CELLULAR DISTRIBUTION OF CYTOCHROMES P-450 IN THE RAT KIDNEY

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ABSTRACT:
The distribution of several cytochrome P-450 (P-450) isoenzymes between proximal tubular (PT) and distal tubular (DT) cells of the rat kidney was determined. Western blot analysis of microsomes prepared from liver and kidney cortical homogenates revealed that CYP2E1 protein was expressed in rat kidney microsomes at approximately 10% of hepatic levels. Microsomes from renal cortical, PT, and DT cells all expressed CYP2E1, with DT microsomes expressing slightly higher levels than PT microsomes. In contrast, chloroxazone hydroxylation activity was markedly higher in microsomes from PT cells than in those from DT cells. Northern blot analysis of total RNA from PT and DT cells exhibited a pattern of CYP2E1 mRNA distribution similar to that of CYP2E1 protein. CYP2C11 protein expression in renal cortical microsomes was approximately 10% of that in liver microsomes but was significantly higher in microsomes from PT cells than in those from DT cells. CYP3A1/2 was not detected in microsomes from either cortical, PT, or DT cells, but was detected in microsomes isolated from total liver or kidney cortical homogenates. CYP2B1/2 expression was detected in all tissues tested. The peroxisomal proliferator clofibrate enhanced the level of CYP2B1/2 in microsomes from both total liver and kidney cortical homogenates but not in microsomes from cortical, PT, or DT cells. CYP4A2/3 protein and CYP4A mRNA expression were detected in microsomes from total liver and kidney cortical homogenates and from renal cortical, PT, and DT cells using Western and Northern blot analyses, respectively. Lauric acid hydroxylation activity, an indicator of CYP4A, was comparable in PT and DT cells. Clofibrate elevation of CYP4A in cortical, PT, and DT microsomes was not as great as that detected in total kidney cortical microsomes. These results establish the distribution of several P-450 isoenzymes between different cell populations of the rat kidney. Furthermore, these results present evidence that the level of induction of certain P-450 isoenzymes in the kidney is cell type-specific.

The cytochromes P-450 (P-450) comprise a large superfamily of enzymes (Nelson et al., 1996) that function as monooxygenases and catalyze the oxidation of a vast array of substrates. The mammalian P-450 families can be divided functionally into two classes. The first class catalyzes the synthesis of steroids and bile acids, whereas the second class is primarily involved in the metabolism of xenobiotics and the degradative metabolism of endogenous lipophilic substances (Gonzalez, 1992). The expression of P-450s in the liver has been well studied (Guengerich et al., 1982; Guengerich, 1989), but much less is known about P-450 expression in the kidney. Recent studies have shown that several renal P-450s are inducible and that the levels of these are likely to play an important role in the maintenance of renal physiological function and susceptibility to toxic agents (Capdevila et al., 1992; Kim et al., 1992; Makita et al., 1994, 1996; Nakamura et al., 1994; Zanelli et al., 1996; Zangar et al., 1996). However, a better understanding of the specific P-450 forms, their distribution, and inducibility within the various renal cell types is necessary to understand the role of P-450s in renal physiology and toxicology.

Isoenzymes of the CYP2C and 4A subfamilies catalyze the oxidation of several hormones, including testosterone and arachidonic acid, respectively (McGiff et al., 1993; Waxman and Chang, 1995; Nelson et al., 1996). Furthermore, P-450s catalyze many of the key reactions involved in the transformation of arachidonic acid to biologically active hydroxyeicosatetraenoic and epoxyeicosatrienoic acids (Escalante et al., 1989; Carroll et al., 1990; Romero et al., 1991; Karara et al., 1993; Makita et al., 1994, 1996; Waxman and Chang, 1995). The epoxide products of these P-450-catalyzed reactions alter tubular transport functions (Hirt et al., 1989; Escalante et al., 1991) and may have an important role in the regulation of systemic blood pressure and other cardiovascular functions. The specific cell types and the P-450 enzymes that catalyze the above reactions are still under investigation, but recent studies indicated that renal proximal tubular (PT) cells may be important sites for many P-450-catalyzed reactions, especially those involving CYP4A isoenzymes (Hirt et al., 1989; Escalante et al., 1991).

Many P-450s that catalyze the oxidation of endogenous substrates are involved in the oxidation and toxicity of many highly nephrotoxic xenobiotics (Pons et al., 1992). Isoenzymes from the CYP4A subfamily metabolize arachidonic and lauric acid and are induced by several peroxisomal proliferator-inducing compounds such as fibrate hypo-
Materials and Methods

Chemicals. All chemicals used, unless otherwise stated, were purchased from Sigma Chemical Co. (St. Louis, MO). Rat CYP4A1 cDNA, which recognizes several CYP4A isoforms, and mouse 7S cDNA were generously provided by Dr. Milton Adesnick (New York University Medical Center, New York, NY), Dr. Frank Gonzalez (National Cancer Institute, Bethesda, MD), and Dr. Allan Balmain (Beatson Institute for Cancer Research, Glasgow, UK), respectively. Antibodies for CYP2E1, CYP2B11, and CYP3A1/2 were purchased from Oxford Biomedical (Rochester Hills, MI). CYP2B1/2 antibody was obtained from Xenotech (Kansas City, KS). The antibody for CYP4A1 was obtained from Gentest (Woburn, MA). [1-14C]Lauric acid (50 mCi/mmol) was purchased from ICN Pharmaceuticals, Inc. (Irvine, CA).

Isolation of Rat Renal PT and DT Microsomes. Animals were housed in the Wayne State University vivarium, were allowed access to food and water ad libitum, and were kept in a room on a 12-h light/dark cycle. For clofibrate induction, rats were given clofibrate (0.2 mg/kg/day) or corn oil control by i.p. injection for 3 days. Renal cortical cells were isolated by collagenase perfusion from male Fischer 344 (F344) rats (200-300 g; 12-15 weeks old; Charles River Breeding Laboratories, Inc., Wilmington, MA), and enriched populations of renal PT and DT cells were isolated by density gradient centrifugation in Percoll as described previously (Lash and Tokarz, 1989). Marker enzyme activities and functional assays were used to confirm the identity and purity of the two cell populations (Lash and Tokarz, 1989). Cell concentrations were determined in a hemacytometer and cell viability was estimated by the trypan blue exclusion method. Microsomes were then prepared from these cells by homogenization of the cells in a Polytron PT1200 ultrasonic homogenizer (Brinkmann Instruments, Westbury, NY) followed by centrifugation at 11,000g for 20 min to spin down nuclei, mitochondria, and cellular debris. Supernatant from this step was centrifuged in a tabletop ultracentrifuge at 105,000g for 90 min at 4°C. The resulting pellet was washed with microsomal buffer (100 mM potassium phosphate, pH 7.4; 1 mM EDTA; and 20% glycerol) and was resuspended by sonication using a microprobe.

CYP2E1 Assays. CYP2E1 activity was determined by measuring 6-chlorozoxazone hydroxylase activity as described previously (Peter et al., 1991), using 6-hydroxylchlorzoxazone (Ultrafine Fine Chemicals, Manchester, UK) as a standard. Rat liver microsomes were used as a positive control. CYP2B1/2, CYP3A1/2, and CYP2C11 activities were determined by the testosterone hydroxylase HPLC assay (Vind et al., 1989). CYP4A activity was determined by measuring the formation of the ω- and (ω-1)-metabolites of lauric acid by HPLC as described previously (Salhab et al., 1987; Miranda et al., 1990). Briefly, 0.5 to 1 mg of tissue was incubated in 0.5 ml 50 mM Tris-HCl buffer with 1 mM NADPH and 100 μM [1-14C]-lauric acid for either 30 or 60 min. Controls included the absence of either NADPH or lauric acid. Liver microsomes were used as positive controls. The reactions were stopped by addition of 100 μL of 10% H2SO4 after which 5 μL of 1 mM tolbutamide was added as an internal standard. Lauric acid and its metabolites were then extracted twice in diethyl ether followed by evaporation to dryness under N2. Samples were resuspended in 150 μL of HPLC grade MeOH and injected onto a reversed phase µBondpak C18 10-μm cartridge (10 × 8 mm; Waters Associates, Milford, MA). Peaks for lauric acid and ω-ω-lauric acid were identified by comparison with authentic standards. The presence of (ω-1)-lauric acid was determined on the basis of both its retention time and radiometric labeling. Retention times for ω-, ω-1-, and lauric acid itself were 8, 16 to 20, and 48 min, respectively. ω-ω-1-Lauric acid was eluted isocratically by a mobile phase consisting of 62% MeOH, 37.5% H2O, and 0.2% acetic acid for 40 min at 0.7 ml/min. Lauric acid was then eluted isocratically by a mobile phase consisting of 100% MeOH for 20 min.

Northern and Western Blot Analyses. Microsomes from total liver or renal cortical homogenates or freshly isolated renal cortical, PT, or DT cells from the rat were analyzed for CYP2E1, CYP1A1, and CYP4A1 mRNA as described previously (Zangar et al., 1995). P-450 mRNA band densities were normalized for load based on 7S RNA band densities. Total RNA (10 μg/lane) was fractionated on a formaldehyde/agarose gel, transferred to a nylon membrane, probed with cDNAs complementary to rat CYP2E1, CYP3A1, or CYP4A1, or stripped and reprobed with mouse 7S RNA to examine mRNA loading, then autoradiographed; band density was determined by scanning laser densitometry.

Detection of CYP2E1, CYP2B1/2, CYP2C11, CYP3A1/2, and CYP4A in male rat liver and kidney cortical, PT, and DT microsomes was determined by Western blot analysis. The method of Okita et al. (1998) was used to achieve separation of CYP4A into its individual isoforms. Microsomes were fractionated by SDS-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and probed with the appropriate antibodies. Alkaline phosphatase-conjugated secondary enzymes and substrate were used to detect protein bands and scanning laser densitometry was used to determine band densities. Protein concentrations were determined by the method of Lowry et al. (1951) and equal amounts of protein were loaded onto each lane.

Statistical Analysis. Significant differences within a study were first determined using a one-way ANOVA and then delineated using a t-test comparison between untreated and treated groups. A significance level of P < .05 was used in all cases.

Results

CYP2E1 Expression. To determine if CYP2E1 expression differed between rat renal PT and DT cells, Western blot analysis was performed. Cortical, PT, and DT cells from at least three rats were isolated and combined for preparation of microsomes. Microsomes from total liver and total kidney cortical homogenates and cortical, PT, and DT microsomes were subjected to Western blot analysis using a polyclonal anti-rat CYP2E1 antibody (Fig. 1A). The expression of CYP2E1 was greater in liver microsomes than in kidney microsomes and cortical, PT, and DT microsomes expressed comparable levels of CYP2E1. The CYP2E1 antibody reacted with an unidentified band with a higher mobility (Fig. 1A, →) in PT and DT microsomes but not...
in cortical, total kidney, or liver microsomes. The identity of this band is unknown. Densitometric analysis of these blots demonstrated that CYP2E1 protein levels in cortical and DT microsomes were approximately equal, but CYP2E1 protein levels were marginally higher (~30%) in DT microsomes than in PT microsomes (Fig. 1B).

To determine if the level of expression of CYP2E1 between PT and DT microsomes paralleled differences in CYP2E1 activity, 6-chlorozoxazone hydroxylase activity was examined in PT and DT microsomes (Table 1). Rates of chlorozoxazone hydrolylation were 5-fold higher in liver microsomes than in renal cortical microsomes and were 2.7-fold higher in microsomes from PT cells than in microsomes from DT cells. This lack of correlation in cell type distribution between chlorozoxazone hydroxylase activity and CYP2E1 expression suggests that other enzymes are contributing to chlorozoxazone hydroxylation activity in PT microsomes.

Northern blot analysis of total RNA from rat renal PT and DT cells revealed that the expression of CYP2E1 mRNA was approximately 3-fold higher in DT cells than in PT cells (Fig. 2). This was not due to differences in loading as there were no apparent differences in 7S RNA levels (Fig. 2B).

**CYP2C11 Expression.** Western blot analysis of kidney and liver tissues using a monoclonal anti-rat CYP2C11 antibody demonstrated that CYP2C11 was expressed in both tissues. CYP2C11 expression was detected in kidney microsomes and cortical and PT microsomes but was undetectable in DT microsomes (Fig. 3A). The level of expression in kidney microsomes was approximately 10% of that detected in the liver. CYP2C11 expression in PT microsomes was twice that of cortical microsomes, as determined by densitometric analysis (Fig. 3B).

**CYP3A1/2 Expression.** Western blot analysis of liver and kidney tissue using a monoclonal anti-rat CYP3A1 antibody, which also recognizes CYP3A2, demonstrated that CYP3A1/2 was expressed in liver and kidney microsomes but not in cortical, PT, or DT microsomes (Fig. 4). The expression of CYP3A1/2 in kidney microsomes was approximately 10% of that seen in liver microsomes (data not shown). CYP3A1 mRNA was not detected in either cortical, PT, or DT cells (data not shown).

**CYP2B Expression.** Western blot analysis of liver and kidney tissues using a polyclonal anti-rat CYP2B antibody, which recognizes both CYP2B1 and CYP2B2, revealed that CYP2B was expressed in both liver and kidney microsomes but not in cortical, PT, or DT microsomes (Fig. 5A). The expression of CYP2B1/2 in kidney microsomes was approximately 4-fold in both liver and kidney microsomes (Fig. 5C). CYP2B1/2 expression was detected in cortical, PT, and DT microsomes (Fig. 5B) as well, but clofibrate did not elevate CYP2B1/2 levels in these microsomes (data not shown). Cortical, PT, and DT microsomes from untreated rats expressed comparable levels of CYP2B1/2 (Fig. 5D). An additional band with increased mobility was detected in kidney, PT, and DT microsomes using this antibody (Fig. 5, A and B). The identity of this band remains unknown.

### TABLE 1

<table>
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<tr>
<th>Sample</th>
<th>6-Chlorozoxazone Hydroxylation (pmol/min/mg protein)</th>
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<tbody>
<tr>
<td>Liver microsomes</td>
<td>682 ± 92</td>
</tr>
<tr>
<td>Kidney cortical microsomes</td>
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</tr>
<tr>
<td>Cortical cell microsomes</td>
<td>104 ± 18</td>
</tr>
<tr>
<td>PT cell microsomes</td>
<td>62.0 ± 3.2</td>
</tr>
<tr>
<td>DT cell microsomes</td>
<td>23.0 ± 8.0*</td>
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*Significant difference (P < 0.05) from activity in PT cells.

**Fig. 1.** Expression of CYP2E1 protein in rat liver and kidney.
A, representative Western blot showing the expression of CYP2E1 in 5 μg of pyridine-induced liver microsomes (Lane 1) and kidney microsomes (Lane 2), or 60 μg of cortical (Lane 3), PT (Lane 4), and DT (Lane 5) microsomes. B, densitometric analysis of CYP2E1 protein expression in cortical, PT, and DT microsomes.

**Fig. 2.** Expression of CYP2E1 mRNA in rat renal PT and DT cells.
A, representative Northern blot from PT and DT cells of 7S RNA and CYP2E1 mRNA. B, densitometric analyses of CYP2E1 mRNA bands from PT and DT cells. Individual band densities were normalized to the 7S RNA band density.
Expression and Distribution of CYP4A. Western blot analysis of liver and kidney microsomes from untreated rats resulted in the detection of CYP4A in both liver and kidney tissues (Fig. 6A). Clofibrate treatment increased the expression of CYP4A in both tissues approximately 5-fold. Using the method of Okita et al. (1998), multiple bands were detected in liver, cortical, PT, and DT microsomes (Fig. 6B). Multiple bands were also detected in kidney microsomes (data not shown). The migration of these bands in cortical, PT, and DT microsomes (Fig. 6B, lanes 1–6) was comparable to that observed in liver microsomes (Fig. 6B, lane 7). Okita et al. (1998), using male F344 rat liver microsomes, identified these bands as CYP4A2 (bottom) and CYP4A3 (top). Expression of CYP4A2/3 appeared to be equal in both untreated cortical and PT microsomes, with slightly lower levels of both isoforms being expressed in untreated DT microsomes (Fig. 6B; lanes 1, 3, and 5). Treatment of rats with clofibrate resulted in a slight increase in expression of these bands in PT microsomes (Fig. 6B, lane 4), an even greater increase in DT microsomes (Fig. 6B, lane 6), and no increase in cortical microsomes (Fig. 6B, lane 2).

There was no significant difference in CYP4A activity between liver and kidney microsomes from untreated rats, as assessed by the hydroxylation of [1-14C]lauric acid (Table 2). The rate of formation of ω-lauric acid in liver microsomes was 1.47 ± 0.23 nmol/min/mg protein and 1.19 ± 0.01 nmol/min/mg protein in kidney microsomes, whereas the formation of the ω-1 metabolite was 0.29 ± 0.07 and 0.22 ± 0.05 in liver and kidney microsomes, respectively. Furthermore, there was no significant difference in lauric acid hydroxylation between cortical, PT, and DT cells.

Northern blot analysis of total RNA extracted from PT and DT cells isolated from untreated rats demonstrated that CYP4A mRNA levels were higher in DT cells than in PT cells (Fig. 7, A and B).

Discussion

A majority of studies characterizing the expression of P-450 in the kidney have been done using microsomes prepared from whole tissue homogenates (Bebri et al., 1995; Okita et al., 1998). Much less work has been devoted to the determination of the distribution of renal P-450s within specific cell types. Some studies have been done using immunohistochemistry on whole kidney slices (Schuetz et al., 1992; Bebri et al., 1995; Hotchkiss et al., 1995) or studying the expression of P-450s in PT cells only (Cui and Douglas, 1997). Very little is known concerning the expression of P-450 forms in renal DT cells. This study demonstrates that several P-450s are indeed expressed in both PT and DT cells, determines the distribution of these P-450s, and studies the effect of clofibrate on the expression of CYP4A and CYP2B isoforms in these cells.

CYP2E1 was expressed in total kidney, cortical, PT, and DT microsomes with slightly higher amounts being measured in microsomes from DT cells (Figs. 1 and 2, Table 1). Studies using immunohistochemistry have shown that CYP2E1 is expressed in PT and DT cells in Sprague-Dawley and F344 rats (Hotchkiss et al., 1995; Ronis et al., 1998) but the differences in expression between these two cell types were not quantitated. Data from this study confirm the above results. This slightly greater expression may play a role in the DT cell-selective toxicity seen with certain nephrotoxins (Lash et al., 1994; Lash and Tokarz, 1995). Caution should be taken when comparing toxicity and metabolism studies of CYP2E1 substrates between the rat and human because recent work with tissue from 18 patients reported that CYP2E1 was not detected in human kidney microsomes (Amet et al., 1997a). Indeed, we were unable to detect CYP2E1 protein in freshly isolated human PT cells by Western blot analysis (B.S.C. and L.H.L., unpublished data).

The difference in the renal cellular pattern of chlorzoxazone hydroxylase activity and levels of CYP2E1 protein and mRNA expression in PT and DT microsomes suggests that CYP2E1 is not the only P-450 form responsible for chlorzoxazone hydroxylase activities in these cells. Rather, recent reports on the selectivity of chlorzoxazone hydroxylation as a marker for CYP2E1 activity showed that both CYP3A isoforms (Jayyosi et al., 1995; Gorski et al., 1997) and CYP1A1 (Jayyosi et al., 1995; Yamazaki et al., 1995) can catalyze this activity. The possibility that enzymes other than CYP2E1 can metabolize chlorzoxazone in PT and DT microsomes is supported by the inability of chlorzoxazone to fully inhibit p-nitrophenol hydroxylation in PT and DT cells (data not shown). It should be noted that the levels of chlorzoxazone hydroxylation reported in this study are within the range reported previously for rat kidney microsomes (Amet et al., 1997b). However, Amet et al. (1997b) did not specifically measure chlorzoxazone hydroxylation in PT and DT cells. A role for both CYP2E1 and CYP3A in the hydroxylation of p-nitrophenol has also been suggested (Zerilli et al., 1997, 1998).

Both kidney microsomes and cortical and PT microsomes expressed CYP2C11 (Fig. 3A), whereas expression was not detectable in DT microsomes. The level of expression of renal CYP2C11 was approximately 10% of that seen in the liver (data not shown). Renal CYP2C11 functions in the metabolism of both exogenous chemicals such as thiophene or cephaloridine, and endogenous chemicals such as...
Activity of CYP2C11 (as measured by the 2α-hydroxylation of testosterone) corresponded to the expression of CYP2C11 in PT and DT microsomes (data not shown). Thus, it is possible that the metabolism of cephaloridine and 4-(2-thienyl)butyric acid by CYP2C11 is responsible for the cell type-selective toxicity demonstrated by these chemicals (Lash et al., 1994; Lash and Tokarz, 1995).

The role of CYP2C11 in the metabolism of testosterone or other endogenous substrates in the kidney has not been extensively studied. Other studies (Sundseth and Waxman, 1992) failed to detect CYP2C11 mRNA in the kidney of male F344 rats.

Testosterone is also metabolized by CYP3A isoforms to 6β-hydroxytestosterone (Waxman et al., 1987). The expression of isoforms of CYP3A in the kidney has been extensively studied and investigators have found that CYP3A1, CYP3A2, and CYP3A5 are expressed in rat and human kidney microsomes (Schuetz et al., 1992; Bebri et al., 1995). Data from this study showed the presence of CYP3A1/2 in both liver and kidney microsomes but not in cortical, PT, or DT microsomes. Thus, it is likely that CYP3A1 and CYP3A2 are expressed in other areas of the kidney such as the glomeruli. Formation of 6β-hydroxytestosterone was also demonstrated in incubations with liver and kidney microsomes and analysis by HPLC (data not shown).

It is possible that other isoforms of CYP3A are expressed in either PT or DT cells and the possible presence and identity of these are currently under investigation.

CYP2B1/2 was detected in liver and kidney microsomes, and low levels of expression were detected in cortical, PT, and DT microsomes (Fig. 5, A and B). CYP2B isoforms hydroxylate testosterone primarily at the 16α and 16β positions. CYP2B1/2 was induced by clofibrate in both liver and kidney microsomes but not in cortical, PT, or DT microsomes. Possible reasons for these differences are difficult to determine because of uncertainties about the mechanism of CYP2B induction in general. Clofibrate or similar compounds have been reported to increase rat hepatic CYP2B (Sundseth and Waxman, 1992; Kocarek et al., 1993; Zangar et al., 1995) but no data could be found examining the effect of these compounds on renal CYP2B. Sundseth and Waxman (1992) found a 13-fold increase in male rat hepatic CYP2B1/2 mRNA after 3 days of treatment with 40 mg of clofibrate/0.2 ml of corn oil/100 g of body weight, but expression of renal CYP2B1/2 was not examined. Data from the present study demonstrated that CYP2B1/2 protein levels were increased in both liver and kidney microsomes as a result of clofibrate treatment. Furthermore, clofibrate-mediated increases in rat renal CYP2B1/2 appeared to be specific to other renal cell types besides those studied, as increases in CYP2B1/2 levels were not detected in microsomes from either cortical, PT, or DT cells.

CYP4A isoforms have been previously reported to be expressed in rat renal PT cells (Escalante et al., 1989; Carroll et al., 1990; Romero et al., 1991; Makita et al., 1996; Ito et al., 1998). Ito et al. (1998) showed significant expression of CYP4A2 and CYP4A3 mRNA in all nephron segments tested, including the glomerulus, proximal convoluted and straight tubules, medullary and cortical thick ascending limbs, cortical collecting duct, and inner and outer medullary collecting ducts, but only found small but detectable levels of CYP4A8 mRNA in cortical, but not in medullary, nephron segments. In contrast, Sundseth and Waxman (1992) and Okita et al. (1998) reported that neither CYP4A1 mRNA nor CYP4A1 protein were expressed in male F344 rat kidneys, although Ito et al. (1998) found CYP4A1 mRNA in total kidney homogenates, but not in single nephron segments of Sprague-Dawley rats. Both Sundseth and Waxman (1992) and Okita et al. (1998) identified CYP4A2 and CYP4A3 protein in kidney microsomes from male F344 rats, with CYP4A2 being present in higher amounts. Ito et al. (1998), using a polyclonal antibody raised...
against CYP4A1 that cross-reacts with CYP4A1, CYP4A2, and CYP4A3, found strong expression of CYP4A proteins in glomerular, PT, and medullary thick ascending limb segments. Using the same antibody and methods used by Okita et al. (1998), we were able to detect multiple bands in all tissues tested. Based on the above studies, it appears that the lower band in Fig. 6B is CYP4A2 and the higher band is CYP4A3. CYP4A2 and CYP4A3 proteins were expressed equally in both PT and DT microsomes (Fig. 6). Thus, although Ito et al. (1998) described the distribution of CYP4A mRNAs and proteins in many, but not all, nephron segments, this is the first time that expression of CYP4A2/3 has been shown in rat renal DT cells.

Similar to the results on clofibrate induction of renal CYP2B1/2, clofibrate induction of renal CYP4A2/3 expression did not occur in all cell types tested. Significant increases in CYP4A2/3 were detected only in DT cells, suggesting that clofibrate induction of CYP4A isoforms in the kidney is cell type-specific. CYP4A2/3 expression correlated well with the activity of lauric acid hydroxylation in these tissues (Table 2). The level of ω- and ω-1 lauric acid hydroxylation in rat kidney microsomes agreed with that reported previously (Amet et al., 1997b; Ronis et al., 1998). The activity of CYP4A4 in rat renal microsomes as measured by lauric acid hydroxylation reported here is within the range of values reported in human kidney microsomes (Amet et al., 1997a), suggesting that the rat may serve as a valid model for the study of CYP4A-dependent substrate metabolism in humans. The distribution of CYP4A isoforms between human renal PT and DT cells has not been studied.

In conclusion, this study showed that there are differences in the expression of P-450 family in the arachidonic acid cascade has been cited previously. A potential role for CYP4A in the subsequent metabolism of reaction products of other P-450 isoenzymes has also been suggested. For example, certain metabolites of trichloroethylene may be substrates for CYP4A (Hanioka et al., 1997) and a role for CYP4A4 in the genesis of trichloroethylene-induced nephrotoxicity has been proposed (Davidson and Beliles, 1991). Thus, a role for renal CYP4A isoforms in the metabolism of both physiological and toxicological substrates should be considered.

In conclusion, this study showed that there are differences in the expression of P-450s between different renal cell populations. CYP2E1, CYP2C11, and CYP2B1/2 were expressed at low levels in the rat kidney relative to rat liver, whereas CYP4A2/3 was expressed at comparable levels in the two tissues. CYP2E1 was expressed at slightly higher levels in DT microsomes than in PT microsomes whereas CYP2C11 expression was highest in PT microsomes. CYP2B1/2 was expressed in comparable amounts in both PT and DT

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**TABLE 2**

Activity of CYP4A in liver and kidney cortical microsomes and in renal cells as measured by lauric acid hydroxylation

Activity of CYP4A was determined by analysis of the appearance of two metabolites of lauric acid (ω and ω-1) by HPLC. Results are the means ± S.E. of measurements from three separate preparations of microsomes or cells. For isolated cells, material from two animals was combined and was used for a single experiment. No significant differences (P < .05) were observed between PT and DT cells.

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<tr>
<th>Sample</th>
<th>ω</th>
<th>ω-1</th>
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<td>Liver microsomes</td>
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<td>0.29 ± 0.07</td>
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<td>Kidney cortical microsomes</td>
<td>1.19 ± 0.01</td>
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<tr>
<td>Cortical cells</td>
<td>1.14 ± 0.15</td>
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<td>PT cells</td>
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<tr>
<td>DT cells</td>
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<td>0.18 ± 0.02</td>
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**FIG. 6.** Expression of CYP4A2/3 in rat liver and kidney.

A, representative Western blot showing the expression of 4A2/3 in 5 μg of liver microsomes (Lanes 1 and 2) and 30 μg of kidney microsomes (Lanes 3 and 4). Lanes 1 and 3 are microsomes from untreated rats; lanes 2 and 4 are microsomes from clofibrate-treated rats. B, representative Western blot showing the expression of CYP4A2/3 in 60 μg of cortical (Lanes 1 and 2), PT (Lanes 3 and 4), DT (Lanes 5 and 6), and 1 μg of liver microsomes (Lane 7). Lanes 2, 4, and 6 represent microsomes from clofibrate-treated animals. C, densitometric analyses of CYP4A2/3 expression in untreated and treated cortical, PT, and DT microsomes.

**FIG. 7.** Expression of CYP4A mRNA in rat renal PT and DT cells.

A, representative Northern blot of CYP4A from homogenates of total PT and DT cells. B, Northern blot for 7S RNA for blots given in (A). C, densitometric analyses of CYP4A mRNA bands from PT and DT cells. Individual band densities were normalized to the 7S RNA band density.
cells; CYP3A1 was not detected in either cell type. CYP4A2/3 expression was increased by clofibrate treatment, but these increases were not observed in all renal cell types tested. The significance of these results in terms of both the nephrotoxicity of several chemicals and renal physiology warrants further investigation.

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References


