Biochemical Pharmacology, Vol. 53, 765–771, 1997.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(96)00821-0

Cytochrome P450 4A and 2E1 Expression in Human Kidney Microsomes

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ABSTRACT. Laurate and arachidonate ω and (ω-1)-hydroxylase activities, cytochrome P450 2E1 (CYP2E1), and CYP4A content were measured in 18 human kidney microsomal samples. The rates of laurate and arachidonate were found to be very different from those measured in human liver samples, with a laurate ω/ω-1 ratio of approximately 22 in human kidney vs 0.75 in human liver. Immunoblot analysis of the 18 human kidney microsomal samples identified 1 CYP4A electrophoretic band, but CYP2E1 was not detectable in human kidney, contrary to liver. Laurate and arachidonate ω-hydroxylase activities were significantly correlated with CYP4A content (r = 0.86 and 0.75, respectively). Polyclonal antirat CYP2E1 antibody did not affect ω-hydroxylase activity, whereas the polyclonal antirat CYP4A1 antibody inhibited it by 60%. These results suggest that, in contrast to other species, human kidney microsomes do not contain significant amounts of CYP2E1, but possess CYP4A and fatty acid ω-hydroxylase activity. BIOCHEM PHARMACOL 53;6:765–771, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. P450; CYP4A; CYP2E1; lauric acid; arachidonic acid; human kidney

CYP¶ [1] and its associated monooxygenase activities are predominantly found in the liver as well as in many other tissues, although usually at lower levels [2]. The distribution of the various P450s differs according to age, gender, tissue, and species [3], and the equilibrium of metabolic activation and detoxification of drugs and other chemicals by individual forms of P450 in different tissues or species is an important factor in explaining organ- or species-specific toxicity. The majority of P450 studies have focused on hepatic forms in rats and humans [4–6]. Much less is known about renal P450s, especially in humans, and the characteristics of rat renal P450s are different from those of rat hepatic P450s [7, 8].

Mammalian renal P450s are involved in ω and (ω-1)-hydroxylations of fatty acids, including medium chain fatty acids, such as lauric acid [9, 10], and polyunsaturated fatty acids, such as arachidonic acid [11, 12], which are converted to ω and (ω-1)-hydroxyarachidonic acid and epoxyeicosatrienoic acid (EET) [12, 13]. The 20-hydroxyarachidonic acid (20-OH-AA or ω-OH-AA) has been suggested to be a potent vasoconstrictor [14, 15]. This finding led to speculation that the induction of renal P450 could modify the metabolism of arachidonic acid and, thus, that renal P450s may contribute to the regulation of renal function [16].

The characterization of P450 in human kidneys has been limited by the difficulty in obtaining surgical samples under good conditions. The specific content of P450s in extrahepatic organs is low [17, 18], and the purification of P450 is long and difficult. Recently, Imaoka *et al.* [7] partially purified a P450 that ω and (ω-1)-hydroxylates laurate from human kidney microsomes.

Human liver and kidney microsomes have been shown to catalyze hydroxylations of lauric acid [19–22]. P450-mediated hydroxylations of medium-chain fatty acids in human kidney microsomes were much less active than in kidneys of other species; furthermore, a stereospecificity for ω-hydroxylation was previously reported [19]. Thus, the nature of P450s involved in fatty acid ω-hydroxylations in a panel of 18 human kidney microsomes was reinvestigated, in view of the dramatic breakthrough in our knowledge of P450 isoforms.

MATERIALS AND METHODS

Chemicals

Lauric and arachidonic acids were obtained from Fluka (Buchs, Switzerland) and their ω and (ω-1)-hydroxylated

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‖In memoriam.

¶ *Abbreviations:* P450 or CYP, Cytochrome P450 (EC 1.14.14.1); 11-OH-LA, 11-hydroxylauric acid or (ω-1)-hydroxylauric acid; 12-OH-LA, 12-hydroxylauric acid or ω-hydroxylauric acid; LA, lauric acid; 19-OH-AA, 19-hydroxyarachidonic acid or (ω-1)-hydroxyarachidonic acid; 20-OH-AA, 20-hydroxyarachidonic acid or ω-hydroxyarachidonic acid; AA, arachidonic acid; EROD, ethoxy resorufin O-deethylase; MROD, methoxy resorufin O-demethylase; CHZ, chlorzoxazone.

metabolites were provided by Dr. J. P. Salaün (Strasbourg, France). NADPH and chlorzoxazone were supplied by Sigma (St Louis, MO). Ethoxyresorufin, methoxyresorufin, and resorufin were purchased from Boehringer (Mannheim, Germany). Electrophoresis reagents were from Biorad (Richmond, CA), nitrocellulose sheets from Amersham (Amersham, UK), and immunoblot antibodies were from Oxygene (Dallas, TX) or Amersham. All other chemicals and solvents were of analytical grade from Merck (Darmstad, Germany) or Sigma.

Human Kidney Samples and Microsomal Preparation

Eighteen patients (13 men and 5 women) were investigated as listed in Table 1. Kidney samples were removed in the University Hospital (Brest, France) for pathological purposes. Tissue containing nontransformed cells was used with the full approval of the local ethical committee for research on human subjects. Samples were immediately frozen at −80°C until analysis, allowing good preservation of P450.

Microsomal fractions were prepared as previously described [23], following the recommendations of Philpot [24] for preparation of microsomes from extrahepatic tissues. The term "microsomes" was applied to the 100,000 *g* particulate fraction derived from kidney homogenates by differential centrifugation.

Protein content of microsomal preparations was determined using the Bradford method, according to the procedure recommended by the supplier (Biorad, Munich, Germany). P450 content was determined by measuring the absorbance of the CO-ferrocytochrome P450 complex at 450 nm, using the second derivative spectrophotometry method [25]. NADPH-P450 reductase was determined using the reduction of exogenous cytochrome c ferric form into the ferrous state by P450 reductase with an absorption coefficient of 19.7 $mM^{-1} \cdot cm^{-1}$ for ferrous cytochrome c at 550 nm [26].

TABLE 1. Identification of the 18 human kidney samples

	Gender	Age	Cause of nephrectomy
RH1	F	49	Renal cell carcinoma
RH2	M	72	Renal cell carcinoma
RH3	M	74	Renal cell carcinoma
RH4	M	69	Renal cell carcinoma
RH5	M	66	Renal cell carcinoma
RH6	F	67	Angiomyolipoma
RH8	M	45	Renal cell carcinoma
RH9	M	66	Renal cell carcinoma
RH10	M	48	Renal cell carcinoma
RH11	F	82	Renal cell carcinoma
RH12	M	63	Renal cell carcinoma
RH13	M	72	Renal cell carcinoma
RH14	F	74	Renal cell carcinoma
RH15	M	37	Renal cell carcinoma
RH16	M	45	Renal cell carcinoma
RH17	M	55	Ureteral stricture
RH18	F	62	Renal cell carcinoma
RH19	M	40	Renal cell carcinoma

Determination of Monooxygenase Enzymatic Activities

EROD and MROD activities were determined spectrofluorimetrically according to the method of Prough *et al.* [27]. Briefly, substrates were added in 2 μL of DMSO solution to 2 mL of 0.1 M potassium phosphate buffer (pH 7.4) to obtain a final concentration of 5 μM. Protein concentration was 0.075 mg/mL. The reaction was initiated by addition of 0.5 mM NADPH. Fluorescence was measured directly in cuvettes under stirring for 2 min. Calibration was carried out by addition of 53 pmol of resorufin to the reaction medium.

CHZ hydroxylation was measured according to Peter *et al.* [28] with slight modifications, as previously described [29]. The detection limit was 0.5 pmol/min/mg.

The ω and (ω-1)-lauric acid hydroxylations were determined by HPLC as previously described in rat [30] and human [22] liver microsomes with a substrate concentration of 0.5 mM for 30 min at 37°C. The detection limit of this procedure was 1 pmol/min/mg of hydroxylaurate products [31]. Arachidonic acid metabolism was evaluated in kidney microsomes by incubating substrate (1 μCi; 0.5 mM) and microsomal protein (0.3 mg) for 30 min at 37°C. The ω and (ω-1)-hydroxyarachidonic acid metabolites were identified on the basis of their retention times and their radiometric and fluorimetric labelings.

To determine the kinetic parameters on 4 kidney microsomal samples, lauric or arachidonic acid was added to the reaction mixture in the range 5–500 μM. After extraction with diethylether, the metabolites were converted into fluorescent coumarinic derivatives, separated by HPLC, and detected by fluorimetry as previously described [31].

Immunoblot Analysis of P450 2E1 and 4A

Aliquots of microsomal samples (40 and 80 μg) were separated by electrophoresis on 9% SDS polyacrylamide gels according to the method of Laemmli [32] and then transferred electrophoretically to nitrocellulose sheets according to Towbin *et al.* [33]. Nitrocellulose sheets were blocked with PBS containing 3% (w/v) bovine serum albumin, 10% fetal calf serum, and 0.05% Tween 20 (v/v) for 30 min at room temperature, and incubated overnight at 4°C with either polyclonal antirat P450 2E1 antibody (Oxygene) or polyclonal antirat P450 4A antibody (Amersham, UK). After washing with PBS, the nitrocellulose sheets were treated with PBS containing either antirabbit IgG conjugated to peroxidase (Dako, Versailles, France) or antisheep IgG conjugated to peroxidase (Amersham) for P450 2E1 and 4A, respectively. Finally, the sheets were washed with PBS and the peroxidase activities detected with 4-chloronapthol and H_2O_2 or by ECL detection with luminol for P450 2E1 and

4A, respectively. The quantification of the blots was performed by means of image processing scan analysis (Bioprofil, Vilbert-Lourmat, France). Linearity of response was previously checked and the integrated peak area of the microsomal preparations was expressed as arbitrary units relative to the amount of protein.

Immuno-Inhibition of Lauric And Arachidonic ω-Hydroxylations

Human kidney (0.4 mg) microsomal sample RH8 in 0.12 M phosphate buffer pH 7.4 containing 5 mM $MgCl_2$ was incubated in the absence of NADPH and substrate (lauric or arachidonic acid) at room temperature for 30 min and in the presence of various concentrations of either polyclonal antirat CYP2E1 (from Dr. B. J. Song, Bethesda, MD), or CYP4A1 antibody (Gentest Co, Woburn, MA), or nonimmune IgG. The reaction was initiated by the addition of 0.1 mM lauric or arachidonic acid and 1 mM NADPH, and was carried out as described above.

Statistical Analysis

Results were expressed as mean ± SD. Correlation coefficients were calculated using an ANOVA table by the least-squares regression method. They were considered to be statistically significant when P was < 0.005.

RESULTS

Kinetic Parameters

Table 2 shows the kinetic parameters of ω and (ω-1)-hydroxylauric and arachidonic acid formation by microsomes from 4 human kidneys (RH5, RH8, RH12, and RH13). The mean K_m of ω and (ω-1)-hydroxylation of lauric acid were 46.7 and 82.1 μM in human kidney vs 22 and 130 μM [21] or 13 and 84 μM [22] in human liver microsomes. These differences were not statistically significant. Furthermore, the double reciprocal plot did not suggest the involvement of two distinct P450 isoforms in the lauric ω-hydroxylation, contrary to liver [21].

Determination of Monooxygenase Enzymatic Activities

Total kidney P450 content was found to be 42 ± 7.9 pmol/mg of microsomal protein (N = 5), and NADPH-P450 reductase, a marker of endoplasmic reticulum, was 122.6 ± 25.8 nmol/min/mg (N = 5).

Table 3 shows that kidney microsomes from different human subjects display significant activities toward the ω and (ω-1)-hydroxylation of lauric and arachidonic acid. Human renal microsomes metabolized lauric and arachidonic acid predominantly at the ω-position. The ω/ω-1 ratios ranged from 7.11 to 67.85 (mean ± SD = 22.85 ± 16.3) for lauric acid, and from 2.86 to 19.4 (mean ± SD = 7.17 ± 4.3) for arachidonic acid. Interindividual variability in the level of ω-lauric or arachidonic acid hydroxylations reached 11.3-fold (ranging from 510 to 5780 pmol/min/mg) for lauric acid and 7.2-fold (ranging from 85.6 to 615 pmol/min/mg) for arachidonic acid. Moreover, the ω and (ω-1) activities were not significantly different in male (N = 13) and female (N = 5) kidney samples (2381 ± 1843 vs 1406 ± 1130 pmol/min/mg for lauric acid ω-hydroxylation in men and women, respectively, and 407.3 ± 107.9 vs 332.1 ± 224 pmol/min/mg for arachidonic acid ω-hydroxylation in men and women, respectively).

The capacity of human kidney microsomes to hydroxylate lauric and arachidonic acid is very different from that determined in liver microsomes (Table 4). The specific activity of the human kidney to ω-hydroxylate lauric acid was 2 times greater than the values found in human liver, but the stereospecificity of laurate hydroxylations was shown to be very different in liver and kidney. The ω/ω-1 ratio was 0.75 ± 0.5 and 22.85 ± 16.3 in human liver and kidney microsomes, respectively.

None of these samples had significant 6-chlorzoxazone hydroxylase (values between 2 and 6.5 pmol/min/mg) or para-nitrophenol hydroxylase (data not shown) activities, both enzymatic activities known to be specific to P450 2E1.

EROD and MROD, activities known to be mediated by CYP1A1/1A2 in rat and human liver microsomes, were very weak, practically at the detection limit in the 8 tested kidney samples. The values were 4.19 ± 2.60 and 4.58 ±

TABLE 2. Kinetic parameters of (ω-1) and ω-hydroxylations of lauric and arachidonic acid in microsomes from 4 human kidney samples

	Lauric acid				Arachidonic acid			
	ω-1		ω		ω-1		ω	
	Km	Vm	Km	Vm	Km	Vm	Km	Vm
RH5	178	0.86	30.8	5.1	199	0.14	127	1.35
RH8	62.1	0.53	65.9	9.6	181	0.22	137	1.85
RH12	50	0.33	75	2.3	95	0.04	75	0.75
RH13	38	0.40	15.2	3.5	105	0.08	87	0.81
Mean	82.1	0.53	46.7	5.1	145	0.12	106.5	1.19
±SD	64.7	0.24	28.4	3.2	52.6	0.08	30.1	0.52

K_m was expressed as μM and V_m as nmol/min/mg of microsomal protein.

TABLE 3. Monooxygenase enzymatic activities in 18 human kidney microsomal samples

Samples	Lauric acid*		Arachidonic acid†		CHZ†	EROD†	MROD†	CYP4A‡	CYP2E1
	(ω-1)	ω	(ω-1)	ω					
RH1	0.12	3.35	56.6	523.3	2.5	5.28	3.84	1.60	ND
RH2	0.15	1.95	43.3	401.6	3.1	3.2	5.64	1.42	ND
RH3	0.027	1.47	21.6	241.6	2	1.86	2.17	0.57	ND
RH4	0.27	5.26	145.5	520.3	2.4	4.1	5.11	1.75	ND
RH5	0.19	3.05	66.6	395	2.8	9.7	5.55	1.44	ND
RH6	0.088	1.41	–	–	–	3.3	4.25	1.21	ND
RH8	0.17	5.07	115	615	3.1	1.3	6.3	2	ND
RH9	0.07	2.45	95	498	2.9	4.8	3.7	1.33	ND
RH10	0.30	5.78	87	495	3.5	–	–	1.35	ND
RH11	0.016	0.51	–	–	–	–	–	–	–
RH12	0.04	0.67	20	388	5.5	–	–	0.76	–
RH13	0.07	0.98	35	372	3.9	–	–	1.05	ND
RH14	0.014	0.95	ND	85.6	–	–	–	–	–
RH15	0.058	0.59	87	425	5.4	–	–	0.43	–
RH16	0.10	0.96	78.8	225.6	6.5	–	–	0.57	–
RH17	0.14	1.45	56.5	342.4	3.3	–	–	0.19	–
RH18	0.114	0.81	97.3	387.3	4.9	–	–	1.02	–
RH19	0.10	1.27	88.5	375	5.4	–	–	2.21	–
Mean	0.113	2.11	68.35	393.17	3.81	4.19	4.58	1.18	–
±SD	±0.08	±1.70	±38.4	±130.1	±1.4	±2.6	±1.33	±0.57	–

* nmol/min/mg microsomal protein; †pmol/min/mg microsomal protein; ‡arbitrary relative units; –, Not determined; ND, Not detectable (below the minimum level of detectability).

1.30 pmol/min/mg for EROD and MROD activities, respectively.

Correlations Between Lauric and Arachidonic ω-Hydroxylase Activities and Immunoquantified CYP4A

Involvement of the CYP4A family enzyme in the ω-hydroxylation of fatty acids was confirmed by comparing the specific activities of ω-hydroxylation reactions with the intensity of individual protein bands obtained from immunoblots using a specific polyclonal antirat CYP4A antibody. As shown in Fig. 1, immunoblot analysis of human kidney and liver microsomes revealed only one electrophoretic band of ca. 52 kDa, and there were no differences in the protein pattern between men and women. The antirat CYP4A antibody used in this experiment did not cross-react with other P450 families, but recognized other rat P450 4A isoforms (4A1/A2/A3).

The variations in the kidney content of CYP4A measured by immunoblot and expressed as relative arbitrary units reflected the changes in lauric and arachidonic ω-hydroxylases activities (Table 3). These activities were significantly correlated with CYP4A content (r = 0.86 and 0.75 for lauric and arachidonic acids, respectively; $P < 0.05$) (Table 5).

Conversely, no immunostained protein could be detected in the immunoblot analysis using polyclonal antirat CYP2E1 antibody, with a detection limit estimated to be 0.5 pmole.

Immuno-Inhibition of Lauric and Arachidonic ω-Hydroxylation

To confirm whether or not the CYP4A family enzyme is the main enzyme involved in lauric and arachidonic acid ω-hydroxylation in human renal microsomes, immuno-inhibition studies were carried out using either polyclonal antirat CYP2E1 or CYP4A1 antibodies. Inhibition experiments were performed using human kidney sample RH8, which had high enzymatic activities towards lauric and arachidonic acid. The (ω-1)-hydroxylase activity was weakly inhibited (ca. 35% of control activity for the two fatty acids) at a concentration of 1.25 mg of anti-CYP2E1/mg microsomal protein, whereas the ω-hydroxylase was not modified. Using the anti-CYP4A1 antibody, the lauric or arachidonic ω-hydroxylases were inhibited by approxi-

TABLE 4. Comparison of the monooxygenase enzymatic activities (expressed as pmol/min/mg) in human liver and kidney microsomal samples

	(ω-1)-OH-LA	ω-OH-LA	ω/ω-1 ratio	CHZ	4-NP
Human liver (N = 29)*	1530 ± 840	1070 ± 640	0.75 ± 0.5	456 ± 352	129 ± 96
Human kidney (N = 18)	113 ± 80	2110 ± 1700	22.85 ± 16.3	3.81 ± 1.4	ND

* from Ref. 22; ND, Not detectable.

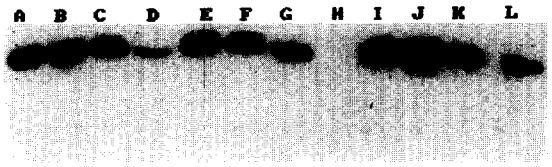

FIG. 1. Immunoblot analysis of 8 human renal (RH) and 1 human liver (Br046) microsomes using a polyclonal antirat P450 4A antibody. Lane A, RH1; lanes B and C, RH2; lane D, RH3; lane E, RH4; lane F, RH5; lane G, RH6; lane H, blank sample; lanes I and J, RH8; lane K, RH9; lane L, Br042. The amount of protein applied was 40 µg human kidney and liver microsomes, except for 80 µg in lanes C and J.

mately 60% at a concentration of 1 mg of anti-CYP4A1/mg microsomal protein. Moreover, the nonimmune serum produced no inhibition of either ω or (ω-1)-hydroxylase activities (data not shown).

DISCUSSION

Many forms of P450s have been purified and characterized from hepatic microsomes [5], but only limited data are available on human kidney P450 [19, 34]. P450 2E1 was not detectable in human kidney samples [35], and it represented approximately 7% of total P450 in human liver [36]. However, Imaoka *et al.* [7] purified 3 different forms of P450 from the renal microsomes of untreated male rats and partially purified human renal cytochrome P450. These P450s were efficient catalysts of the ω and (ω-1)-hydroxylation of fatty acids or prostaglandins. More recently, Kawashima *et al.* [37] purified to homogeneity a P450 form of the CYP4A subfamily from human kidney microsomes, termed $P450_{HK\omega}$. Following this, they purified and cloned a cDNA of human liver CYP4A fatty acid ω-hydroxylase [38], classified as CYP4A11 [39].

In the present study, 18 human kidney samples were examined for their ability to catalyze laurate and arachidonate hydroxylations. Immunochemical assays and enzymatic activities were assessed to estimate the levels of P450.

Total P450 content measured in the human kidneys was in agreement with results previously reported [2, 19, 34]. This mean value is approximately 8 or 10 times lower than that observed in human liver or rat kidney microsomes [2]. The level of NADPH-P450 reductase measured in human kidney microsomes is close to that reported in human liver samples [40], suggesting a good quality of the microsomal preparation.

The levels of CYP2E1 catalytic activity, measured using chlorzoxazone and paranitrophenol hydroxylations, were very weak and often close to the detection limit. This result was confirmed by the immunoblot analysis of CYP2E1 content using a polyclonal antirat CYP2E1 antibody that produced no visible electrophoretic band (limit of detection of 0.5 pmole), data that are in agreement with previous results [35]. Previous studies have reported that (ω-1)-hydroxylation of lauric acid was a useful and highly specific probe of CYP2E1 activity in rat [30] and human [21, 22] liver microsomes. Our results suggest that, although CYP2E1 was undetectable by immunoblot in kidney, specific monooxygenase activities such as (ω-1)-hydroxylation of fatty acids could be detected and slightly immunoinhibited.

The levels of CYP1A1/1A2 catalytic activity, evaluated using EROD and MROD activities, appeared to be undetectable because they were less than 8 pmol/min/mg. These results are in concordance with those of Kawashima *et al.* [37], who found that the $P450_{HK\omega}$ enzyme, isolated and purified from human kidney microsomes, had negligible or no activity towards 7-ethoxycoumarin or ethoxyresorufin activities mediated by CYP2E1 and 1A2, respectively.

Among the P450 isoforms (1A1/1A2, 2C8/C9/C18, 2D6, 3A4/A5, 2E1) produced by cDNA expression in human B-lymphoblastoid cells, only P450 2E1 was shown to be able to (ω-1)-hydroxylate lauric acid [20, 22], but any P450 isoform was able to ω-hydroxylate lauric acid, suggesting that there are two distinct P450 isoforms for the ω and (ω-1)-hydroxylations of fatty acid, i.e. CYP2E1 for the (ω-1) and CYP4A for the ω-hydroxylations.

The present study has shown that ω-hydroxylation of fatty acids is the major P450 enzymatic activity found in human kidney microsomes compared to fatty acid (ω-1)-hydroxylation, chlorzoxazone, and 4-nitrophenol hydroxylations, or EROD and MROD activities. Immunoblot analysis using a polyclonal CYP4A showed that only one protein band was recognized in the human kidney microsomes. The presence of this band was in agreement with the observations of previous studies [21, 37], where the authors reported the presence of a 52 kDa protein band not only in rat liver microsomes but also in human liver and kidney microsomes. CYP4A11 was reported to be the major P450 found in human kidney [39].

The (ω-1)-hydroxylation of lauric or arachidonic acid

TABLE 5. Correlation coefficients between lauric and arachidonic acid hydroxylase activities and CYP4A content in the human kidney samples

	ω-OH-LA	(ω-1)-OH-AA	ω-OH-AA	CYP4A content
(ω-1)-OH-LA	0.85	0.64	0.58	0.72
ω-OH-LA		0.57	0.68	0.86
(ω-1)-OH-AA			0.68	0.46
ω-OH-AA				0.75

N = 16.

was very weak when compared to the ω-hydroxylation, and was barely inhibited when using the polyclonal antirat CYP2E1 antibody. Immunoinhibition experiments with the CYP4A1 antibody indicated a 60% decrease in human kidney microsomal ω-hydroxylase activity, whereas the (ω-1)-hydroxylase activity was not significantly modified. Recent studies using the expressed P450 4A1 protein [39, 41] have indicated that the P450 involved in this reaction was predominantly a fatty acid ω-hydroxylase with only minor (ω-1)-hydroxylase activity. Rat P450 4A1 and human P450 4A11 were demonstrated to catalyze the ω-hydroxylation of lauric acid with turnover of 300 [42] or 15 min^{-1} [data not shown]. However, caution must be taken to resolve 11-hydroxy-dodecanoic acid and dodecanedioic acid, which had the same mobility when assayed by reverse-phase HPLC [42]. The chromatographic method used during this study, based upon analysis of fluorescent derivatives of fatty acids, was demonstrated to be able to prevent such interferences.

The physiological significance of kidney fatty acid ω-hydroxylase is not yet clearly understood. The finding that lauric and arachidonic acid ω-hydroxylases predominate over other forms of P450s in kidney microsomes of several species such as human [7, 19], rat [7, 37, 43, 44] and rabbit [45–48] suggests that the ω-hydroxylation of fatty acids plays an important role in renal function.

In conclusion, renal P450 contents were shown to be very different from those determined in human liver microsomes. First, human kidney does not contain significant amounts of CYP2E1 and, second, the ω-hydroxylation of fatty acids involves predominantly, if not singular, the CYP4A family in human kidney, contrary to liver. The physiological significance of this renal isoenzyme merits further investigation.

The authors thank the "Ligue contre le cancer" (Comité du Finistère) for a grant to Y. A. and Dr. J. P. Salaün (Université Louis Pasteur, Strasbourg, France) and Dr. B. J. Song (NIH, Bethesda, MD) for the gift of 11- and 12-OH-LA and rat polyclonal anti-CYP2E1 antibody, respectively.

References

1. Nelson DR, Koymans L, Kamataki T, Stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC and Nebert DW, P450 super family: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* **6:** 1–42, 1996.
2. Guengerich FP and Mason PS, Immunological comparison of hepatic and extrahepatic cytochromes P-450 in rats. *Mol Pharmacol* **15:** 154–164, 1979.
3. Guengerich FP, Wang P and Davidson NK, Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits, and human using immunochemical staining coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Biochemistry* **21:** 1698–1706, 1982.
4. Guengerich FP, Characterization of human microsomal cytochrome P450 enzymes. *Annu Rev Pharmacol Toxicol* **29:** 241–264, 1989.
5. Ryan DE and Lewin W, Purification and characterization of hepatic microsomal P-450. *Pharmacol Ther* **45:** 153–239, 1990.
6. Guengerich FP, Human cytochrome P-450 enzymes. *Life Sci* **50:** 1471–1478, 1992.
7. Imaoka S, Nagashima K and Funae Y, Characterization of three cytochrome P450s purified from renal microsomes of untreated male rats and comparison with human renal cytochrome P-450. *Arch Biochem Biophys* **276:** 473–480, 1990.
8. Imaoka S, Terrano Y and Funae Y, Expression of four phenobarbital inducible cytochrome P-450s in liver, kidney and lung of rats. *J Biochem* **105:** 939–945, 1989.
9. Ellin A and Orrenius S, Fatty acid hydroxylation in rat kidney cortex microsomes. *Mol Cell Biochem* **8:** 69–79, 1975.
10. Yoshimoto M, Kusunose E, Yamamoto S, Maekawa M and Kusunose M, Purification and characterization of two forms of P-450 from rat kidney cortex microsomes. *Biochem Int* **13:** 749–755, 1986.
11. Capdevila JL, Parkhill N, Chacos N, Okita R, Masters BSS and Estabrook RW, The oxidative metabolism of arachidonic acid by purified cytochromes P-450. *Biochem Biophys Res Commun* **101:** 1357–1363, 1981.
12. Oliw EH, Lawson JA, Brash AR and Oates JA, Arachidonic acid metabolism in rabbit renal cortex: formation of two novel dihydroxyeicosatrienoic acids. *J Biol Chem* **256:** 9924–9931, 1981.
13. Schwartzman ML, Abraham NG, Carroll MA, Levere RD and McGiff JC, Regulation of arachidonic acid metabolism by cytochrome P450 in rabbit kidney. *Biochem J* **238:** 283–290, 1986.
14. Schwartzman ML, Falck JR, Yadagiri P and Escalante B, Metabolism of 20-hydroxyeicosatetraenoic acid cyclooxygenase. *J Biol Chem* **264:** 11658–11662, 1989.
15. Chacos N, Falck JR, Wixtrom C and Capdevila J, Novel epoxides formed during the liver cytochrome P-450 oxidation of arachidonic acid. *Biochem Biophys Res Commun* **104:** 916–922, 1982.
16. Mayer RD, Berman S, Coclett ATK and Maines MO, Differential effect of cyclosporin on hepatic and renal heme, cytochrome P-450 and drug metabolism. *Biochem Pharmacol* **38:** 1001–1007, 1989.
17. Prough RA, Patrizi VW, Okita RT, Masters BSS and Jakobsson SW, Characteristics of benzo(a)pyrene metabolism by kidney, liver and lung microsomal fractions from rodents and humans. *Cancer Res* **39:** 1199–1206, 1979.
18. Jakobsson SW, Okita RT, Mock NI, Masters BSS, Buja LM and Prough RA, Monooxygenase activities of human liver, lung and kidney microsomes—a study of 42 post mortem cases. *Acta Pharmacol Toxicol* **50:** 332–341, 1982.
19. Okita RT, Jakobsson SW, Prough RA and Masters BSS, Lauric acid hydroxylation in human liver and kidney cortex microsomes. *Biochem Pharmacol* **28:** 3385–3390, 1979.
20. Castle PJ, Merdink JL, Okita JR, Wrighton SA and Okita RT, Human liver lauric acid hydroxylase activities. *Drug Metab Dispos* **23:** 1037–1043, 1995.
21. Clarke SE, Baldwin SJ, Bloomer JC, Ayrton AD, Sozio RS and Chenery RJ, Lauric acid as a model substrate for the simultaneous determination of cytochrome P450 2E1 and 4A in hepatic microsomes. *Chem Res Toxicol* **7:** 836–842, 1995.
22. Amet Y, Berthou F, Baird S, Dréano Y, Bail JP and Ménez JF, Validation of the (ω-1)-hydroxylation of lauric acid as an *in vitro* substrate probe for human liver CYP2E1. *Biochem Pharmacol* **50:** 1775–1782, 1995.
23. Berthou F, Ratanasavanh D, Riché C, Picart D, Voirin T and Guillouzo A, Comparison of caffeine metabolism by slices, microsomes and hepatocyte cultures from adult human liver. *Xenobiotica* **19:** 401–417, 1989.
24. Philpot RM, Characterization of human P450 in extrahepatic tissues. In: *Methods in Enzymology, Cytochrome P450* (Water-

man MR and Johnson EF, Eds.) Vol. 206 pp. 623–631, New York, Academic Press, 1991.
25. Ghersi-Egea JF, Walther B, Minn A and Siest G, Quantitative measurement of cerebral cytochrome P-450 by second derivative spectrophotometry. *J Neurosci Meth* **20:** 261–269, 1987.
26. Gibson GG and Skett P, Techniques and experiments illustrating drug metabolism. In: *Introduction to drug metabolism*. pp. 231–281. Chapman and Hall, London, 1986.
27. Prough RA, Burke MD and Mayer RT, Direct fluorimetric methods for measuring mixed-function oxidases. In: *Methods in Enzymology, Biomembranes*. (Fleischer S and Packer L, Eds.) Vol. 52 pp. 372–377. New York, Academic Press, 1978.
28. Peter R, Böcker R, Beaune P, Iwasaki M, Guengerich FP and Yang CS, Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P450 IIE1. *Chem Res Toxicol* **3:** 566–573, 1990.
29. Carrière V, Goasduff T, Ratanasavanh D, Morel F, Gautier JC, Guillouzo A, Beaune P and Berthou F, Both cytochrome P450 2E1 and 1A1 are involved in the metabolism of chlorzoxazone. *Chem Res Toxicol* **6:** 852–857, 1993.
30. Amet Y, Berthou F, Goasduff T, Salaün JP, Le Breton L and Ménez JF, Evidence that cytochrome P450 2E1 is involved in the (ω-1)-hydroxylation of lauric acid in rat liver microsomes. *Biochem Biophys Res Commun* **203:** 1168–1174, 1994.
31. Amet Y, Berthou F and Ménez JF, Simultaneous radiometric and fluorimetric detection of lauric acid metabolites using HPLC following esterification with 4-bromomethyl-6,7-dimethoxycoumarin in human and rat liver microsomes. *J Chromatogr B* **681:** 233–239, 1996.
32. Laemmli UK, Change of structural proteins during the assembly of the bacteriophage T4. *Nature* **227:** 680–685, 1970.
33. Towbin H, Staehelin T and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gel to nitrocellulose sheets: procedure and source applications. *Proc Natl Acad Sci USA* **76:** 4350–4354, 1979.
34. Jakobsson S and Cinti DL, Studies on the cytochrome P-450 containing monooxygenase system in human kidney cortex microsomes. *J Pharmacol Exp Ther* **185:** 226–234, 1973.
35. De Waziers I, Cugnenc PH, Yang CS, Leroux JP and Beaune PH, Cytochrome P-450 isoenzyme, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *J Pharmacol Exp Ther* **253:** 387–394, 1990.
36. Shimada T, Yamazaki H, Mimura M, Inui Y and Guengerich FP, Interindividual variations in human liver cytochrome P 450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 japanese and 30 caucasians. *J Pharmacol Exp Ther* **270:** 414–423, 1994.
37. Kawashima H, Kusunose E, Kubota I, Maekawa M and Kusunose M, Purification and NH_2-terminal amino acid sequences of human and rat kidney fatty acid ω-hydroxylase. *Biochim Biophys Acta* **1123:** 156–162, 1992.
38. Kawashima H, Kusunose E, Kikuta Y, Kinoshita H, Tanaka S, Yamamoto S, Kishimoto T and Kusunose M, Purification and cDNA cloning of human liver CYP4A fatty acid ω-hydroxylase. *J Biochem* **116:** 74–80, 1994.
39. Imaoka S, Ogawa H, Kimura S and Gonzalez FJ, Complete cDNA sequence and cDNA-directed expression of CYP 4A11, a fatty acid ω-hydroxylase expressed in human kidney. *DNA Cell Biol* **12:** 893–899, 1993.
40. Distlerath LM and Guengerich FP, Enzymology of human liver cytochromes P-450. In: *Mammalian cytochromes P-450*. (Guengerich FP, Ed) vol. 1, pp. 133–188. CRC Press, Boca Raton, FL, 1989.
41. Palmer CNA, Richardson TH, Griffin KJ, Hsu MH, Muerhoff AS, Clark JE and Johnson EF, Characterization of a cDNA encoding a human kidney cytochrome P-450 4A fatty acid ω-hydroxylase and the cognate enzyme expressed in *Escherichia coli*. *Biochim Biophys Acta* **1172:** 161–166, 1993.
42. Shet MS, Fisher CW, Holmans PL and Estabrook RW, The omega-hydroxylation of lauric acid: oxidation of 12-hydroxylauric acid to dodecanedioic acid by a purified recombinant fusion protein containing P450 4A1 and NADPH-P450 reductase. *Arch Biochem Biophys* **330:** 199–208, 1996.
43. Ellin A, Jakobsson SW, Schenkman JB and Orrenius S, Cytochrome P 450K of rat kidney cortex microsomes: its involvement in fatty acid ω and (ω-1)-hydroxylation. *Arch Biochem Biophys* **150:** 64–71, 1972.
44. Sharma R, Lake BG, Makowski R, Bradshaw T, Dale JW and Gibson G, Differential induction of peroxisomal and microsomal fatty-acid-oxidising enzymes by peroxisome proliferators in rat liver and kidney. Characterization of a renal cytochrome P-450 and implications for peroxisome proliferation. *Eur J Biochem* **184:** 69–78, 1989.
45. Sawamura A, Kusunose E, Satouchi K and Kusunose M, Catalytic properties of rabbit kidney fatty acid ω-hydroxylase cytochrome P450 Ka2(CYP4A7). *Biochim Biophys Acta* **1168:** 30–35, 1993.
46. Ogita K, Kusunose E, Yamamoto S, Ichihara K and Kusunose M, Multiple forms of cytochrome P-450 from kidney cortex microsomes of rabbits treated with phenobarbital. *Biochem Int* **6:** 191–198, 1983.
47. Yamamoto S, Kusunose E, Kaku M, Ichihara K and Kusunose M, Occurence of cytochrome P-450 with prostaglandin omega-hydroxylase activity in rabbit placental microsomes. *J Biochem* **100:** 1449–1455, 1986.
48. Yoshimura R, Kusunose E, Yokotani N, Yamamoto S, Kubota I and Kusunose M, Purification and characterization of two forms of fatty acid ω-hydroxylase cytochrome P-450 from rabbit kidney cortex microsomes. *J Biochem* **108:** 544–548, 1990.