Formation of 20-Hydroxyeicosatetraenoic Acid, a Vasoactive and Natriuretic Eicosanoid, in Human Kidney

ROLE OF CYP4F2 AND CYP4A11*

(Received for publication, September 2, 1999, and in revised form, October 16, 1999)

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20-Hydroxyeicosatetraenoic acid (20-HETE), an ω-hydroxylated arachidonic acid (AA) metabolite, elicits specific effects on kidney vascular and tubular function that, in turn, influence blood pressure control. The human kidney’s capacity to convert AA to 20-HETE is unclear, however, as is the underlying P450 catalyst. Microsomes from human kidney cortex were found to convert AA to a single major product, namely 20-HETE, but failed to catalyze AA epoxygenation and midchain hydroxylation. Despite the monophasic nature of renal AA ω-hydroxylation kinetics, immunological studies revealed participation of two P450s, CYP4F2 and CYP4A11, since antibodies to these enzymes inhibited 20-HETE formation by 65.9 ± 17 and 32.5 ± 14%, respectively. Western blotting confirmed abundant expression of these CYP4 proteins in human kidney and revealed that other AA-oxidizing P450s, including CYP2C8, CYP2C9, and CYP2E1, were not expressed. Immunocytochemistry showed CYP4F2 and CYP4A11 expression in only the S2 and S3 segments of proximal tubules in cortex and outer medulla. Our results demonstrate that CYP4F2 and CYP4A11 underline conversion of AA to 20-HETE, a natriuretic and vasoactive eicosanoid, in human kidney. Considering their proximal tubular localization, these P450 enzymes may partake in pivotal renal functions, including the regulation of salt and water balance, and arterial blood pressure itself.

The kidney plays a central role in the regulation of salt and water balance and in the control of blood pressure. Fluid and electrolyte homeostasis by this organ involves diverse mechanisms, including the localized formation of substances that affect renal tubular function and/or blood flow (1). One of these substances may be 20-HETE, an ω-hydroxylated derivative of arachidonic acid (AA) that is a potent inhibitor of renal tubular Na+/K+-ATPase activity as well as a powerful constrictor of kidney microvessels (2–4). Previous studies have localized 20-HETE formation in rat and/or rabbit kidney to proximal tubules (5), TALH (6), and renal microvessels (7) in the cortex and outer medulla. Inhibition of 20-HETE formation in rat kidney in vivo has been reported to block autoregulation of renal blood flow and tubuloglomerular feedback (8–10), perturb chloride (Cl–) transport within TALH (11), and interfere with the long term control of arterial blood pressure (12, 13). As a physiological correlate, there is considerable evidence suggesting that 20-HETE plays a role in the pathogenesis of hypertension in the spontaneously hypertensive rat (5, 12, 14–16).

The formation of 20-HETE requires hydroxylation of AA at the primary carbon-hydrogen bond, and is catalyzed by P450 enzymes belonging to the CYP42 gene family (17). While the liver contains the largest amounts of CYP4A enzymes, these P450s are also expressed at significant levels in the kidney cortex, where they have been localized to the proximal tubules, TALH, and microvessels (7, 18–20). Renal cortical microsomes from rats and rabbits convert AA mainly to 20-HETE, although other oxidative metabolites are also formed, including 19-HETE, EETs, and di-HETEs (21–23). In fact, due to their vasodilatory nature and inhibitory effects on ion transport, a role for EETs (e.g. 5,6-EET and 11,12-EET) in the regulation of renal function has been proposed (3, 24, 25). With regard to 20-HETE, three different CYP4A P450s capable of AA hydroxylation, namely CYP4A1, CYP4A2, and CYP4A3, are expressed in rat kidney (19, 26–28), although more recent studies have attributed the bulk of renal 20-HETE formation in this species to CYP4A2 (18, 19, 29). The predominant catalyst of AA ω-hydroxylation in rabbit kidney has been identified as CYP4A6 and/or CYP4A7 (30–32). In contrast, there have been few studies characterizing P450-mediated AA oxygenation in the human kidney (33, 34), and the renal P450 enzymes underlying formation of 20-HETE or any other AA metabolite have yet to be identified in humans. It is known, however, that the CYP4A11 cDNA, which was cloned from a human kidney cDNA library, catalyzes AA 20-hydroxylation upon its heterologous expression in Escherichia coli (35).

We recently found that AA ω-hydroxylation to 20-HETE in human liver was mediated not by CYP4A11 but rather by CYP4F2, another member of the CYP4 gene family (36). While...
both of these enzymes exhibited extensive AA ω-hydroxylase activity in reconstituted systems, only antibodies to CYP4F2 proved capable of inhibiting 20-HETE formation by intact human liver microsomes. Moreover, it was revealed that CYP4F2 and CYP4A11 were expressed in human renal microsomes at levels nearly equivalent to those found in hepatic microsomes (36). In this investigation, which extends our prior findings, we show that microsomes derived from the human kidney cortex convert AA to a single major metabolite, namely 20-HETE. Other than CYP4F2 and CYP4A11, no other P450 enzyme, including those capable of either AA epoxidation (e.g. CYP2C8, CYP2C9, and CYP2C19) or ω-1 hydroxylation (CYP2E1), was expressed in human kidney microsomes at appreciable levels. Kinetic analyses and immunoinhibition studies demonstrated that renal 20-HETE formation was mediated by both CYP4F2 and CYP4A11. Finally, immunohistochemical techniques revealed that these two CYP4 family members were localized chiefly in the S2 and S3 segments of the proximal tubule, a region in the kidney nephron where most salt and water reabsorption occurs.

**EXPERIMENTAL PROCEDURES**

**Human Kidney and Liver Microsomes**—Microsomes derived from samples of human renal cortex were obtained from the Human Cell Culture Center (Laurel, MD), from the International Institute for the Advancement of Medicine (Scranton, PA), and from Dr. Barbara Haehner-Daniels (Indiana University, Indianapolis, IN). The donor organs had been removed within 30 min of death, frozen in liquid nitrogen, and stored at −80 °C until microsomes were prepared from the samples. The source and properties of the human liver microsomes employed herein have been described elsewhere (36–38). Microsomal protein concentration was determined using the biocinchonic acid procedure (39).

**Microsomal Enzyme Purification**—CYP4F2, CYP4A11, b5, and P450 reductase were purified to electrophoretic homogeneity from human liver microsomes as reported elsewhere (38, 40, 41). The specific contents of the hemoproteins were 7.2 (CYP4F2), 12.6 (CYP4A11), and 29.6 (b5) nmol/mg of protein, whereas the specific activity of P450 reductase was 25,800 units/mg; 1 unit of P450 reductase activity was defined as that amount catalyzing reduction of 1 nmol of ferricytochrome c/min at 22 °C in 300 mM KPO4 buffer (pH 7.4).

**AA Hydroxylation Assay**—The conversion of AA to oxygenated metabolites was assessed according to Powell et al. (36) in incubation mixtures (0.25 ml) containing 100 mM KPO4 buffer (pH 7.4), 100 μM AA, 1 mM NADPH, and one of the following enzyme sources: human kidney microsomes (0.5 mg of protein), human liver microsomes (0.25–0.4 mg of protein), or reconstituted P450 enzymes. Reconstituted systems consisted of 25 pmol of purified P450, 250 units of P450 reductase, 100 pmol of b5, and 7.5 μg of synthetic diaroylphosphatidylcholine. All reactions were initiated with NADPH and were terminated after 10 min at 37 °C with 10 μl of 2.0 N HCl and vigorous mixing. In antibody inhibition studies, renal microsomes were first incubated with either anti-human CYP4A11, anti-human CYP4F2, or preimmune IgG (described below) for 3 min at 37 °C and then for 10 min at room temperature, followed by the addition of the remaining reaction components. In chemical inhibition studies, microsomes or reconstituted P450 enzymes were preincubated with or without 17-ODYA in the presence of NADPH for 15 min at 37 °C. After cooling on ice, 100 μM AA and additional NADPH were added. AA and its metabolites were isolated from incubation mixtures by extraction with 4 volumes of ethyl acetate, then separated, evaporated to dryness with nitrogen gas at room temperature, resolubilized in 15 μl of 100% acetone containing 0.1% acetic acid, and subjected to HPLC analysis (36). Rates of 20-HETE formation were determined from standard curves prepared by adding varying amounts of authentic standard to incubations conducted without AA, whereas 19-HETE production rates were estimated by applying the same standard curve as that used for 20-HETE. Enzyme kinetic results were analyzed by nonlinear regression using weighted (1/ν) transformed data (Grafit; Erithacus Software Ltd., Cambridge, UK); Michaelis-Menten parameters were determined using either a one- or two-enzyme model.

**Immunohistochemical Methods**—Polyclonal antibodies to human liver CYP4F2 and CYP4A11 were raised in male New Zealand White rabbits as described previously (36, 38). Preimmune (control) IgG was prepared from rabbit sera obtained prior to immunization. Anti-CYP4F2 and anti-CYP4A11 were essentially monospecific as isolated but required back-adsorption against human epidermal keratin covalently linked to Sepharose 4B to remove the keratin cross-reactivity that interfered with immunoassay performance. The characteristics of anti-CYP2E1, anti-CYP3A4, anti-CYP1A2, anti-CYP2A6, and anti-CYP2C9 have been reported elsewhere (37, 42–46). Protein blotting of microsomal proteins and purified P450 enzymes to nitrocellulose and subsequent immunostaining with anti-CYP4A11 IgG or anti-CYP4F2 were performed as described previously (40, 43).

20-HETE and CYP4A11 enzyme levels were first quantitated in our reference human kidney sample, HK-31, by applying various amounts of purified CYP4F2 (0.25–0.65 pmol), purified CYP4A11 (0.13–0.5 pmol), and HK-31 kidney microsomes (10–20 μg) to the same polycrylamide gel, followed by staining of the ensuing Western blots with either anti-CYP4F2 or anti-CYP4A11 IgG. The blots were scanned with an Agfa Arcus II flat bed scanner interfaced to a computer, and immunoreactive areas on the image were measured using ImageQuant software (Molecular Dynamics, Sunnyvale, CA). CYP4F2 and CYP4A11 enzyme content were then assessed in the other kidney specimens (applied at 10 and/or 15 μg/gel lane) by comparing immunostaining intensities to that of the HK-31 reference sample. All immunostaining was performed under conditions where the peroxidase reaction density was directly proportional to the amount of protein applied to the original polycrylamide gels. The contents of CYP4F2 and CYP4A11 in human liver microsomes were determined as described elsewhere (36, 38).

For immunohistochemistry, normal human kidneys suitable for transplantation were perfused with ice-cold basic salts solution, dissected into small pieces, and fixed in 4% paraformaldehyde solution in phosphate-buffered saline for 4–12 h at 4 °C. After extensive washing with phosphate-buffered saline, the kidney samples were embedded in low temperature paraffin wax, and 4-μm thick sections were then prepared. The sections were incubated with polyclonal rabbit anti-human CYP4F2 or rabbit anti-human CYP4A11 IgG (5–20 μg of IgG/ml of blocking reagent) as the primary antibody, followed by biotinylated goat anti-rabbit IgG and avidin-biotinylated peroxidase (Vectastain; Vector Laboratories, Burlingame, CA) or directly applied to the original polycrylamide gels. The blots of CYP4F2 and CYP4A11 in human liver microsomes were determined as described elsewhere (36, 38).

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**RESULTS**

**Expression of Human Renal P450 Enzymes**—Our initial studies involved the assessment by Western blotting of the various P450 proteins expressed in human kidney. As shown in Fig. 1, renal cortical microsomes from subjects HK860828 and HK861212 contained anti-CYP4F2 and anti-CYP4A11 immunoreactive proteins with the same molecular weights as human liver CYP4F2 and CYP4A11, respectively. Extensive CYP4F2 and CYP4A11 expression was likewise noted in the nine other kidney samples examined (see Table I and Fig. 8). These kidney specimens also expressed CYP3A5 (but not CYP3A4), as described previously (47), albeit at substantially lower levels than the CYP4 proteins. In contrast, expression of CYP2A6, CYP2E1, and the CYP2C450s (CYP2C8, CYP2C9, and/or CYP2C19) was observed in neither the depicted kidney samples nor in the nine other renal specimens tested. Furthermore, the low level of renal CYP1A2 expression observed in subject HK860828 was not replicated among any of the other subjects. Fig. 1 also reveals that each of the antibodies used for these experiments recognized their corresponding immunogen in human liver microsomes (see lane 1 in each of the panels). In the case of anti-CYP2C9, this antibody also weakly cross-reacted with the structurally related P450s CYP2C8 and CYP2C19 (46), whereas anti-CYP3A4 exhibited strong cross-reactivity with...
Measurement of aggregate P450 content in human renal microsomes was precluded here due to sample size constraints.

We next examined the capacity of microsomes derived from human kidney cortex to catalyze AA metabolism, particularly the ω-hydroxylation of this essential long chain fatty acid to 20-HETE. In the presence of NADPH, renal cortical microsomes converted AA to 20-HETE in a time- and protein-dependent manner (linear up to 10 min of reaction time at 37 °C with up to 1.0 mg of microsomal protein) (Fig. 2A). Rates of renal 20-HETE formation among nine different subjects were 0.42 ± 0.2 nmol of product formed/min/mg of protein (range of 0.18–0.74 nmol/min/mg), or 4.5-fold less than rates observed with human liver microsomes (Table I) (36). As shown in Fig. 2A, renal microsomes also formed 19-HETE (0.09 ± 0.08 nmol of product formed/min/mg of protein), yet three of the nine subjects failed to generate measurable amounts of this AA ω-1 hydroxylation product (data not shown). 20-HETE and 19-HETE were the only UV-detectable metabolites of AA formed by the human kidney samples, although the HPLC assay employed herein allows for adequate resolution and detection of other P450-mediated AA oxygenation products, including di-HETEs, mid-chain HETEs, and EETs (36). Had renal microsomes, like hepatic microsomes, converted AA to 14,15-EET, for example, at least the corresponding hydration product 14,15-di-HETE would have been observed upon HPLC analysis (Fig. 2, compare A with B).

Kidney microsomes from subject HK013197 were used to examine the kinetics properties of renal 20-HETE formation. Over the range of substrate concentrations utilized (5–240 μM), AA metabolism to 20-HETE exhibited simple Michaelis-Menten kinetics (Fig. 3A), which were consistent with reaction

![Fig. 1. Comparison of P450 enzyme expression in human kidney versus liver.](image)

**TABLE I**

| AA ω-hydroxylation | CYP4A11 content | CYP4F2 content |
|--------------------|-----------------|----------------|
| Human kidney Mx (n = 9) | 0.42 ± 0.2 | 32.4 ± 18.4 | 82.0 ± 15.4 |
| Human liver Mx (n = 9) | 1.81 ± 1.0 | 47.6 ± 27.6 | 154.6 ± 47.7 |

a nmol of 20-HETE formed/min/mg of microsomal protein.

b pmol of enzyme/mg of microsomal protein.

CYP3A5 (45). Measurement of aggregate P450 content in human renal microsomes was precluded here due to sample size constraints.

**Fig. 2. HPLC analysis of AA oxidation by human kidney and liver microsomes.** A, representative chromatogram of the metabolites produced upon incubation of human kidney cortical microsomes (0.5 mg of protein) from subject 010597 with 100 μM AA. B, representative chromatogram of the metabolites produced upon incubation of human liver microsomes (0.4 mg of protein) from subject UC9408 with 100 μM AA. Further details of the reactions are given under “Experimental Procedures.”

AA Metabolism by Human Kidney Microsomes—We next examined the capacity of microsomes derived from human kidney cortex to catalyze AA metabolism, particularly the ω-hydroxylation of this essential long chain fatty acid to 20-HETE. In the presence of NADPH, renal cortical microsomes converted AA to 20-HETE in a time- and protein-dependent manner (linear up to 10 min of reaction time at 37 °C with up to 1.0 mg of microsomal protein) (Fig. 2A). Rates of renal 20-HETE formation among nine different subjects were 0.42 ± 0.2 nmol of product formed/min/mg of protein (range of 0.18–0.74 nmol/min/mg), or 4.5-fold less than rates observed with human liver microsomes (Table I) (36). As shown in Fig. 2A, renal microsomes also formed 19-HETE (0.09 ± 0.08 nmol of product formed/min/mg of protein), yet three of the nine subjects failed to generate measurable amounts of this AA ω-1 hydroxylation product (data not shown). 20-HETE and 19-HETE were the only UV-detectable metabolites of AA formed by the human kidney samples, although the HPLC assay employed herein allows for adequate resolution and detection of other P450-mediated AA oxygenation products, including di-HETEs, mid-chain HETEs, and EETs (36). Had renal microsomes, like hepatic microsomes, converted AA to 14,15-EET, for example, at least the corresponding hydration product 14,15-di-HETE would have been observed upon HPLC analysis (Fig. 2, compare A with B).

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catalysis by a single enzyme (or by two or more enzymes with similar kinetic properties). Nonlinear regression analysis was used to derive an apparent $K_m$ of 43.0 ± 2.8 μM and a $V_{max}$ of 0.84 ± 0.02 nmol 20-HETE formed/min/mg of protein for this particular kidney sample (Fig. 3B). In fact, identical $K_m$ values were derived when the data was subjected to a two-component Michaelis-Menten equation. Interestingly, such monophasic-type kinetics of 20-HETE formation by renal microsomes from subject HK013197 more closely resembled that observed with liver samples (12.9 ± 9%) (Fig. 5B). In contrast to these results, anti-CYP4F2 was without effect on renal 19-HETE formation, while anti-CYP4A11 completely inhibited generation of this $\omega$-1 hydroxylated AA metabolite (data not shown).

Acetylenic fatty acid analogs such as 11-dodecynoic acid and 17-octadecynoic acid (17-ODYA) have proven rather potent and specific inhibitors of laurate, prostaglandin E1, and AA $\omega$-hydroxylation catalyzed by rat and rabbit CYP4A enzymes (8–10, 48, 49). Moreover, in vivo experimental studies with 17-ODYA have provided perhaps the strongest evidence to date that 20-HETE plays an essential role in tubuloglomerular feedback and in the regulation of renal vascular tone (8–10). As shown in Fig. 6A, the catalytic activity of human CYP4 enzymes was also potently inhibited by 17-ODYA, since CYP4F2-mediated and CYP4A11-mediated AA $\omega$-hydroxylation were decreased 71 and 27%, respectively, at a 17-ODYA concentration of only 5 μM. The enhanced susceptibility of CYP4F2 activity to 17-ODYA inhibition compared with CYP4A11 was more obvious at low 17-ODYA concentrations, whereas at higher concentrations (25 μM), both enzymes were equally affected by this mechanism-based P450 inhibitor (Fig. 6A). Prominent inhibition of AA $\omega$-hydroxylase activity (55–88% inhibition) was also observed upon preincubation of 25 μM 17-ODYA with native renal microsomes from subjects 063097 and 010597, although 20-HETE formation was decreased to a greater extent in the former sample (Fig. 6B). Indeed, the capacity of 17-ODYA to inhibit renal 20-HETE formation to a greater extent in subject 063097 versus subject 010597 may have stemmed from the 8-fold higher ratio of CYP4F2 to CYP4A11 content in the former sample (see below). Of obvious interest is the fact that 17-ODYA-mediated inhibition of 20-HETE formation by renal microsomes from subject 063097 paralleled that observed with liver microsomes from subject UC9410 (Fig. 6B).

**CYP4F2 and CYP4A11 Expression in Human Kidney Microsomes**—Polyclonal CYP4F2 and CYP4A11 antibodies were also utilized to quantitate levels of their corresponding antigens in human renal microsomes. Enzyme immunoquantitation was performed on Western blots similar to those shown in Fig. 1.
that were subjected to scanning densitometry. Among the nine subjects studied, renal CYP4F2 content varied only 1.7-fold (71.5–121.6 pmol/mg), with a mean enzyme content of 82.0 ± 15.4 pmol/mg (Table I). In contrast, kidney CYP4A11 content varied 8-fold (7.5–60 pmol/mg) among these same individuals, with a mean enzyme content of 32.4 ± 18.4 pmol/mg.

These renal CYP4F2 and CYP4A11 protein levels were 53 and 68%, respectively, of those found in human liver microsomes (Table I), despite the fact that aggregate renal P450 content is nearly 20-fold lower than hepatic P450 content (34, 50).

Associations between P450 enzyme content and rates of substrate metabolism in microsomes are often used to establish involvement of a particular P450 enzyme in that substrate’s metabolism. In fact, we utilized this type of approach to demonstrate that the capacity of human liver microsomes to convert AA to 20-HETE was strongly correlated (r = 0.78; p < 0.02; n = 9) with their content of CYP4F2 (36). In the present study, however, a much weaker correlation (r = 0.21; p > 0.6; n = 8) was obtained between rates of renal microsomal 20-HETE formation and CYP4F2 content. The relationship between AA ω-hydroxylase activity and CYP4A11 expression in

**Fig. 4.** Inhibition of AA ω-hydroxylation in human kidney microsomes by CYP4A11 and CYP4F2 antibodies. A, AA ω-hydroxylation was assessed in incubation mixtures containing renal microsomes from subject 010597 (0.5 mg of protein), 100 μm AA, 0.5 mm NADPH, 100 mm KPO₄ buffer (pH 7.4), and anti-CYP4A11, anti-CYP4F2 and/or rabbit preimmune (control) IgG. Immune-specific and/or rabbit preimmune (control) IgG were added in various ratios so that the total IgG concentration remained constant (0.4 mg of IgG/mg of microsomal protein). Reactions were performed as described under “Experimental Procedures” except that microsomes were preincubated with antibodies for 3 min at 37 °C, followed by 10 min at ambient temperature before initiating the reactions. 100% of control activity was 0.58 nmol of 20-HETE formed/min/mg of protein. B, conditions identical to those described for A except that renal microsomes from subject 063097 were used. 100% of control activity was 0.33 nmol of 20-HETE formed/min/mg of protein. Values denote the average of triplicate determinations.

**Fig. 5.** Inhibition of AA ω-hydroxylation in human kidney and liver microsomes by CYP4A11 and CYP4F2 antibodies. A, conditions identical to those described in Fig. 4 except that the amount of anti-CYP4A11, anti-CYP4F2, and preimmune IgG added to the incubation mixtures was 0.1 mg. The human kidney samples examined were as follows. A, subject HK010597; B, subject HK063097; C, subject HK013197; D, subject HK022395; E, subject HK20; F, subject HK30. Values denote the average of triplicate determinations. B, inhibition (mean ± S.D.) of AA ω-hydroxylation by anti-CYP4A11 and anti-CYP4F2 IgG in the six human kidney samples shown in A compared with the inhibition of AA ω-hydroxylation by the same P450 antibodies in five different human liver samples (36).
these kidney samples was also rather poor ($r = 0.14; p > 0.7; n = 8$) (data not shown). Such weak correlations may have resulted from the unknown quality of the commercially obtained kidney microsomes employed here, since extensive denaturation of CYP4F2 and/or CYP4A11 to the P420 state during microsomal preparation would markedly decrease their AA ω-hydroxylase activity but would not influence their immunochmical detection. Unfortunately, the limited availability of these kidney samples obviated more detailed characterization, particularly the spectroscopic measurement of P450 (and P420) concentrations.

Localization of CYP4F2 and CYP4A11 in Human Kidney Tissue—Immunohistochemical studies were performed to determine the region(s) of the human kidney where CYP4F2 and CYP4A11 expression occurred (Fig. 7). For these studies, the CYP4F2 antibody utilized had been back-adsorbed against a partially purified P450 fraction enriched in the cross-reacting 70-kDa non-P450 protein (but not in CYP4F2). In all six subjects examined (age range of 15–48 years), intense CYP4F2 immunostaining was observed specifically in the S2 and S3 segments of proximal tubule epithelia in the cortex and outer medulla (Fig. 7, A and B). Expression of CYP4F2 was not noted in glomeruli, loops of Henle, or collecting tubules. CYP4A11 exhibited a similar pattern of cortical and outer medullary localization...
expression, i.e. abundant immunostaining in proximal tubule pars convoluta and pars recta portions (Fig. 7, C and D). Like CYP4F2, CYP4A11 was not found in glomeruli, loops of Henle, or collecting tubules. Examination of the immunostained sections at higher magnification revealed that the cellular localization of both CYP4F2 and CYP4A11 was predominantly cytoplasmic in nature, although some dilated proximal tubules showed enzyme staining associated with the apical plasma membrane (Fig. 7, B and D). In control experiments where the primary antibody was omitted, specific CYP4F2 and/or CYP4A11 immunostaining was clearly not evident (Fig. 7, E and F).

**DISCUSSION**

We have demonstrated herein that microsomes from human kidney cortex metabolize AA to a single major product, namely 20-HETE, an eicosanoid with potent effects on renal ion transport, vascular tone, and cellular proliferation. This capacity of the human kidney to convert AA to 20-HETE was found to stem from renal expression of the CYP4 gene family members CYP4F2 and CYP4A11. Both of these P450 enzymes are potent AA \( \omega \)-hydroxylases, although CYP4F2 is the more effective catalyst due to an apparent \( K_m \) (24 \( \mu \)M) for AA nearly 10-fold lower than that of CYP4A11 (228 \( \mu \)M) (36). While kinetic studies with human kidney microsomes suggested the involvement of only a single P450 enzyme in AA \( \omega \)-hydroxylation (Fig. 3), subsequent experiments with inhibitors revealed participation of CYP4F2 as well as CYP4A11 in renal 20-HETE formation. Indeed, polyclonal antibodies to CYP4F2 were found to inhibit renal 20-HETE formation by 65.9 ± 17.6%, while antibodies to CYP4A11 inhibited this reaction by 32.5 ± 14.4% (n = 6). Similarly, 17-ODYA, an acetylenic fatty acid analog that is a powerful mechanism-based inhibitor of CYP4A-catalyzed reactions (3, 8, 48), gave greater inhibition of CYP4F2-mediated 20-HETE formation than of the CYP4A11-mediated reaction, substantiating the former P450’s role as the prevailing AA \( \omega \)-hydroxylase in renal cortical microsomes. Finally, we utilized immunocytochemistry to establish that in human kidney, CYP4F2 and CYP4A11 proteins are localized exclusively in the S2 and S3 (pars recta) segments of proximal tubule epithelia in cortex and outer medulla. As such, 20-HETE formation would occur mainly in these regions of the nephron, which are the same regions where extensive electrolyte transport and water reabsorption occurs.

Western blot analysis of microsomes derived from the human kidney cortex revealed expression of three members of the CYP1–CYP4 gene families (Fig. 1). Two of these P450s, CYP4F2 and CYP4A11, were found at substantial, albeit variable, levels in each of the nine renal specimens analyzed (Fig. 7 and Table I). The other P450 enzyme detected in human renal microsomes to predominantly 20-HETE formation. Indeed, polyclonal antibodies to CYP4F2 were found to inhibit renal 20-HETE formation by 65.9 ± 17.6%, while antibodies to CYP4A11 inhibited this reaction by 32.5 ± 14.4% (n = 6). Similarly, 17-ODYA, an acetylenic fatty acid analog that is a powerful mechanism-based inhibitor of CYP4A-catalyzed reactions (3, 8, 48), gave greater inhibition of CYP4F2-mediated 20-HETE formation than of the CYP4A11-mediated reaction, substantiating the former P450’s role as the prevailing AA \( \omega \)-hydroxylase in renal cortical microsomes. Finally, we utilized immunocytochemistry to establish that in human kidney, CYP4F2 and CYP4A11 proteins are localized exclusively in the S2 and S3 (pars recta) segments of proximal tubule epithelia in cortex and outer medulla. As such, 20-HETE formation would occur mainly in these regions of the nephron, which are the same regions where extensive electrolyte transport and water reabsorption occurs.

The pattern of AA metabolism noted with renal microsomes, which differs markedly from that observed with hepatic microsomes (36, 58–60), exemplifies the complement of P450 enzymes found in the human kidney. That 20-HETE was the major AA metabolite formed by kidney cortical microsomes indeed reflected the extensive expression of renal CYP4F2 and CYP4A11 in this tissue (Fig. 1 and Table I). In fact, although the aggregate P450 content of human kidney microsomes is only 12% of that of liver microsomes (40 versus 340 pmol of P450/mg) (34, 50, 61), CYP4A11 and CYP4F2 levels in these two tissues were rather similar (Table I). Conversion of AA by human renal microsomes to predominantly 20-HETE has been described previously (33, 34) and, as reported by Amet et al. (34), at rates (0.39 ± 0.13 nmol of product/min/mg of protein; n = 16) nearly identical to those given here. While six of the nine subjects examined here also converted AA to 19-HETE, albeit at low rates, renal \( \omega \)-1 hydroxylation activity was catalyzed not by CYP2E1 but rather by CYP4A11, which metabolizes this long chain unsaturated fatty acid to both 20-HETE and 19-HETE at a ratio approaching 5:1 (36). The capacity of anti-CYP4A11 but not of anti-CYP4F2 to completely inhibit formation by human kidney microsomes of 19-HETE, an eicosanoid that stimulates Na\(^+\)K\(^{-}\)-ATPase activity (6), further supports this conclusion (see “Results”). CYP2E1 plays a much greater role in 19-HETE generation in human liver, since rates of this eicosanoid’s formation are inhibited only 18 ± 12% (n = 5) by optimal amounts of anti-CYP4A11.

Like the CYP4 proteins, the renal P450 epoxygenases (e.g. rat CYP2C23, rabbit CYP2C2 and P4502C2A, and mouse CYP2C29 and CYP2C38) have been purported to play a pivotal role in controlling blood pressure and body fluid volume composition and in regulating the adaptive response of the kidney to excess dietary salt intake (2, 4, 62). However, the capacity of the human kidney to oxidize AA primarily to 20-HETE, and not to EETs, suggests that only the CYP4 proteins are involved in regulating these renal functions and, perhaps, in the pathogenesis of hypertension itself. The inability of renal microsomes to convert AA to EETs (Fig. 2; see “Results”) most likely stems from their lack of CYP2C enzyme expression. Indeed, CYP2C8, CYP2C9, and CYP2C19 catalyze the epoxygenation of AA to any of three EETs, including 8,9-EET, 11,12-EET, and 14,15-EET (58–60, 63), and underlie most EET formation occurring in human liver (59). Although Zeldin and co-workers (64–66) have reported that CYP2J2, an AA epoxygenase, is present in human kidney as well as heart, liver, jejunum, and lung, comparative Western and Northern blotting (64) shows equivocal expression of this P450 protein and its corresponding mRNA in the former tissue. While inaccessibility to the appropriate antibody obviated immunochemical screening for CYP2J2 in our kidney samples, we can still rule out extensive renal expression of this P450 epoxygenase due to the lack of significant EET and/or di-HETE formation by these specimens. EET detection in human kidney tissues by gas chromatography/mass spectrometry has been presented as evidence for in

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4  P. K. Powell and J. M. Lasker, unpublished observations.
vivo AA metabolism by a renal P450 epoxygenase(s) (67, 68). While the presence of EETs, and especially their stereochemical isomers, as endogenous constituents of a given organ or tissue do indeed indicate that AA has undergone epoxygenation in vivo, it remains unclear whether the EETs actually originated in that tissue or were formed in a different one (e.g., liver), followed by transport via the circulation to the organ in question. Indeed, there appears to be little relationship between EET levels and CYP2J2 content in those tissues where this P450 functions as the predominant epoxygenase (64–66).

In a previous study (36), we found that the kinetics of 20-HETE formation by human liver microsomes were biphasic in nature, which led us to hypothesize that at least two enzymes were involved in hepatic AA ω-hydroxylation. That premise led to the identification of a hepatic AA hydroxylase other than CYP4A11, namely CYP4F2, which ultimately proved to be the principal AA ω-hydroxylase in human liver. It was thus surprising to find that 20-HETE formation by human kidney microsomes exhibited monophasic Michaelis-Menten kinetics (Fig. 3), considering the extensive expression of CYP4F2 and CYP4A11 in this tissue. In fact, the apparent $K_m$ and $V_{max}$ (43.0 μM and 0.84 nmol of 20-HETE formed/min/mg of protein) for AA ω-hydroxylation by renal microsomes from subject HK013197 (Fig. 3) were quite similar to the kinetic parameters derived for the high affinity component of the liver microsomal reaction ($K_m$ = 23.3 μM, and $V_{max}$ = 0.71 nmol of 20-HETE formed/min/mg of protein) as well as to the Michaelis constant (24 μM) derived for AA with purified CYP4F2 (36). Such differences in AA ω-hydroxylation kinetics may indicate the presence in human kidney of a CYP4A11 enzyme with catalytic properties (e.g. decreased $K_m$ for AA) somewhat distinct from the corresponding liver enzyme. Alterations in CYP4A11 (or CYP4F2) AA-metabolizing properties would not necessarily require a change in enzyme primary structure but could arise from variations in the microsomal phospholipid environment or in the $b_5$ to CYP4A11 ratio.

Despite the kinetic results obtained, other evidence presented here indicated that CYP4F2 and, albeit to a lesser extent, CYP4A11 contributed to renal AA ω-hydroxylation. First, anti-CYP4F2 inhibited renal microsomal AA ω-hydroxylase activity by 66% among the six subjects we examined, while anti-CYP4A11 inhibited the reaction by 33% in the same subjects (Fig. 5). This extent of inhibition of 20-HETE formation by CYP4F2 antibodies was less than that observed with human liver microsomes, while the inhibition elicited by CYP4A11 antibodies was greater (Fig. 5) (36). The metabolic specificity of the anti-CYP4F2 and anti-CYP4A11 IgGs utilized for these studies was confirmed by their ability to inhibit AA ω-hydroxylation catalyzed by only the corresponding antigen and not by the heterologous antigen (36), while immunospecificity was demonstrated by recognition of only the analogous P450 on Western blots (Fig. 1) (37, 38). Second, we found that CYP4F2-mediated 20-HETE formation was more sensitive to inhibition by the fatty acid analog 17-ODYA than was the CYP4A11-catalyzed reaction (Fig. 6). The enhanced capacity of 17-ODYA to inhibit AA ω-hydroxylation by CYP4F2 was found to extend to intact renal (and hepatic) microsomes, where this powerful mechanism-based P450 inhibitor (3, 8, 48) decreased rates of 20-HETE formation in CYP4F2-enriched samples to a greater extent than in CYP4A11-enriched samples (Fig. 6; see “Results”). It should be noted that in vivo experimental studies with 17-ODYA have provided the best evidence to date that 20-HETE plays an essential role in tubuloglomerular feedback and in the regulation of renal vascular tone (8, 9, 13). While it has been assumed that 17-ODYA decreases renal 20-HETE formation via its potent inhibitory effects on the CYP4A proteins, the data presented here suggest that a role for the CYP4F proteins in formation of this bioactive eicosanoid must also be considered. Indeed, the P450s designated CYP4F4, CYP4F5, and CYP4F6 recently cloned from rat brain display extensive fatty acid ω-hydroxylation activity upon their heterologous expression in E. coli and are expressed at much higher levels in liver and kidney than in brain (69).

Among the most salient findings made in this study was the localization of both CYP4F2 and CYP4A11 to the S2 and S3 segments of proximal tubular epithelia in cortex and outer medulla (Fig. 7). Interestingly, these two human P450s exhibited the same highly specific pattern of distribution within the nephron and were not expressed in cells comprising the glomeruli, loops of Henle, or collecting tubules. Thus, the proximal tubular pars convoluta and pars recta segments represents the principal site for 20-HETE formation in the human kidney, an observation similar to that made in experimental animals (18, 19, 26, 29). This pattern of CYP4F2 and CYP4A11 distribution in human kidney has, in all likelihood, important implications with regard to effects of AA-derived eicosanoids on integrated renal function. As already mentioned, 20-HETE is a potent constrictor of renal and extrarenal vessels (7, 70, 71), a property that has been attributed to the ability of this eicosanoid to inhibit opening of the large conductance Ca$^{2+}$-activated K$^+$ channel in vascular smooth muscle cells (72, 73). Thus, it is relevant that most CYP4 protein expression and, hence, 20-HETE production, would occur in portions of the nephron (i.e. proximal tubular S2 and S3 segments), where, due to uncomplicated access to the systemic circulation, this eicosanoid could contribute to renovascular tone regulation and, ultimately, arterial blood pressure control. Furthermore, since the proximal tubule is where most electrolyte and water reabsorption occurs (1), the capacity of 20-HETE to potentely inhibit Na$^+$/$K^+$-ATPase activity in this region of the nephron probably contributes to its known natriuretic and diuretic effects (4, 62, 74). In rats, CYP4A expression and 20-HETE formation have also been noted in renal microvessels (19, 29, 75), another proposed site where this eicosanoid influences vascular tone, autoregulation of renal blood flow, and/or tubuloglomerular feedback (9, 10). However, the absence of CYP4 enzymes from the human renal vasculature suggests that synthesis of 20-HETE exclusively within the proximal tubules is sufficient for this compound to elicit its potent effects on kidney function. Finally, the hepatic and renal expression of several CYP4A P450 enzymes has proven inducible in animals by peroxisomal proliferator-type agents (30, 76), chronic alcohol consumption (77, 78), diabetes (79, 80), hypertension (81), and pregnancy (82). Whether the CYP4 enzymes in human kidney are also inducible by these same agents or treatments is not yet known, but, in the affirmative, the attendant increase in 20-HETE formation could have important consequences on integrated renal function.

Acknowledgments—We thank Dr. Barbara Haehner-Daniels (Indiana University School of Medicine) for the generous gift of human kidney cortical microsomes.

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