific and that the asymmetry of oxygen addition is P-450 isoform-specific (5). To date, arachidonic acid epoxygenase activity has been demonstrated in numerous tissues, including kidney, liver, brain, pituitary, adrenal, and endothelium (6, 7 and references therein). The demonstration of a role for P-450 in the in vivo metabolism of endogenous arachidonic acid established the epoxygenase as a member of the arachidonic acid cascade and documented a novel endogenous role for this hemoprotein in the generation of bioactive eicosanoids (6, 7 and references therein).

The potent biological activities associated with several metabolites of the arachidonic acid epoxygenase have stimulated interest in the role of P-450 in the control of cell and organ physiology (6, 7 and references therein). Among the biological activities attributed to the EETs or to their hydration products, the DHETs, those of significance to renal physiology include (a) changes in the permeability of the rabbit isolated cortical collecting tubule to Na+ and K+ (8) and in the transport of Na+ by the proximal tubule (9); (b) inhibition of vasopressin-stimulated water reabsorption (10, 11); and (c) enantioselective renal vasoconstriction (12). Studies with the spontaneous hypertensive rat model indicated that P-450-derived arachidonic acid metabolites may play a role in the pathophysiology of hypertension (6). This view has recently been strengthened by (a) the demonstration, in humans, of increased urinary excretion of DHETs during pregnancy-induced hypertension (13); (b) the preferential expression of the P-450 4A2 gene in spontaneous hypertensive rats (14); and (c) the regulation of the renal epoxygenase by dietary salt loading (15).

Although there have been extensive biochemical and structural studies of the epoxygenase metabolites, little is as yet known with regard to the molecular properties of the renal cytochrome P-450 4A2 protein isoform(s) active in arachidonic acid epoxidation. A member of the P-450 2C gene family was recently purified from rabbit kidney and shown to catalyze arachidonic acid epoxidation and, to a lesser extent, ω-1 oxidation (16). Inhibition studies utilizing a panel of polyclonal antibodies to several rat liver P-450 isoforms indicated that the rat kidney epoxygenase belongs to the hemoprotein 2C gene family (15). Thus, it became apparent that further progress in delineating the functional role of the epoxygenase enzyme system requires, on the one hand, a detailed knowledge of the molecular properties of the enzyme and, on the other hand, access to biospecific probes for studies of its regulation at the gene and/or protein level. We report here the molecular cloning of the rat kidney P-450 epoxygenase; demonstrate that its nucleic acid sequence is nearly identical to that reported for P-450 2C23 (17); and establish unequiv-
ocally that a single recombinant protein can catalyze, albeit with different efficiencies, the highly asymmetric epoxidation of the arachidonic acid 8,9-, 11,12-, and 14,15-olefins.

MATERIALS AND METHODS

Isolation of Rat Kidney Poly(A)+ RNA, Synthesis, and Screening of the cDNA Library—Whole kidneys were removed from anesthetized (Nembutal, 50 mg/kg body weight) adult male Sprague-Dawley rats (300-350 g body weight), immediately frozen in liquid N₂, and stored at -80°C. Within the next 48 h, poly(A)+ RNA was prepared by the guanidinium isothiocyanate/oligo(dT)-Sepharose method using an mRNA extraction kit from Pharmacia LKB Biotechnology Inc. An oligo(dt)-primed Uni-Zap cDNA library was synthesized using a Lambda-Zap cDNA synthesis kit from Stratagene (La Jolla, CA) following the manufacturer's instructions. For screening, the plaque was plated, employing XL-1-Blue Escherichia coli as host, at a density of 10⁶ plaque-forming units/plate. A total of approximately 2 x 10⁹ clones were screened with a 1-kb fragment probe containing the 3' sequence of rat liver cytochrome P-450 2C11 cDNA (18), labeled by nick translation with 3²PdATP. Nucleic acid hybridizations were done at 65°C in 0.9 M NaCl, 90 mM sodium citrate (pH 7.0) containing 1% SDS. Double-stranded cDNA was isolated using the Invitrogen Lambda-Zap cDNA synthesis kit from Stratagene (La Jolla, CA) following the manufacturer's instructions. The phage DNA isolated from positive clones were plaque-purified, the phage DNA isolated and rescued into pBluescript SR(+)(Stratagene).

DNA Sequencing and Nucleic Acid Blot Hybridization—Plasmid DNAs were replicated in DH5α competent Escherichia coli (In Vitro- gen, San Diego, CA) grown in Luria Broth containing ampicillin (100 μg/ml). DNA Sequencing and Nucleic Acid Blot Hybridization—Plasmid DNAs were replicated in DH5α competent Escherichia coli (In Vitrogen, San Diego, CA) grown in Luria Broth containing ampicillin (100 μg/ml). For plasmid purification, the cells were lysed in the presence of 0.2 N NaOH containing 1% sodium dodecyl sulfate and the plasmid DNA purified using Qiagen Columns (Qiagen Inc. Chatsworth, CA) following the manufacturer's instructions. The plasmid DNA insert (1.9 kb) was sequenced by the dideoxy chain termination method (19) using Sequenase (U. S. Biochemical Corp., 25 oligonucleotides (15-20-mer, each) spanning the length of the cDNA were utilized as primers for the sense and/or antisense cDNA strands. Nucleotide sequences were analyzed utilizing the IntelliGenetics Suite Molecular Biology Software and the GenBank On-line Service Genetics, Inc. Mountain View, CA).

Samples of poly(A)+ RNA (2-5 μg), isolated from rat liver, heart, and brain, were denatured and electrophoresed in agarose gels (13%) containing 0.2% formaldehyde as described (20). After transfer to a nitrocellulose membrane, the blots were hybridized with either a 1-kb fragment probe containing a region of the 3' sequence of P-450 2C11 cDNA (18), a 1.3-kb fragment containing a region of the 3' sequence of P-450 2C27 (21), the cloned P-450 2C23, or alternatively, a P-450 2C23-specific 51-mer oligonucleotide (5'-ctctctataatggatattctcaagtctgctcacc- atccattcgc-3'; residues 118-165 of the RD3 cDNA). Double-strand probes were labeled by nick translation using E. coli polynucleotide kinase and [γ-3²P]ATP. Single-strand probes were end labeled using T4 polynucleotide kinase and [γ-3²P]ATP. After exposure, the blots were rehybridized to a cDNA probe encoding rat liver tubulin.

Transfection and Expression in COS-1 Cells—For transfection, the cDNA fragment was released from the pBluescript SK(+)/phagemid and subcloned into pBluescript II (Stratagene), after adding an EcoRI site at the 3' end. This cDNA was released from the pBluescript II plasmid by digestion with KpnI and XhoI and cloned into the pCMV2 vector (a gift from Dr. Michael Waterman, Vanderbilt University) as described (22). The presence of an XhoI site in the 3'-untranslated region of the cloned cDNA yielded a 1,680-base pair insert containing the reading frame for a polypeptide of 184 amino acids. After propagation, the pCMV2 plasmid DNA was purified by centrifugation in CsCl. For transfection, 20 μg of plasmid DNA and 40-50 μg of Lipofectin (GIBCO), each in 250 μl of OPTI MEM (GIBCO/BRL) were mixed and used within the next 10 min.

Canine cells were grown to 60-70% confluence (100-mm dishes) in Dulbecco's modified eagle's Medium (Sigma) containing 10% (v/v) fetal calf serum, washed twice with serum-free medium, and then 2.5 ml of OPTI MEM and 0.5 ml of the DNA/Lipofecting mixture were added. After 5 h at 37°C, 7 ml of Dulbecco's modified Eagle's medium containing 10% (v/v) fetal calf serum was added and the incubation continued for a total of 72 h with medium changes every 24 h. Cells were washed with serum-free medium, scraped from the plates into 10 ml Tris-Cl (pH 7.5) containing 0.5 M sucrose, 1 mm EDTA, 1 mm phenylmethylsulfonyl fluoride, leupeptin (0.1 μg/ml), and antipain (0.02 μg/ml). The homogenized cell suspension was subjected to glass/Teflon cell disruption. Microsomal fractions were prepared by differential centrifugation exactly as described (22). Microsomal fractions were isolated from nontransfected cells or from cells transfected with the pCMV2 vector either lacking the coding insert or containing an antisense cDNA insert.

Microsome Incubations and Product Characterization—Microsomal fractions were suspended (1 mg of protein/ml) in 50 mM Tris-Cl buffer (pH 7.5) containing 150 mM KCl, 10 mM MgCl₂, 8 mM sodium isocitrate and isocitrate dehydrogenase (0.5 IU/ml) and 1 mm argon and quantified by liquid scintillation. The resolved [l-14C]EET antipodes were dried under N₂ and rehydrated with 1 ml of OPTI MEM and 0.5 ml of the DNA/Lipofecting mixture exactly as described (23). Microsomal fractions were prepared exactly as described (23). Microsomal fractions were added, followed by NADPH (1 mM final concentration). Aliquots of the reaction mixture were withdrawn at different time periods, the organic soluble products extracted into ethyl ether and analyzed by TLC (SiO₂ hexane/ether/CH₃Cl/CH₃COOH, 5:2:1:0.1). Prior to TLC analysis, excess [1,14C] arachidonic acid was removed by chromatography in a SiO₂ column (0.4 x 1 cm) equilibrated with hexane/CH₃COOH (2:1). Organic products, dissolved in equilibration solvent, were loaded onto the column, and after elution of excess arachidonic acid with the same solvent, oxygenated metabolites were eluted with ethyl ether/hexane/CH₃COOH (4:1:6). Products were detected and identified using synthetic [-1,14C] EETs and [1,14C] DHETs by exposing the plates to x-ray film (Eastman Kodak). For radiochemistry studies, reaction products were first purified by reversed-phase HPLC (23) and then resolved by normal phase HPLC as described (23). For chiral analysis the EETs were collected from the HPLC eluant, derivatized, purified, and resolved into the corresponding antipodes by chiral phase HPLC as described previously in (24). The resolved [1,14C] EET antipodes were dried under argon and quantified by liquid scintillation.

RESULTS AND DISCUSSION

Isolation and Characterization of the Rat Kidney Arachidonic Acid Epoxidease cDNA—Inhibition studies utilizing polyclonal antibodies against several rat liver P-450 isoforms showed that anti-cytochrome P-450 2C11 was a selective and potent inhibitor of kidney epoxidease (15). These results suggested that the rat kidney arachidonate epoxidease belongs to the P-450 2C gene family (25 and references therein). Furthermore, comparisons of the regio- and stereoselectivity of the rat kidney microsomal epoxidease with those of purified P-450 2C11 (5) indicate that although these proteins exhibited common immunocommunetchemical properties, they are nonetheless catalytically different proteins with distinct active site geometries (5). The high enantiofacial selectivity of the rat kidney microsomal enzyme as well as the inability of typical P-450 inducers to alter either its activity, regioselectivity, and/or stereoselectivity indicated that, different from the liver, the molecular heterogeneity of the renal epoxidease is limited (5). Based on the immunological evidence, the rat kidney cDNA library was screened with a 1-kb nucleotide fragment containing the 3' sequence of rat liver P-450 2C11 (18). Approximately 100 positive clones were identified of which 40 clones, selected at random, were plaque purified and rescued into pBluescript. Insert sizes were determined by agarose electrophoresis after EcoRI and XhoI digestion of the purified plasmids. Initial sequence analysis, utilizing T3 and T7 primers, demonstrated that (a) the majority of the positive clones (27 out of 40, 68%) shared sequence identity with rat liver P-450 2C23 (17); (b) 12 clones contained non-cytochrome P-450 inserts; and (c) the remaining two clones contained nucleotide sequence homologous to P-450 2E1 (26). One clone (RD3) contained part of the published 5' end and the entire coding sequence for P-450 2C23, and it was therefore selected for further study.

Sequence analysis demonstrated that RD3 contained an insert of 1,924 nucleotides with 99% sequence similarity to the published sequence for rat liver P-450 2C23 (17). An open reading frame, coding for a polypeptide of 494 amino acids, was flanked by initiation and termination codons (ATG and TAA) positioned between nucleotides 6 and 1488, respectively. Compared with the cDNA for P-450 2C23 (17), the 3' end of RD3 was 230 nucleotides shorter and did not contain
a poly(A) tail. Additionally, RD3 lacked a nucleotide (G) at position 111 and had an extra base (C) at position 168 (17). The deduced amino acid sequence for RD3 contained a putative heme-binding FSLGKRACVGESLAR peptide with the underlined conserved residues and the invariant cysteine heme-ligand at position 439 (27 and references therein). As a consequence of the base deletion and insertion mentioned above, between nucleotides 110 and 167 the coding sequences for RD3 and P-450 2C23 were different (17). Only the RD3 region codes for a peptide that is conserved among most of the members of this P-450 gene family.

A comparison of the RD3 nucleotide and/or amino acid sequences with those of other members of the P-450 2C subfamily indicated that with the exception of P-450 2C23, the extent of similarity was limited. Thus, human P-450 2C18 (21) exhibited the second highest degree of sequence identity with RD3 (67 and 61% for nucleotide and amino acid sequence, respectively). Moreover, the differences in nucleotide sequence between RD3 and other members of the 2C gene subfamily were randomly distributed along the entire length of the cDNA, indicating that the gene for P-450 2C23 or RD3 diverged from the members of the 2C subfamily at an early stage. Importantly, many of the differences in the amino acid sequence between P-450 2C23 or RD3 and other members of this subfamily represent conservative changes, i.e. replacements with residues with overall similar chemical properties.

Expression and Enzymatic Characterization of the Recombinant Arachidonic Acid Epoxygenase—The use of COS-1 cells for the transient expression of cloned P-450s is well documented (22). COS-1 cells have an endogenous NADPH-cytochrome P-450 reductase, fully capable of effective redox coupling to several expressed P-450s (22). Additionally, expression requires minimal manipulations of the cDNA, and the cells are easily grown and maintained. To characterize the catalytic properties of the protein molecule coded by RD3, COS-1 cells were transfected with a pCMV2 plasmid containing either a sense or an antisense 1,680-base pair insert spanning the RD3 5' end and open reading frame. Microsomal fractions were then isolated from the cultured cells and incubated with [1-14C]arachidonic acid in the presence of NADPH and an NADPH-regenerating system (23). As shown in Fig. 1, microsomes isolated from cells containing the antisense RD3 plasmid did not metabolize arachidonic acid to a significant extent. On the other hand, microsomal fractions isolated from cells transfected with the sense RD3 plasmid metabolized the fatty acid to products with chromatographic properties similar to those of synthetic EETs and DHETs. Furthermore, as shown in Fig. 2, the catalysis of EET and DHET formation was incubation time-dependent. It is apparent from the data in Figs. 1 and 2 that either, once formed, the EETs underwent extensive chemical hydration or that COS-1 microsomal fractions contained an active epoxide hydrolase activity, responsible for their rapid hydration to the corresponding DHETs (28). Inasmuch as DHET formation must be preceded by EET formation, we concluded that RD3 coded for a renal arachidonic acid epoxygenase. The translated, primary structure of the kidney arachidonate epoxygenase was nearly identical to that of the protein coded by a cDNA isolated previously from a rat liver cDNA library and designated P-450 2C23 (17). To the best of our knowledge, this is the first time that the P-450 2C23 gene product has been expressed and a catalytic activity for the recombinant protein determined.

The analysis of product regio- and stereoechemical properties provided important information concerning the P-450 isoform heterogeneity of the rat liver arachidonic acid epoxygenase (5). Studies utilizing purified hemoproteins and/or microsomal fractions, isolated from animals treated with selected P-450 inducers, demonstrated the coexistence in liver of several protein isoforms capable of epoxidizing arachidonate with distinct regio- and/or enantiofacial selectivities (5). To ascertain the role of the recombinant enzyme in the catalysis of arachidonic acid epoxidation by rat kidney, mi-

![Fig. 1. TLC analysis of the metabolites generated by incubates containing the microsomal recombinant epoxygenase and arachidonic acid.](image1)

![Fig. 2. Time course of EET and DHET formation by incubates containing the microsomal recombinant epoxygenase and arachidonic acid.](image2)
crosomal fractions isolated from transfected COS-1 cells were incubated with radiolabeled arachidonic acid, NADPH, and 4-fluorochalcone oxide (10 μM, final concentration), an inhibitor of epoxide hydrolase (29). After extraction, the reaction products were resolved by reversed HPLC as described (23). The data in Fig. 3 confirm that the RD3 cDNA clone coded for an arachidonate epoxygenase. Thus, microsomal fractions prepared from COS-1 cells containing recombinant rat kidney P-450 2C23 metabolized arachidonic acid to generate EETs and DHETs as their only oxygenation products (Fig. 3). As it is the case with the kidney microsomal enzyme (15), 11,12-EET was the predominant epoxygenase metabolite generated by the recombinant cytochrome P-450 2C23 epoxygenase (54% of the total), followed by 8,9- and 14,15-EET (27 and 14% of the total, respectively). The recombinant enzyme generated small quantities of the labile 5,6-DHET regioisomer as revealed by the presence of radioactive material eluting with an HPLC retention time similar to that of authentic 5,6-DHET. Importantly, the enantiofacial selectivity of the recombinant epoxygenase closely matches that reported for the kidney microsomal enzyme (15) and generated 8(R),9(S)-, 11(R),12(S)-, and 14(S)15(R)-EETs with optical purities of 95, 85, and 75%, respectively (Table I). We concluded, therefore, that the RD3 cDNA codes for the predominant P-450 epoxygenase of rat kidney and that the renal hemoprotein is nearly identical to liver P-450 2C23 (17).

A corollary of the studies with the recombinant enzyme was the demonstration that a single protein catalyst mediates, albeit with different efficiencies, the asymmetric epoxidation at the fatty acid 8,9-, 11,12- and 14,15-olefins. Since with the recombinant protein the asymmetry of the epoxygenase is under the control of a single protein catalyst, we propose that the P-450 2C23 active site molecular coordinates responsible for heme-fatty acid spatial orientation allow for a limited degree of substrate lateral displacement but, on the other hand, are remarkably rigid in defining the olefin enantiotropic face exposed to the heme-bound active oxygen (5).

**Expression Levels of the Cytochrome P-450 2C23 Arachidonic Acid Epoxygenase Gene**—The relative abundance of the P-450 2C23 epoxygenase mRNA in the rat kidney was evaluated by nuclear acid blot hybridization (20). Poly(A)* RNA was isolated from a male rat kidney, electrophoresed, and hybridized to several P-450 2C family probes, cloned from our cDNA library, and to a P-450 2C23 specific oligonucleotide (for sequence details see “Materials and Methods”). The labeled cDNA probes utilized contained sequences for P450 2C11 (RD1, 1 kb), 2C7 (RD2, 1.3 kb), and 2C23 (RD3, 1.9 kb). The proposal that the predominant arachidonic acid epoxygenase isoform in rat kidney is P-450 2C23 is further substantiated by the data in Fig. 4. As shown in this figure the organ steady-state concentrations of P-450 2C23 mRNA, measured by hybridization with the RD3 cDNA or with a P-450 2C23-specific nucleotide, were markedly higher than those for P-450 2C11 (RD1) and 2C7 (RD2). Previous studies have demonstrated that in addition to P-450 2C23, several other purified P-450 isoforms can actively catalyze the epoxidation of arachidonic acid (5–7, 16). Additionally, no information is yet available concerning the catalytic activity of P-450 2C23 toward substrates other than arachidonic acid.

The levels of expression of the P-450 2C23 arachidonic acid epoxygenase in different organs were studied in poly(A)* mRNA samples extracted from whole liver, kidneys, heart, and brain obtained from a single male rat. Blot hybridization using the RD3 cDNA clone demonstrated that although the levels of expression for liver and kidney were similar, the mRNA was undetectable in brain and heart (Fig. 4). The members of the P-450 2C gene family show extensive sequence homology, and therefore cross-hybridization is a common problem (30). The P-450 2C23-specific 51-base oligonucleotide was designed to minimize cross-hybridization between members of this gene subfamily. Blot hybridization with the 51-base oligonucleotide indicated the predominant expression of the mRNA coding for P-450 2C23 in the kidneys (Fig. 4). Compared with liver, the kidney is functionally and anatomically a highly heterogeneous organ. Several correlations between enzymatic stratification and the anatomical and/or...
TABLE I

Regio and stereoselectivity of the recombinant rat kidney arachidonic acid epoxygenase

Microsomal fractions, isolated from COS-1 cells transfected with the pCMV2 plasmid containing the cloned RD3 cDNA, were incubated with [1^14C]arachidonic acid in the presence of NADPH and 4-fluorochalcone oxide, an epoxide hydrolase inhibitor. After 20 min at 30 °C, the reaction products were extracted, resolved by reversed phase HPLC, and derivatized as described under “Materials and Methods.” The optical antipodes of derivatized 8,9-, 11,12-, and 14,15-EET were resolved by chiral phase HPLC as described (24).

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Fig. 4. Nucleic acid blot hybridization analysis of male rat mRNAs. 5 μg of poly(A)^+ RNA, isolated from the kidneys (KD), liver (LV), heart (HT), and brain (BR) of a male Sprague-Dawley rat (320 g, body weight), was submitted to electrophoresis and blot hybridized either to a 51-mer oligonucleotide specific for cytochrome P-450 2C11 (RD1) or 2C7 (RD2). After exposure to x-ray film (RD1, RD2, or RD3 probe, 2 h; 51-mer, 12 h), the blots were stripped and probed with [35S]tubulin cDNA. Densitometric analysis showed that the percent relative abundance of the tubulin mRNA with respect to the liver sample (100%) was 260, 530, and 126% for kidney, brain, and heart, respectively.

functional segmentation of the nephron are known (31 and references therein). The data in Fig. 4, in conjunction with the reported segment specific biosynthesis of some P-450 isofoms in the rat and rabbit nephron (32–34), suggest the potential for localized high level expression of the P-450 2C23 arachidonate epoxygenase.

Recently, it was demonstrated that a high dietary salt intake induces a kidney P-450 isoform active in the in vitro epoxidation of arachidonic acid (15). Moreover, the salt-induced hemoprotein(s) appeared immunologically different from that present in control rat kidney (15). The effect of dietary salt on the kidney levels of P-450 expression was studied by blot hybridization utilizing poly(A)^+ RNA samples extracted from the kidneys of control and salt-treated rats (15) and full-length cDNA probes coding for P-450s 2C23 (RD3) and 4A1 (a gift from Dr. Gordon Gibson, University of Surrey, Guildford, Surrey) (25, 27 and references therein), the RD1 (2C11) cDNA clone, as well as, the 51-mer 2C23-specific oligonucleotide described above. As shown in Fig. 5, high dietary salt intake resulted in a marked reduction in the levels of a kidney P450 mRNA that hybridized to the 2C11 probe (RD1). This marked reduction in the organ levels of 2C11 mRNA was evident within the first 12 h of salt treatment, and it was maintained for at least 12 days (not shown). On the other hand, salt treatment did not significantly alter the steady-state levels of kidney cytochromes P450 2C23 or 4A1 mRNAs. The results in Fig. 5 as well as published data obtained utilizing immunological techniques (15) are consistent with the proposal that the P-450 isoform induced by excess dietary salt is not P-450 2C23. Moreover, the data also indicate that salt induces a P-450 isoform either absent or present at very low levels in the kidneys of control animals (15).

In conclusion, we report here the molecular cloning, the initial structural characterization, and the expression of the major form of rat kidney cytochrome P-450 active in the highly asymmetric epoxidation of arachidonic acid. Additionally, we demonstrate unequivocally that a single protein can catalyze epoxidation at the fatty acid 5,6-, 8,9-, 11,12-, and 14,15- olefins. These results indicate that as a member of the renal arachidonic acid metabolic cascade, the cytochrome P-450 2C23 epoxygenase plays a central role in the biosynthesis of the biologically active EETs and, consequently, may have important role(s) in renal function.

REFERENCES

Molecular Cloning of a Renal Arachidonic Acid Epoxygenase