Increased Epoxyeicosatrienoic Acid Formation in the Rat Kidney during Liver Cirrhosis

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Abstract. Vascular complications during liver cirrhosis are often severe, particularly in the kidney. These complications are the result of complex and poorly understood interactions between the injured liver and other organs such as the lungs, heart, and kidney. The purpose of this study was to investigate the alterations to renal hemodynamics during cirrhosis, focusing on the actions of epoxyeicosatrienoic acids (EET), known to be potent regulators of renal hemodynamics. Cirrhosis was induced in rats by common bile duct ligation (CBDL), and they were compared with sham rats. Experiments were conducted 4 wk after either the sham or CBDL surgery. Vasoreactivity was assessed in isolated perfused kidneys. cPLA2 expression and cytochrome P450 (CYP450) expression were measured using Western blot. cPLA2 enzymatic activity was measured by radioenzymatic assay. EET production was measured using rpHPLC analysis. The major findings were that kidneys from CBDL rats had significantly greater acetylcholine-induced vasodilation that was partially blocked by nitric oxide (NO) and prostaglandin inhibition and fully blocked by the combined inhibition of NO, prostaglandins, and CYP450 metabolites. Expression and activity of cPLA2 in CBDL kidneys was increased, providing arachidonic acid substrate to the CYP450 enzymes. Finally, expression and activity of CYP450 enzymes was elevated in CBDL kidneys, resulting in significantly greater production of the vasodilating 11,12-EET and 14,15-EET. While it is well documented that renal vasoconstriction leading to impaired renal function occurs during cirrhosis, our data clearly demonstrate that endogenous production of EET is increased in cirrhotic kidneys. This may be a homeostatic response to preserve renal perfusion.

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endothelial-dependent vasodilation, much of which was NO-independent. To support our physiologic observations, we performed Western blot analysis to measure expression of cPLA2 and CYP450 isoforms and HPLC analysis to measure production of CYP450 metabolites. These biochemical analyses revealed that CBDL kidneys had significantly greater expression and production of cPLA2 and arachidonic acid, as well as the CYP vasodilating metabolites 11,12-EET and 14,15-EET.

Materials and Methods

Animal Model of Liver Cirrhosis

Biliary cirrhosis was induced in rats by common bile duct ligation (17). The surgical procedures were approved by the Animal Care and Use Committee at the University of Colorado Health Sciences Center (Denver, CO). Male Sprague-Dawley rats (body wt, 200 to 250 g) were allowed to acclimate to Denver’s altitude (1500 m) for 1 wk before any experimental protocols were performed. Animals had continuous access to food and water. Laparotomy was performed under anesthesia with intramuscular ketamine (100 mg/kg) and xylazine (4 mg/kg). The bile duct was isolated, doubly ligated with 3-0 silk, and resected between the two ligatures. The abdominal wall was closed with 4-0 silk sutures. Sham animals underwent laparotomy, bile duct isolation with no ligation and resection, followed by surgical closure. Buprenorphine (0.25 mg/kg) was given subcutaneously twice to both sham surgery and CBDL surgery rats during the first 24 h after surgery to alleviate postsurgical discomfort. Animals were studied 4 wk after surgery. Liver injury was evaluated by measuring serum levels of bilirubin and CYP450 isoforms and HPLC analysis to measure production of CYP450 metabolites. These biochemical analyses revealed that CBDL kidneys had significantly greater expression and production of cPLA2 and arachidonic acid, as well as the CYP vasodilating metabolites 11,12-EET and 14,15-EET.

Histologic Analysis

Livers were perfused free of blood with heparinized PBS via the aorta, followed by perfusion fixation with 10% buffered Formalin. The intact tissues were placed in Formalin for 24 h. Small cubes of tissue were paraffin-embedded. Paraffin sections (5 μm thick) were serially mounted onto glass slides. Hematoxylin and eosin staining was performed on tissue sections from both sham and CBDL rats 4 wk following surgery.

Isolated Perfused Kidney Preparation

After pentobarbital anesthesia (65 mg/kg intraperitoneally), the abdomen was opened through a midline incision and the left renal and superior mesenteric arteries were exposed. To maintain continuous renal perfusion, the superior mesenteric artery was cannulated with a blunt 21-gauge needle that was advanced into the left renal artery and secured with ligature. The kidney was perfused with Tyrode’s solution (37°C) of the following composition (in mM): 137 NaCl; 2.7 KCl; 1.8 CaCl2; 1.1 MgCl2; 12 NaHCO3; 0.42 NaH2PO4; and 5.6 D (+)-glucose, 6.7% (wt/vol) bovine serum albumin-fraction V, and complete amino acids. This perfusate was gassed with 95% O2:5% CO2.

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The kidney was then excised from its surrounding tissues and placed in a chamber containing Tyrode solution at 37°C. Renal perfusion pressure (RPP) was adjusted at constant pressure (100 mmHg) by means of a roller pump (Minipulse; Gilson, Middleton, WI). Renal vascular responses were recorded (MP100 BioPac System, Santa Barbara, CA) as changes in RPP downstream from the pump. The

Experimental Protocols for Isolated Perfused Kidneys

Experiment 1. After a 20-min stabilization period, the renal vasculature was preconstricted with phenylephrine (Sigma) titrated and infused at a rate to produce a final concentrate of 1 × 10^{-6} M in the perfusate. After 20 min of stabilization, dose-response curves to ACh (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) were constructed by a bolus injection. The minimum time between successive doses was 10 min. When necessary, these periods were extended until the previous response had disappeared.

Experiment 2. To elucidate whether NOS and cyclooxygenase products contribute to vascular response in the cirrhotic rat kidney, the COX inhibitor indomethacin (10^{-5} M dissolved in 0.5 M Na2CO3) was added to the perfusion solution; then, after a 20-min stabilization period, Nω-nitro-L-arginine methyl ester (L-NAMe, 100 μM) was infused. After a 30-min stabilization period, the renal vasculature was preconstricted with phenylephrine titrated and infused at a rate to produce a final concentrate of 0.5 × 10^{-6} M in the perfusate. After 20 min of stabilization, dose-response curves to ACh (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) were constructed by a bolus injection. The minimum time between successive doses was 10 min. When necessary, these periods were extended until the previous response had disappeared.

Experiment 3. To further define the contribution of EET to the renal vascular effect, experiments were performed with CYP450 epoxygenase inhibitors, miconazole and N-methylsulphonyl-6(2-propargyloxyphenyl) hexanamide (MS-PPOH, gift from J.R. Falck, University of Texas) that predominantly inhibit epoxygenase activity. Indomethacin (10^{-5} M) was added to the perfusion solution, then after 20 min stabilization period, L-NAMe (100 μM) was infused in 30 min. Then either miconazole (1 μM) or MS-PPOH (50 μM) was administered to selectively inhibit the epoxygenase pathway. After a 30-min stabilization period, the renal vasculature was preconstricted with phenylephrine (Sigma) titrated and infused at a rate to produce a final concentrate of 0.5 × 10^{-6} M in the perfusate. After 20 min of stabilization, dose-response curves to ACh (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) were constructed by a bolus injection. The minimum time between successive doses was 10 min. When necessary, these periods were extended until the previous response had disappeared.

For all of the above conditions, changes in RPP in response to vasodilators were expressed as percentage of the vasconstriction obtained with phenylephrine.

Western Blot Analysis of cPLA2 and CYP450 Isoform Protein Expression

Standard techniques that we have previously described were used to evaluate protein expression (17). Protein (15 μg) from renal cortical homogenates was electrophoresed through a 10% acrylamide gel. cPLA2, CYP2C11, CYP2C23, CYP2E1, CYP4A1, and CYP2J2 protein expression were detected using the following antibodies (cPLA2: monoclonal from Santa Cruz Biotech, Inc.; CYP2C11, CYP2E1, and CYP4A1 polyclonal from Daiich Pure Chemical Tokyo, Japan; CYP2C23 polyclonal from Dr JH Capdevila, Vanderbilt University, Nashville, TN; and CYP2J2 polyclonal from Dr. DC Zeldin, Research Triangle Park, NC). Western blots were incubated at the following dilutions: 1:100 (cPLA2); 1:1000 (CYP2C11); 1:5000 (CYP2C23); 1:1000 (CYP2E1); 1:1000 (CYP4A1); or 1:2000 (CYP2J2) in Tris-buffered saline-Tween 20 containing 5% dry milk. The secondary antibody (anti-mouse, anti-rabbit, or anti-goat IgG-conjugated to horse radish peroxidase) was diluted 1:1000 to 1:10,000 in Tris-buff-
ered saline-Tween 20 containing 5% dry milk. Antigenic detection was done using enhanced chemiluminescence (Amersham, Arlington Heights, IL) with exposure to x-ray film. Densitometry was performed using a densitometer from Kodak (Kodak 1D v 3.5 and Kodak digital science Image Station 440 CF, Tokyo, Japan).

**Assay of cPLA2 Activity**

cPLA2 activity was measured as described previously (18). Briefly, a small amount of frozen kidney cortex was homogenized in 1 to 2 ml of assay buffer containing 50 mM HEPES, pH 7.4, 250 mM sucrose, 1 mM EDTA, 1 mM EGTA, 20 μM leupeptin, 20 μM pepstatin, 0.1 mM phenylmethylsulfonyl fluoride, and 1000 U/ml aprotinin. The homogenate was spun at 100,000 × g for 1 h in an ultracentrifuge. The resulting supernatants were matched for protein and assayed for cPLA2 activity. The substrate for cPLA2 activity was [14C]-arachidonyl-phosphatidylethanolamine (DuPont-New England Nuclear, Boston, MA). Substrate was dried down under N2 and resuspended in DMSO. Activity was assayed in triplicate in a total volume of 40 μl containing 2 μl of substrate (final concentration, 15 μM), 5 μl CaCl2 (final concentration, 5 mM), and 33 μl of the soluble extract (1 to 3 mg prot/ml). The reaction mixture was incubated for 30 min at 37°C. Reactions were terminated by the addition of one volume (40 μl) of ethanol/acetic acid (98:2) containing 0.5 mg/ml arachidonic acid (Sigma). Release of free fatty acid was analyzed using silica gel ethanol/acetic acid (98:2) containing 0.5 mg/ml arachidonic acid (Sigma). Determination of free fatty acid was performed on the plate, and the plates were developed in the organic phase of the ethyl acetate/thin-layer chromatography (Whatman L5KD, Maidstone, England). Activity was assayed in triplicate in a total volume of 40 μl containing 2 μl of substrate (final concentration, 15 μM), 5 μl CaCl2 (final concentration, 5 mM), and 33 μl of the soluble extract (1 to 3 mg prot/ml). The reaction mixture was incubated for 30 min at 37°C. Reactions were terminated by the addition of one volume (40 μl) of ethanol/acetic acid (98:2) containing 0.5 mg/ml arachidonic acid (Sigma). Release of free fatty acid was analyzed using silica gel ethanol/acetic acid (98:2) containing 0.5 mg/ml arachidonic acid (Sigma). Determination of free fatty acid was performed on the plate, and the plates were developed in the organic phase of the ethyl acetate/thin-layer chromatography (Whatman L5KD, Maidstone, England).

**HPLC Analysis of EET Production.**

Microsomes from renal cortex were prepared by sequential centrifugations as described previously (19) and incubated at a final protein concentration of 300 mg/ml (final volume, 200 μl) for 30 min at 37°C with [14C]-arachidonic acid (2 μM), 1 mM NADPH, and an NADPH regenerating system containing 10 mM isocitrate and 0.1 U/ml isocitrate dehydrogenase. Reactions were terminated by acidification and extracted for resolution by HPLC.

Products from microsomal reactions were loaded onto a Nucleosil 5 silica, 4.5 × 250–mm column. Solvent A was water and solvent B was acetonitrile: 0.1% acetic acid. The program was a 40 min linear gradient from 50% solvent B in A to 100% solvent B at a flow rate of 1 ml/min. Identification of products was based upon co-elution with authentic standards.

**Data Presentation and Statistical Analyses**

Physiologic experiments using isolated kidneys were repeated a minimum of four times using different animals for each experiment. For all Western blot experiments, tissue from the same eight rats (4 sham, 4 CBDL) was used on repeated gels. An additional set of eight rats (4 sham, 4 CBDL) was used for the HPLC analysis and cPLA2 activity experiments. Group means were compared using either unpaired t test or ANOVA, as statistically appropriate. For significant comparisons within the ANOVA, Tukey’s post-hoc analysis was used.

**Results**

**Characterization of CBDL Model and Perfused Kidney**

Several parameters were assessed to monitor the health of the rats and development of liver disease. These data are summarized in Table 1. The elevated serum bilirubin indicated the presence of liver injury in the CBDL rats. Injury was confirmed by histopathologic analysis of liver sections, which revealed significant proliferation of cholangiocytes, structural reorganization, and ductal expansion in the CBDL livers (Figure 1). White blood cell counts revealed that neutrophils were elevated in CBDL rats, a common finding after CBDL (20).

To verify the stability and viability of the isolated perfused kidney preparation, RPP and flow rate were monitored in a series of sham and CBDL kidneys over a 90-min period in the absence of any vasoactive mediators. Neither RPP or flow rate changed significantly over this 90-min period (Table 1).

**Role of NO, COX, and Epoxygenase Pathway in Renal Vasodilation in Response to ACh**

In kidneys preconstricted with phenylephrine, ACh decreased RPP dose dependently in both groups. However, this vasodilator response was significantly greater in the CBDL kidneys compared with sham (Figure 2). The initial vasoconstriction to phenylephrine was not different between the sham and CBDL kidneys (Figure 2, inset). In contrast to the data in Figure 2, following NOS and COX inhibition, the initial phenylephrine contraction was significantly lower in the CBDL kidneys compared with sham (Figure 3, inset). While ACh still elicited a dose-dependent vasodilatation, the CBDL kidneys still had a small but significantly greater vasodilator response compared with sham kidneys (Figure 3). These results were concordant with a previous report (16) and indicated to us that an NO-independent and prostaglandin-independent vasodilator pathway was active in the kidneys from CBDL rats.

To determine the role of CYP450 in NO-independent and prostaglandin-independent vasodilation in CBDL kidneys, we used two inhibitors that have been reported to block production of CYP450 metabolites: miconazole (Figure 4; reference 21) and MS-PPOH (Figure 5; reference 22), in combination with L-NAME and indomethacin (NOS and COX inhibitors). The epoxygenase inhibitor miconazole, in combination with L-NAME and indomethacin nearly eliminated the elevated vasodilator response to ACh in the CBDL rat kidney compared to sham (Figure 2, inset).

**Table 1. Physical characteristics and isolated kidney perfusion conditions**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>CBDL</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>320 ± 6</td>
<td>294 ± 9</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>1.089 ± 0.01</td>
<td>1.139 ± 0.047</td>
</tr>
<tr>
<td>Serum bilirubin (mg/dl)</td>
<td>0.12 ± 0.01</td>
<td>13.47 ± 1.86b</td>
</tr>
<tr>
<td>White cell counts (10^9/L)</td>
<td>5.62 ± 1.15</td>
<td>21.7 ± 3.2b</td>
</tr>
<tr>
<td>Renal flow rate (ml/min; at 100 mmHg RPP)</td>
<td>15.7 ± 0.2</td>
<td>16.5 ± 1.0</td>
</tr>
<tr>
<td>Baseline drift after 90 min (mmHg)</td>
<td>+3.6 ± 1.2</td>
<td>+3.2 ± 0.9</td>
</tr>
</tbody>
</table>

* All values are mean ± SEM.

b P < 0.05 compared to Sham.

Sample sizes ranged from 4 to 16 depending on experimental group and parameter measured. RPP, renal perfusion pressure.
with sham kidney (Figure 4). Because miconazole might have nonspecific effects (21), we elected to repeat these experiments using MS-PPOH, a highly selective epoxygenase inhibitor. The CBDL induced-vasodilation was completely abolished with MS-PPOH (Figure 5). These results indicated that epoxygenase metabolites contributed to the greater vasodilation in response to ACh in CBDL rat kidney.

Renal CYP450-Derived Eicosanoids

Since the results from the physiologic measurements indicated that the arachidonic acid/phospholipase A_2 and EET/epoxygenase pathway might be activated in the kidney during cirrhosis, we used several biochemical approaches to measure both enzyme amounts and activities and metabolite production.

Western blot analysis demonstrated that amounts of cPLA_2 enzyme were increased in the kidneys of CBDL rats (Figure 6A). This was accompanied by increased activity of the cPLA_2 enzyme leading to increased production of arachidonic acid (Figure 6B).

Several isoforms of the CYP family of enzymes are reported to generate the renal vasodilating eicosanoids, 11,12-EET and 14,15-EET. Western blot analysis showed that expression of CYP 2C23 and 2E1 were both higher in the CBDL kidneys (Figure 7, A and C). Although not thought to be a source of 11,12-EET or 14,15-EET, CYP 4A1 was also increased in CBDL kidneys (Figure 7D). The increased CYP protein expression was not uniform; there was no increase in expression of CYP 2C11 (Figure 7B), and CYP 2J2 was not detectable in kidneys from either group (data not shown).

rpHPLC analysis was used to evaluate EET and HETE production (Figure 8). Consistent with increased amounts of
CYP protein expression, 11,12-EET and 14,15-EET were both substantially increased by 6-fold and 11-fold, respectively, in the CBDL kidneys compared with sham.

**Discussion**

The extrarenal and intrarenal factors that control renal hemodynamics during cirrhosis are under active investigation but...
not completely understood. Systemic production of NO is increased during liver cirrhosis (23), and it is central to the development of peripheral vasodilation and hyperdynamic circulation (24,25). In the present study, we sought to separate the intrarenal and extrarenal factors that control renal hemodynamics during cirrhosis. We used isolated perfused kidneys to examine the endogenous vasoactive properties during CBDL-induced cirrhosis in rats. Our primary observations are that CBDL kidneys have (1) reduced vasoconstriction in response to phenylephrine in the presence of NOS, cyclooxygenase, and CYP 450 inhibition; (2) exaggerated vasodilation to ACh; and (3) increased production of vasodilating arachidonic acid, 11,12-EET and 14,15-EET.

Figure 5. Effect of combined COX NOS and specific CYP450 inhibition on ACh-induced relaxation in isolated perfused kidneys from Sham and CBDL rats. (A) Representative recording of perfusion pressure showing basal perfusion pressure, elevation of perfusion pressure with phenylephrine (PE, 10^{-6} M) and vasodilator responses to ACh (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) in isolated perfused Sham and CBDL rat kidney treated with L-NAME, indomethacin, and MS-PPOH to block NO, prostaglandin, and eicosanoid production, respectively. (B) Effects of MS-PPOH (50 μM) on vasodilator responses to ACh treated with L-NAME (100 μM) and indomethacin (10^{-5} M) in isolated perfused kidney of Sham and CBDL rats. The initial increase in RPP following COX NOS and CYP450 inhibition was slightly lower in CBDL kidneys compared to Sham (inset) * P < 0.05 statistically different between Sham and CBDL of same group.

Figure 6. cPLA2 expression and activity in sham and CBDL rat whole kidney. (A) Kidney homogenates (15 μg total protein) from Sham and CBDL rat kidney were separated on 10% Tris-glycine gel, transferred to PVDF membrane, and blotted with a monoclonal antibody against cPLA2 (upper). Densitometric analysis was performed to quantitate differences in expression (lower). (B) cPLA2 activity was measured radioenzymatically using 14C-arachidonyl-phosphatidylcholine conversion to 14C-arachidonic acid by cPLA2 in renal cortex from sham and CBDL rats. See Materials and Methods for specific details. * P < 0.05, statistically greater than Sham samples.
NO-independent and prostaglandin-independent component remained. This prompted us to examine the cytochrome P-450 mono-oxygenase pathway.

Although the structure of EDHF is unresolved and under active investigation, evidence implicates a CYP-450 arachidonic acid metabolite (27), perhaps an EET. Several of the EET are potent renal vasodilators (28,29). In the rat, 11,12-EET dilates renal arteries preconstricted with phenylephrine and activates Ca\(^{2+}\)-activated K\(^{+}\) channels in vascular smooth muscle isolated from renal arteries (28). 14,15-EET vasodilates interlobular and afferent arterioles, presumably also by the activation of Ca\(^{2+}\)-activated K\(^{+}\) channels, although the exact mechanism of 14,15-EET–dependent vasodilation has not been confirmed (29). The vasoconstrictor action of EET persists during NOS and COX blockade, similar to what we observed in the present study. Several additional pieces of data in our study demonstrate that the EET/EDHF pathway is upregulated in the kidneys of cirrhotic rats. Our Western blot analyses showed a modest but reproducible increase in expression of EET-forming CYP-450 isoforms, 2C23 and 2E1. More strikingly, production of 11,12-EET and 14,15-EET levels were indisputably increased by 6-fold and 11-fold, respectively, in CBDL kidneys compared with sham kidneys. In our model of cirrhosis, eicosonoid production and CYP-450 isoform expression in the kidney were specific and not globally unidirectionally regulated. For example, CYP2C11 levels were unchanged or even slightly decreased (Figure 7B) and CYP2J2 levels were not detectable (data not shown). Production of EET known to be vasoconstrictors, namely 5,6-EET (data not shown), was unchanged, as were levels of di-HETE and 20-HETE (Figure 8A).

The generation of EET requires arachidonic acid produced from cPLA\(_2\) activity. Two forms of cPLA\(_2\) are known to exist.

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**Figure 7.** Western blot analysis of renal CYP450 isoform expression in sham and CBDL rats. Whole kidney homogenates (15 mg) were separated on 10% Tris-glycine gel, transferred to PVDF membrane, and blotted with specific CYP450 isoform antibodies. (A) Blot probed with CYP 2C23 antibody (upper) and corresponding densitometry analysis (lower). (B) Blot probed with CYP 2C11 antibody (upper) and corresponding densitometry analysis (lower). (C) Blot probed with CYP 2E1 antibody (upper) and corresponding densitometry analysis (lower). (D) Blot probed with CYP 4A1 antibody (upper) and corresponding densitometry analysis (lower). For densitometry analyses, *P* < 0.05, statistically greater than Sham samples.
in nine different groups: secretory cPLA\(_2\) (sPLA\(_2\); groups I, II, III, V, VII, and IX) and cytosolic PLA\(_2\) (cPLA\(_2\); groups IV, VI, and VII). The physiologic or pathophysiologic roles for many of these different groups of enzymes remain undefined. Potential roles for groups I and II sPLA\(_2\) include platelet aggregation, inflammation in rheumatic diseases, and proliferative effects in cancer cells (30,31). Group IV PLA\(_2\) (cPLA\(_2\)) is an intracellular form, selective for arachidonic acid, and regulated by changes in intracellular calcium and protein phosphorylation (2–4). In most cell types, it is therefore the dominant enzyme controlling eicosanoid production. Because sPLA\(_2\) enzymes show low selectivity for arachidonic acid, their role in eicosanoid production is less clear. By using the high-speed supernatant and phospholipid substrate with arachidonic acid in the sn2 position, our assay conditions were specific for measuring cPLA\(_2\) activity, thus complementing our Western blot data using a cPLA\(_2\) specific monoclonal antibody.

While one previous study reported that renal cPLA\(_2\) activity was increased during cirrhosis (18), ours is the first report to conclusively link the entire arachidonic acid/cPLA\(_2\) and EET/CYP-450 pathway to the exaggerated ACh-induced renal vasodilation during cirrhosis. We showed that both cPLA\(_2\) expression and activity are upregulated in the kidneys of CBDL rats. This presumably provides increased substrate in the form of arachidonic acid to the CYP 450 mono-oxygenase pathway, which underlies the generation of 11,12-EET and 14,15-EET. The upstream mechanisms responsible for controlling the expression and activity of this entire pathway are currently under investigation.

**Figure 8.** Metabolism of \(^{14}\)C-labeled arachidonic acid by representative renal microsomes from Sham operated and CBDL rats. (A) Reverse-phase HPLC chromatograms depicting the metabolites formed from Sham (left) and CBDL (right) renal microsomes incubated with \(^{14}\)C-labeled arachidonic acid (AA; 0.2 \(\mu\)Ci/ml, 2 \(\mu\)M) for 30 min at 37\(^\circ\)C in a 0.1 M KPO\(_4\) buffer containing 1 mM NADPH. The peak eluting at 36 min co-eluted with AA standard. Those appearing at 25 and 27 min co-eluted with 14,15- and 11,12-dihydroxyeicosatetraenoic acids, respectively. Peaks appearing around 14 min co-eluted with Di-HETEs. The large peak at approximately 17 min represented 20-HETE, which was not different between Sham and CBD tissues. The metabolite that eluted around 8 min was unidentified. (B) Summary of 14,15-EET production by Sham and CBDL kidney microsomes. (C) Summary of 11,12-EET production by Sham and CBDL kidney microsomes. Sample size was 6 Sham and 6 CBDL. * \(P < 0.05\), statistically greater than Sham samples.
investigation in our laboratory. While many factors (e.g., NO, angiotensin II, epidermal growth factor, dopamine, phenobarbital, etc) and conditions (e.g., high salt intake, hypertension, diabetes, pregnancy, chronic renal disease, etc) regulate either cPLA₂ or CYP-450 function and expression (reviewed in references 5 and 32), we did not directly address what factors are mediating the observations we reported here. It is apparent that, as yet unidentified factors possibly arising from the injured liver are activating the arachidonic acid/cPLA₂ and EET/CYP-450 pathways.

Based on the profound functional impact that the cPLA₂/CYP-450 pathway has on renal hemodynamics and tubular function, it would be predicted that expression would exhibit some degree of axial heterogeneity across the renal tubules and microvasculature. In fact, EET biosynthesis occurs throughout the nephron (33–35). EET contribute to integrated renal function, either by directly affecting tubular transport process, vascular tone, and cellular proliferation or by mediating the actions of renal hormones including renin, angiotensin II, and arginine vasopressin (7,10,20,35,36). While the CYP-450 pathway has been demonstrated to be involved in several renal pathologies, such as hypertension (11,37–39), our report is the first to biochemically and functionally implicate this pathway to the renal dysfunction during cirrhosis.

An interesting observation from the HPLC analysis of eicosanoid production in sham and CBDL kidney cortex was the peak with a retention time of approximately 8 min that was substantially increased in the CBDL kidneys. We do not know the identity of this peak, but we speculate that it is a vasodilator like 7,8-dihydroxy-hexadecadienoic acid (DHHD) (40). Due to different chromatographic conditions, it is not possible to compare absolute retention times of 7,8-DHHD with the unknown peak at 8 min in our present study. However, 7,8-DHHD comes off the column earlier than 11,12-EET (40). Of further interest is that 7,8-DHHD relaxes porcine coronary artery to a similar magnitude as 11,12-EET does, although the temporal relaxation action is different (40). These observations indicate that redundant eicosanoid-based vasodilators may be activated in the kidney during cirrhosis.

Renal vasoconstriction leading to reduced renal function and renal failure is a common observation of cirrhosis. We recently reported that the important vasodilator enzymes eNOS, HO-1, and HO-2 were decreased in the kidneys of CBDL rats (17). This was functionally linked to decreased renal function (both RPF and GFR) measured in vivo in CBDL rats. Decreased production of both CO generated from HO-1 and HO-2 and NO from eNOS were critical to the renal vasoconstriction and impaired renal function. One possible mechanism to explain our present results showing increased NO- and COX-independent vasodilation in CBDL kidneys is that HO and CYP-450 are reciprocally regulated (41). Therefore, a reduction in HO expression in CBDL kidney (17) leads to an upregulation of CYP-450 isoforms, presumably in an attempt to maintain renal perfusion. The relationship between HO and CYP450 during liver cirrhosis warrants further investigation.

In conclusion, the present study demonstrates increased production of 11,12-EET and 14,15-EET are critical events in the NO- and prostaglandin-independent vasodilation in CBDL kidneys. We speculate that increased production of vasodilating EET may be a homeostatic response to offset renal vasoconstriction that frequently occurs during liver cirrhosis.

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