Renal Cytochrome P450 Oxygenases and Preglomerular Vascular Response to Arachidonic Acid and Endothelin-1 Following Ischemia/Reperfusion

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ABSTRACT
This study tested the hypothesis that cytochrome P450 (P450) metabolites of arachidonic acid (AA) contribute to the vascular changes in ischemia/reperfusion (I/R) injury in the rat. In this study, P450-dependent ω-hydroxylase-mediated vascular reactivity of the rat renal interlobular and arcuate vessels (preglomerular vessels [PGMV]) was measured in left kidneys subjected to I/R. Clipping the left renal artery and vein for 30 min followed by reperfusion (I/R) for 3, 6, and 24 h markedly reduced renal microsomal ω-hydroxylase-mediated conversion of [14C]AA to 20-hydroxyeicosatetraenoic acid (HETE) that amounted to 34, 37, and 58% of the control enzyme activity, respectively. CYP4A protein expression was also reduced. There was no significant change in epoxygenase activity. Despite these changes, constriction of the rat PGMV by AA or endothelin-1 (ET-1) was not different in vessels from the clipped and nonclipped (contralateral) kidney. Clofibrate (250 mg/kg i.p.), an inducer of CYP4A protein and ω-hydroxylase enzymes, did not increase 20-HETE production but selectively enhanced the vasoconstriction produced by AA and ET-1 in the clipped but not the contralateral kidney without affecting the constriction produced by 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin F2α. On the other hand, administration of 2% NaCl (w/v, orally for 7 days) to induce P450-dependent epoxygenase activity attenuated AA-induced vasoconstriction but enhanced ET-1-induced vasoconstriction only in the clipped kidney. These data indicate that the reduction in CYP4A protein expression and enzyme activity in I/R is an adaptive mechanism to preserve renal vasculature from excessive vasoconstriction. Moreover, the increase in epoxygenase activity following salt loading may account for the diminished vasoconstriction evoked by AA. However, the enhancing effect of salt on ET-1-induced vasoconstriction in I/R appears to result from an overwhelming effect of salt-induced sensitization of the renal vasculature to ET-1 over the enhanced production of dilator epoxygenase products.

Acute renal failure (ARF) is a clinically important problem. The ARF observed after renal ischemia is characterized by reduced glomerular filtration rate, tubular necrosis, and increased renal vascular resistance. However, the pathophysiological changes responsible for the postischemic renal injury and the profoundly depressed renal function in experimental ARF induced by occlusion of renal artery followed by reperfusion (I/R) are incompletely understood. It has been suggested that abnormalities in the renal circulation persist in the postischemic period after the reflow, especially in the outer medulla, and may contribute to the impaired renal function (Mason et al., 1984; Vetterlein et al., 1986; Conesa et al., 2001). The involvement of mediators, such as oxygen-derived free radicals (see Conesa et al., 2001), eicosanoids (including thromboxane A2 and prostaglandins; Ruschitzka et al., 1998), endothelin (Shibouta et al., 1990), angiotensin II (Magnusson et al., 1983), and adenosine (Lin et al., 1988), has been suggested. The release of free radicals during reperfusion could lead to endothelial dysfunction and a consequent diminution in nitric oxide (NO) production (Raab et al., 1997; Liu et al., 1998). This could result in increased generation of cytochrome P450 (P450)-derived eicosanoids from endogenous arachidonic acid (AA), as NO has been shown to inactivate these enzymes in the kidney (Oyekan et al., 1999). In addition, activation of phospholipase A2 plays a role in I/R injury (Nakamura et al., 1991) and affects NADPH-dependent monoxygenase systems, including the P450 system, a source of reactive oxygen species (Tamura et al., 1997). Despite this plausible scenario, the contribution of P450-AA enzymes in renal injury/failure is not clearly understood, as there have been contradictory results from various laborato-

ABBREVIATIONS: ARF, acute renal failure; AA, arachidonic acid; P450, cytochrome P450; HETE, hydroxyeicosatetraenoic acid; PGMV, pregglomerular vessel; ID, intraluminal diameter; I/R, ischemia/reperfusion; U46619, 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin F2α; NO, nitric oxide; HPLC, high-performance liquid chromatography; ET, endothelin.
ET-1 is an important and widely studied mediator that is implicated in vasoconstriction characteristic of ARF, as ET-1 levels increase with ischemia (Shibouta et al., 1990) and anti-ET antibodies or endothelin receptor antagonists protected against I/R injury (see Sheridan and Bonventre, 2000). However, a link has not been established between ET-1 and P450 enzymes in ARF. ET-1 stimulates phospholipases A2 and C, releasing AA (Simonson and Dunn, 1990) that is metabolized to eicosanoids (McGiff and Quilley, 1999), which could potentially mediate the constriction characteristic of ARF. 20-Hydroxyeicosatetraenoic acid (HETE) is the predominant renal eicosanoid and a potent vasoconstrictor of renal microvessels (McGiff and Quilley, 1999), which mirrors the biologic effects of ET-1 (Oyekan and McGiff, 1998) and could well be the mediator of the characteristic persistent vasoconstriction that was ascribed to ET-1 in ARF (Shibouta et al., 1990). Most of the earlier studies that evaluated the role of P450 enzymes in renal failure examined biochemical and histological endpoints. Here, we evaluated a physiological endpoint-vascular reactivity in the renal microvessel, a major site for 20-HETE production (Imig et al., 1996), and determined vascular reactivity to ET-1 and AA in rats subjected to I/R injury. The effects of clofibrate, an inducer of CYP4A (Lenart et al., 1998), the gene responsible for 20-HETE synthesis, or 2% NaCl, an inducer of P450 epoxygenase enzymes (Makita et al., 1994; Oyekan et al., 1999) on vascular responses to ET-1 and AA were also tested. The schematic in Fig. 1 depicts the proposed enzyme pathways involved in ET-1 and AA responses and the proposed sites of actions of clofibrate and NaCl.

**Materials and Methods**

Sodium pentobarbital was obtained from Abbott Laboratories (North Chicago, IL). Arachidonic acid (NuChek Prep Inc., Elysian, MN) was dissolved in normal saline, and [14C]AA (PerkinElmer Life Sciences, Boston, MA) supplied in ethanol was stored at −70°C before use. Endothelin-1 (Peninsula Laboratories, Belmont, CA) was prepared in 0.1% acetic acid and stored at −20°C. U46619 (9,11-dideoxy-9α,11α-methanoepoxy prostaglandin F2α) (Cayman Chemical Co., Ann Arbor, MI) supplied in methyl acetate was stored at −20°C in 5-mg/ml ethanol aliquots. NADPH was obtained from Sigma-Aldrich (St. Louis, MO) and was dissolved in normal saline. The Western blotting kit for rat CYP4A was obtained from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK) and was stored at 4°C until use.

**Animals.** Adult male Sprague-Dawley rats (275–300 g) were purchased from Charles River Laboratories, Inc. (Wilmington, MA). The animals were placed in a room with lighting that was adjusted to produce a normal day-night cycle. They were maintained on a standard diet of Purina chow and allowed ad libitum access to water and food and at least 3 days to become acclimatized to the housing conditions before use in experiments. All protocols were approved by the Institutional Animal Care Facility Committee. Rats were divided into groups that were treated with clofibrate (250 mg/kg, i.p., for 1 day) to induce ω/ε-1 hydroxylase (Lenart et al., 1998) or 2% NaCl (w/v) ad libitum for 7 days to induce epoxygenase (Makita et al., 1994; Oyekan et al., 1999). Respective control rats received olive oil (vehicle for clofibrate, 1 ml/kg, i.p. for 1 day) or tap water (for rats treated with 2% NaCl).

**Induction of Ischemia/Reperfusion.** To induce ischemia reperfusion, the two-kidney one-clip model was employed. Briefly, rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and a left lateral flank incision was made. The left kidney was exposed, and the left renal artery and vein were occluded for 30 min with a nontraumatic clamp. At the end of the ischemic period, the clamp was removed to allow reperfusion (I/R), the incision was closed, and animals were returned to their cages to recover. In sham-operated controls, the rats were treated identically except that the kidneys were exposed but not clamped.

**Measurement of Biochemical Parameters.** Plasma creatinine and urea nitrogen were measured using commercially available kits (Sigma-Aldrich). Urinary sodium excretion (UNaV) was measured by flame photometry (Jenway PP7, Beckman Coulter, Inc., Fullerton, CA). To measure creatinine and urea nitrogen, blood (−600 μl) was collected from the tail vein, mixed with 3.2% sodium citrate (1:10 v/v), and centrifuged immediately. Plasma samples were frozen at −20°C until use. To measure UNaV, rats were put in metabolic cages, and urine production was collected over 12 and 24 h in rats that underwent renal ischemia for 30 min. UNaV values were corrected for 24 h. Urine production was not made at 3 h, because the rats were mainly under anesthesia during this period.

**Preparation of Renal Microsomes.** Microsomes were prepared as we described previously (Oyekan et al., 1999). Briefly, ice-cold normal saline (0.9% NaCl) was injected through the aorta to flush the kidneys of anesthetized (sodium pentobarbital, 60 mg/kg, i.p.) adult male Sprague-Dawley rats. Kidneys were subjected to a 30-min period of ischemia followed by reperfusion for 3, 12, or 24 h. In some experiments, kidneys were removed from rats after treatment with clofibrate, 2% NaCl, or their respective vehicles. In all cases, kidneys were homogenized in 0.01 M Tris containing 0.25 M sucrose (pH 7.4), and microsomes were prepared by standard differential centrifugation technique. Briefly, homogenates were centrifuged at 1000g for 30 min, and the supernatant subsequently centrifuged at 10,000g for 15 min. Microsomes were obtained by centrifugation of the 10,000g supernatant at 100,000g for 60 min and resuspended in 0.1 mol/L
potassium phosphate buffer (pH 7.6). Microsomal protein concentration was determined by the Bradford method using a kit from Sigma-Aldrich, with bovine serum albumin as the standard.

**Microsomal AA Metabolism.** AA metabolism was evaluated as we described previously (Oyekan et al., 1999). Briefly, whole-kidney AA metabolism was measured in incubations with a total reaction volume of 1 ml containing microsomal protein (1.5 mg of protein), cold AA (7 μM), [14C]AA (0.2–0.4 μM; 13 μM), and indomethacin (10 μM) in the presence of NADPH (10 μM) for 30 min at 37°C. The reaction was terminated by addition of 500 μl of 5% acetic acid (pH 3–4). AA and its metabolites were extracted twice with ethyl acetate. After evaporation of the organic solvent layer under nitrogen, the dry residue was stored at −70°C until HPLC analysis.

**HPLC Analysis of AA Metabolites.** A reverse-phase HPLC system (Agilent Technologies, Waldbronn, Germany) and a Packard Radionics 500TR series scintillation analyzer with FLO-One software (Packard Bioscience, Meriden, CT) were used for the separation and quantification of AA metabolites. Metabolites were separated on a C18 5-μm column (250 × 4.5-mm) with a C-18 guard column and in-line filter (Alltech Associates Inc., Deerfield, IL). Epoxide activity is reported as the sum of epoxide and dihydroxyeicosatrienoic acid formation.

**Isolated Microvessel Preparation.** Preglomerular arterioles, interlobular and arcuate (intraluminal diameter (ID), 80–130 μm), were microdissected and mounted on glass micropipettes in a water-jacketed perfusion chamber as described previously (Hercule and Oyekan, 2000). The vessels were pressurized to 80 mm Hg and equilibrated for 45 to 60 min in oxygenated (95% O2/5% CO2) Krebs-Henseleit buffer (37°C, pH 7.2). Vascular diameters were measured 1 to 3 min after the extraluminal addition of an agonist to the bath with the use of a video system composed of a stereomicroscope (Olympus BX40; Olympus, Tokyo, Japan), charge-coupled digital television camera (model JE7826), and television monitor and video measuring system (video micrometer) model JV6000T (Javelin Systems).

**Immunodetection of CYP4A.** The immunodetection procedure was performed according to the protocol provided by Amersham Biosciences and as previously described (Oyekan et al., 1999). The method uses an anti-rat CYP4A primary antibody raised in sheep that binds specifically to the immobilized CYP4A1 isozyme. The isozyme localized to renal microsomes (as prepared above) was immobilized on nitrocellulose membranes. Electrophoretic separation (SDS-polyacrylamide gel electrophoresis) was done using a MiniPROTEAN II dual slab cell (Bio-Rad Laboratories Inc., Hercules, CA) followed by transfer to nitrocellulose membranes. Immunodetection consisted of using the primary antibody to CYP4A1 isozyme, a biotinylated secondary antibody (anti-sheep Ig), a streptavidin-horseradish peroxidase conjugate, and enhanced chemiluminescence Western blotting detection reagents. Autoradiographs were quantitated by densitometric scanning using SigmaScan software (SPSS Science, Chicago, IL).

**Protocol.** Renal microsomal metabolism of AA was determined in adult male Sprague-Dawley rats (n = 6–7) that had clamps placed on the left renal artery for 30 min followed by 3-, 12-, and 24-h reperfusion (I/R). Microsomes prepared from the right (nonclipped) kidney served as the control. In another set of rats (n = 5–12), pregglomerular vascular responses to ET-1 (0.1, 1, 3, and 10 ng/ml), AA (1, 3, and 10 μg/ml), or U46619 (1 and 10 ng/ml) were determined at 24 h post-I/R in microvessels harvested from clipped (left) and nonclipped (right) kidneys following treatment of rats with vehicle (control), clofibrate (250 mg/kg, i.p. single bolus injection), or 2% NaCl (w/v orally) ad libitum for 7 days. Vascular responses in microvessels harvested from nonclipped (right) kidneys served as controls.

**Statistical Analysis.** Renal microsomal data were presented as percentage of control values. Vascular responses were expressed as absolute changes in ID of the renal microvessel. All data are presented as mean ± S.E.M. and were analyzed using analysis of variance followed by the Newman-Keuls’s test when appropriate. In other cases, the Student’s t test for unpaired data was used to determine significant difference between control and treated groups. In all cases, P < 0.05 was regarded as significant.

**Results**

**Effects of Ischemia Reperfusion on Plasma Creatinine and Urea Nitrogen and Urinary Sodium Excretion.** Table 1 shows that plasma urea nitrogen was elevated as early as 3 h post-reperfusion in rats that were subjected to 30 min of renal ischemia. The levels of urea nitrogen were further increased at 12 and 24 h post-ischemia reperfusion to values that are 80 and 89% greater, respectively, than that in the respective sham-operated rats. Unlike plasma urea nitrogen, plasma creatinine levels were not significantly different at any of the time points compared with their respective sham-operated rats. Similarly, UNaV was unchanged at 12 h post-ischemia reperfusion but was 2.5-fold greater at 24 h post-ischemia reperfusion compared with the respective sham-operated rats.

**Effects of Ischemia Reperfusion on Renal Microsomal P450-AA Metabolism.** The specific activities of renal ω-hydroxylase and epoxygenase enzymes in vehicle-treated (control, n = 6–7) rats is 68.7 ± 7.3 and 1.7 ± 0.3 pmol/mg protein/30 min, respectively. Figure 2 shows that I/R induced by clipping the renal artery for 30 min reduced renal microsomal conversion of [14C]AA to HETE but not epoxides at the time points studied. Thus, at 3, 12, and 24 h post-I/R, 20-HETE formation in the clipped kidney was reduced to 34, 37, and 58%, respectively, of the enzyme activity in the nonclipped (contralateral) kidney. However, conversion of [14C]AA to epoxides was not significantly affected at any of the time points evaluated.

**Effects of Ischemia Reperfusion on CYP4A1 Protein Expression.** The autoradiograph in Fig. 3 shows that CYP4A1 protein (molecular weight, 51 kDa) is constitutively expressed in renal microsomes of the nonclipped kidney as demonstrated by Western blot analysis. In microsomes from clipped kidneys, expression of CYP4A1 protein decreased at 3, 12, and 24 h of reperfusion following ischemia. The decrease in expression was highest at 24 h, decreasing the blot density

### Table 1

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<th>Sham 3 h</th>
<th>Sham 12 h</th>
<th>Sham 24 h</th>
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<tr>
<td>Plasma urea nitrogen (mg/dl)</td>
<td>17.4 ± 1.0</td>
<td>24.5 ± 2.4*</td>
<td>14.5 ± 1.9</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>1.1 ± 0.4</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.2</td>
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<tr>
<td>UNaV (µmol/day)</td>
<td>N.D.</td>
<td>N.D.</td>
<td>98.2 ± 12.3</td>
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N.D., not determined.

* p < 0.05 versus sham.
from (44 ± 5) × 10^3 intensity units (nonclipped kidney) to (22 ± 3) × 10^3 intensity units in the clipped kidney (p < 0.05).

**Effects of Ischemia Reperfusion on Preglomerular Vascular Reactivity.** The vasoconstrictor effects of AA and ET-1 in sham-operated rats were not significantly different from those obtained in the nonclipped kidneys. For the data presented, data obtained from the nonclipped (contralateral) kidney represent control data. Figure 4a shows that AA (1–30 μg/ml) elicited a dose-dependent vasoconstriction that was not different in vessels from the clipped (n = 5) or nonclipped (n = 12) kidney. Similarly, vasoconstriction elicited by ET-1 (0.1–10 ng/ml) was not different between the vessels from the clipped and nonclipped kidney (Fig. 4b). Vasoconstriction elicited by U46619 at 1 and 10 μg/ml also was not affected (data not shown). Thus, U46619 at 1 and 10 μg/ml reduced the ID of the PGMV by 20 ± 7 and 48 ± 12 μm, respectively, in the nonclipped kidney, values that were not different from 28 ± 7 and 51 ± 10 μm, respectively, in the clipped kidney.

**Effects of Clofibrate on P450-AA Metabolism and Preglomerular Vasoconstriction Following Ischemia Reperfusion.** Compared with vehicle-treated (n = 4) rats, clofibrate did not affect the renal microsomal conversion of [14C]AA to 20-HETE or epoxides (Table 2). Similarly, conversion of [14C]AA 20-HETE was not different between the clipped and nonclipped kidney harvested from clofibrate-treated (n = 5) rats. However, compared with kidney vessels from vehicle-treated (n = 6) rats, clofibrate enhanced AA-induced vasoconstriction in the clipped (n = 6) kidney by 44 ± 5% (p < 0.05; Fig. 5a) and amplified the vasoconstriction elicited by ET-1 by 37 ± 4% (p < 0.05; Fig. 5c). Clofibrate enhancement of AA- and ET-1-induced vasoconstriction was observed only in clipped kidneys but not in the nonclipped kidney. For ET-1, the reductions in ID in vessels from clipped kidneys from clofibrate-treated rats were 1.5 to 2-fold greater than that obtained in vessels from vehicle-treated rats. However, in the nonclipped kidney, ET-1-induced reductions in ID were not different between vehicle- and clofibrate-treated rats. On the other hand, clofibrate was without effect on the vasoconstriction elicited by U46619 either in the control or the clipped kidney. Thus, U46619 at 1 and 10 μg/ml reduced the ID of the PGMV by 20 ± 7 and 27 ± 8 μm, respectively, in the control kidney, values that were not different from 10 ± 2 and 24 ± 7 μm, respectively, in the clipped kidney (data not shown).

**Effects of Salt Treatment on P450-AA Metabolism and Preglomerular Vasoconstriction Following Ischemia Reperfusion.** Two percent NaCl enhanced renal microsomal eoxypgenase activity in rats (Makita et al., 1994; Oyekan et al., 1999) and could potentially modulate vasoactivity. In renal microsomes harvested from rats (n = 6)
Effects of ARF on preglomerular vascular responses to AA (1–30 μg/ml) (a) and ET-1 (1–10 ng/ml) (b) in the pressurized PGMV. Vasoconstrictor responses were not different in vessels from the clipped (■) and nonclipped (contralateral) (▲) kidney. Data are presented as mean ± S.E.M of the absolute reduction in internal diameter.

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<tr>
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<th>20-HETE</th>
<th>Epoxides and Diols</th>
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<tr>
<td>Control</td>
<td>5.3 ± 1.0</td>
<td>3.2 ± 1.1</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>5.4 ± 1.0</td>
<td>5.1 ± 1.7</td>
</tr>
<tr>
<td>2% NaCl</td>
<td>5.2 ± 1.1</td>
<td>9.4 ± 1.7*</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>7.1 ± 1.5</td>
<td>8.8 ± 1.5</td>
</tr>
<tr>
<td>Nonclipped</td>
<td>8.2 ± 1.2</td>
<td>9.7 ± 0.9</td>
</tr>
<tr>
<td>Clipped</td>
<td>8.3 ± 1.7</td>
<td>12.4 ± 1.5</td>
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<tr>
<td>NaCl</td>
<td>9.1 ± 2.4</td>
<td>17.4 ± 1.6#</td>
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*p < 0.05 vs control, *p < 0.05 versus nonclipped.

Discussion

ARF is a syndrome defined by persistent preglomerular vasoconstriction, which may result from local vasoconstriction due to the release of several vasoconstrictors, including ET-1 (Ruschitzka et al., 1998; Shibouta et al., 1990). Oxidative stress resulting from reperfusion can activate phospholipase A₂ (Nakamura et al., 1991) and promote the formation of eicosanoids, including cysteinyl leukotrienes and the F₂α isoprostanes (Bomzon et al., 1997).

The present study provides evidence that ARF reduced renal microsomal metabolism of AA and the expression of CYP4A, the protein responsible for ω-hydroxylation of AA to 20-HETE. P450-dependent ω-hydroxylase activity and CYP4A protein expression were reduced following I/R as early as 3 h following reperfusion and remained further reduced at 24 h post-I/R. These observations are in agreement with those reported by Tamura et al. (1997) and Portilla et al. (2000) and are consistent with the fact that renal failure is associated with decreased drug metabolism (Leblon et al., 2001). Indeed, the 54% reduction in CYP4A enzyme activity in this study is of similar magnitude to that obtained after 45 min of ischemia and 24-h reperfusion in other studies (Gulati et al., 1993; Tamura et al., 1997; Portilla et al., 2000).

AA metabolism is important in the kidney for the generation of P450-derived metabolites that maintain renal vascular tone and ion transport (Imig, 2000). Indeed, the specific activity of the kidney is greater than that of the liver (Schwartzman et al., 1986), and changes in the levels of monoxygenase activity and P450 isoforms in the kidneys following I/R are faster than those in the liver (Tamura et al., 1997). We therefore evaluated the implications of the reduced renal P450-AA metabolism on renal vasomotor responses of the PGMV to AA after 24 h of reperfusion. AA and ET-1 elicited renal vasoconstrictor responses in PGMV that may be accounted for by 20-HETE production since it is the major eicosanoid produced from AA in the kidney, the levels of 20-HETE being greater than those of PGE₂ (McGiff and Quilley, 1999). In addition, we and others have demonstrated that P450 ω-hydroxylase AA metabolites contribute to the

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<td>2% NaCl</td>
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<td>Clofibrate</td>
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<td>Nonclipped</td>
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<td>NaCl</td>
<td>9.1 ± 2.4</td>
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*p < 0.05 vs control, *p < 0.05 versus nonclipped.
preglomerular vasoconstriction elicited by ET-1 (Hercule and Oyekan, 2000; Imig et al., 2000). In the present study, neither AA- nor ET-1-induced vasoconstriction was affected by ARF despite a 54% reduction in renal microsomal conversion of [14C]AA to 20-HETE. The lack of corroboration between vascular reactivity and renal microsomal metabolic data is surprising and suggests that extrapolation of microsomal to vascular functional data should be treated circumspectly. From data obtained in these studies, we speculate that the diminution in \( \omega \)-hydroxylase activity in ARF affects the availability of either from endogenous pool or exogenous source, such that there is a substrate shunt to other competing renal oxygenases yielding products with opposing biological effects on the vasculature. This is supported by the fact that P450-dependent epoxygenation of AA yields dilator epoxides, whereas cyclooxygenase metabolism of AA generates constrictor prostanoids (e.g., PGH₂, PGF₂α, or TXA₂) or dilator prostanoids (e.g., PGI₂ and PGE₂) (Imig, 2000). This hypothesis was tested by treating with clofibrate on the premise that the increase in \( \omega \)-hydroxylase activity and a predominant 20-HETE production will override the effect of any other eicosanoid that can be generated by other oxygenases from AA. Clofibrate and other hypolipidemic agents have been demonstrated to enhance CYP4A expression and \( \omega \)-hydroxylase activity (Lenart et al., 1998) and reverse the deleterious effects of I/R in the rat (Portilla et al., 2000). The selective enhancement by clofibrate of the vasoconstrictor actions of AA and ET-1 in vessels from clipped kidneys, although consistent with this hypothesis, was not supported by the microsomal P450-dependent AA metabolic data. In addition, the lack of effect of clofibrate, despite its reported effect as an inducer of soluble epoxide hydrolase (Lundgren and DePierre, 1989), rules out the possibility that the enhancing effect of clofibrate on vascular responses to AA and ET-1 is related to a P450-AA mechanism. It therefore appears that a distinct mechanism that is yet to be identified may be involved in the clofibrate effect, producing a permissive effect that enhances vasoconstrictor responses to AA and ET-1 but not U46619. However, the fact that the population of vascular smooth muscle cells in microsomal preparations does not adequately reflect the overall contribution to P450-AA metabolism in both tissues militates against making a correlational inference between both tissues. Apart from \( \omega \)-hydroxylase metabolites, epoxides constitute the other major class of P450-dependent eicosanoids that may impact vasomotor responses in the kidney. However, unlike 20-HETE, epoxides generally elicit vasodilation. Indeed, an epoxygenase- and endothelium-derived hyperpolarizing factor appears to mediate renal dilator responses in the

![Figure 5](https://api.jpet.aspetjournals.org/doi/10.1124/jpet.170.017082)
kidney (Fulton et al., 1992). Studies from our laboratory and others have reported increased epoxygenase activity following salt treatment (Makita et al., 1994; Oyekan et al., 1999). In this study, 2% NaCl produced a 3-fold increase in epoxygenase activity compared with untreated rats and a modest increase in epoxygenase activity in the clipped compared with the nonclipped kidney. However, salt treatment did not affect 20-HETE synthesis. This is at variance with our previous study, in which we reported reduced renal cortical production of 20-HETE (Oyekan et al., 1999). The reason for this is not clear but is not due to strain difference, since we used the same strain in both studies. Based on salt-induced increase in epoxygenase activity, we speculate that elevated levels of dilator epoxides in rats treated with 2% NaCl should diminish the capacity of AA and ET-1 to produce preglomerular vasoconstriction. This was indeed the case, as salt loading attenuated AA-induced vasoconstriction in vessels from the clipped but not the nonclipped kidney. The diminished vasoconstriction by AA is consistent with enhanced conversion of [14C]AA to dilator epoxides. The lack of effect in the nonclipped kidney following salt loading is akin to that obtained in clofibrate-treated rats and causes us to further question the validity of extrapolating renal microsomal data to vascular functional studies.

Unlike with AA, salt loading enhanced ET-1 vasoconstriction in the clipped kidney, and as usual, salt loading had no effect in the nonclipped kidney. It is generally known that salt loading is accompanied by enhanced vascular reactivity to constrictors, including ET-1. The enhanced vasoconstriction to ET-1 in the present study is consistent with studies that demonstrated increases in ETA-mediated increase in blood pressure during high salt intake (Pollock and Pollock, 2001). In the study by Giardina et al. (2001), the enhanced vascular reactivity to ET-1 was observed despite demonstrable ETB-mediated increase in NO production, which was suggested to protect against excessive vasoconstriction and increased blood pressure during high salt intake. When considered with the enhanced epoxygenase activity following salt loading, these data suggest that the increased ET-1 production in the clipped kidney sensitizes the renal vasculature to ET-1, producing an effect that outweighs the vaso-dilation consequent to enhanced production of epoxides. With

Fig. 6. Effect of treatment with 2% NaCl on AA- and ET-1-induced vasoconstriction in vessels from the clipped (a and c) or nonclipped kidney (b and d). Salt treatment blunted AA-induced vasoconstriction but enhanced ET-1-induced vasoconstriction. Salt treatment had no effect on vasoconstriction produced by AA or ET-1 in the nonclipped kidney. Data are represented as mean ± S.E.M of the absolute reduction in ID. *, p < 0.05 versus vehicle (n = 4–9). ■, vehicle; ▲, 2% NaCl.
the nonclipped kidney, it appears that both effects match each other, hence there was no difference in ET-1 effect.

In conclusion, data from the present study suggest that P450 metabolites of AA contribute to the pathophysiology of ischemia reperfusion. The diminution of α-hydroxylase activity and CYP4A1 protein expression in ischemia reperfusion may serve to protect against the reduction in blood flow and impairment of renal function. However, these experiments do not support extrapolating microsomal to vascular functional data.

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References


