Induction of Renal Arachidonate Cytochrome P-450 Epoxygenase After Uninephrectomy: Counterregulation of Hyperfiltration


K. Takahashi, R.C. Harris, J.H. Capdevila, A. Karara, K. Makita, H.R. Jacobson, K.A. Munger, K.F. Badr, Department of Medicine, Vanderbilt University Medical School, Nashville, TN
J.H. Capdevila, A. Karara, Department of Biochemistry, Vanderbilt University Medical School, Nashville, TN

ABSTRACT

After unilateral nephrectomy (UNx) in the rat, cytochrome P-450 (cP-450)-linked arachidonate enzymatic activity was markedly and specifically induced in microsomal fractions from the remaining kidney. The enzymatic activity reached 200% at 1 wk and 285% at 2 wk post-UNx as compared with non-UNx controls. Mean baseline values for GFR and RPF rate in the remaining kidney 2 wk after UNx were 1.56 ± 0.10 and 6.47 ± 0.35 mL/min, respectively. In these rats, the administration of ketoconazole, a cP-450 inhibitor, led to 75% inhibition of renal cP-450 arachidonate metabolism and was associated with acute augmentations in both GFR and RPF to 1.82 ± 0.18 (P < 0.05 versus baseline) and 7.54 ± 0.37 mL/min (P < 0.05 versus baseline), respectively. Because vasoconstrictor arachidonate epoxygenase products are endogenously generated in the rat kidney, these findings suggest that the stimulation of renal cP-450-mediated oxygenation of arachidonic acid may subserve an important counterregulatory function in mitigating the renal hyperperfusion and hyperfiltration that follow reductions in renal mass.

Key Words: RBF, GFR, ketoconazole, glomerulosclerosis

We have reported the transformation of arachidonic acid (AA) to epoxyeicosatrienoic acids (EET) and 19-OH-AA and 20-OH-AA derivatives by cytochrome P-450 (cP-450)-linked epoxygenase and monoxygenase enzyme systems in the rat kidney (1). Biochemical characterization of the metabolites resulting from the incubation of rat renal cortical and medullary microsomal fractions in the presence of NADPH revealed epoxygenase and monoxygenase activities in the cortex and predominant monoxygenase activity in the medulla. Importantly, endogenous generation of EET was demonstrated by mass spectrometric analysis of rat renal extracts. More recently, by a recently developed method for EET chiral resolution (2), the enantiomeric composition of endogenous EET present in rat kidney has been reported (3). Furthermore, intrarenal arterial administration of 5,6-EET and 8(S), 9(R)-EET was associated with dose-dependent falls in the GFR and RPF rate, whereas 20-OH-AA elicited natriuretic responses without affecting renal hemodynamics (1,3). These data, as well as a study demonstrating regulation of rabbit renal epoxygenase/monoxygenase activity during alterations in sodium balance (4), suggested a potential role for this pathway of AA metabolism in the regulation of renal hemodynamics and excretory functions (5,6). In view of the established induction of NADPH-linked metabolic activity in the remaining kidney after uninephrectomy (UNx) (7) and the altered rates of glomerular perfusion, filtration, and solute excretion in this condition, we sought to determine whether cP-450–linked arachidonate metabolites played a role in mediating these alterations.

METHODS

Experimental Model

UNx was performed as follows: adult male Munich-Wister rats (220 to 260 g) were lightly anesthetized (Brevital, 100 mg/kg; Eli Lilly & Co., Indianapolis, IN). The right kidney was exposed through a dorsal right midline incision and was removed after its feeding vessels were ligated. The incision was closed with 3-0 silk. Removed kidneys were subjected to biochemical analysis as controls.

Experimental Groups

Experiments were performed on five groups of rats as follows.
**Group 1 (control, N = 8)**: In this group, a dorsal incision was made and closed without UNx (sham operation). Whole-kidney clearance studies were performed at specified times after sham surgery. Measurements were performed during baseline conditions and again after the administration of ketoconazole (0.5 mg/kg), a CYP450 inhibitor (Janssen Biotech N.V., Brussels, Belgium) (8,9). Because time-dependent changes in the values of each parameter were minimal in Group 1 (i.e., the values in GFR, RPF, and other parameters obtained 1 wk post-sham operation were similar to those obtained 2 wk post-sham operation and also to those obtained from non-operated normal rats), animals on which experiments were conducted at various times were considered as one group, which served as a control to both Groups 2A and 3A.

**Group 2A (N = 9)**: In this group, whole-kidney clearance studies were performed 1 wk after UNx. Measurements were performed in the same protocol as that for Group 1. Kidneys were removed immediately after clearance studies, and the activity of the microsomal CYP450 enzyme system was assayed.

**Group 2B (N = 10)**: In these animals, kidneys were removed 1 wk after UNx, and the activity of the microsomal CYP450-enzyme system was assayed. Clearance studies were not performed.

**Group 3A (N = 9)**: In this group, whole-kidney clearance studies were performed 2 wk after UNx. Measurements were performed in the same protocol as that for Groups 1 and 2A.

**Group 3B (N = 7)**: In these animals, kidneys were removed 2 wk after UNx, and the activities of the microsomal CYP450-enzyme system, renal cortical alkaline phosphatase, and Na⁺-K⁺-ATPase were assayed. Clearance studies were not performed.

**Clearance Studies**

Clearance studies were performed under Inactin (Andrew Lockwood and Associates, Sturtevant, WI) anesthesia (100 mg/kg, ip) according to protocols described previously (1). Inulin and para-aminohippurate clearances were used to measure GFR and RPF, respectively. Measurements were performed during baseline conditions and, in most cases, were repeated after ketoconazole administration as described for the experimental groups. Urinary sodium and potassium concentrations were measured on an emission flamephotometer (Instrumentation Laboratories Inc., Lexington, MA).

**Microsomal Metabolism of Arachidonic Acid**

Rat kidneys were minced and homogenized in 10 mM Tris-chloride (pH 7.4) and 0.25 M sucrose. Microsomal fractions were isolated from combined cortex and medulla homogenates by differential centrifugation as previously described (10). The microsomal, NADPH-dependent metabolism of AA was studied by incubating, at 30°C, the microsomal fractions isolated from kidney with [1-14C]sodium arachidonate (1 to 2 μCi/mmol, 100 μM final concentration) in the presence of NADPH (1 mM, final concentration) and an NADPH-regenerating system, exactly as described (11). Samples of the reaction mixtures were withdrawn at several time points, and the organic soluble products were extracted into ethyl acetate and analyzed by reverse-phase HPLC as described previously (11). Quantifications were done by liquid scintillation.

**Renal Cortical Alkaline Phosphatase and Na⁺-K⁺-ATPase Activity**

Renal cortical activities of these two marker enzymes were measured in cortical homogenates from control kidneys in Group 1 and those from kidneys 2 wk after contralateral UNx in Group 3B. The brush border membrane marker enzyme, alkaline phosphatase, was assayed with p-nitrophenyl phosphate (pNPP) as substrate (Sigma Chemical Co., St. Louis, MO), as described previously (12). Na⁺-K⁺-ATPase was measured as ouabain-sensitive K⁺ phosphatase (13).

**Statistical Analyses**

Within-group comparisons were carried out by paired t test, and intergroup multiple comparisons were made with one-way analysis of variance followed by the Newman–Keuls test. A value of P < 0.05 was required for statistical significance. All values are reported as mean ± SE.

**RESULTS**

**Physiologic Measurements**

UNx-Induced Changes in Systemic Parameters. Mean values for baseline mean arterial pressure (MAP), hematocrit (Hct), GFR, RPF, filtration fraction, urine flow rate, urinary sodium (UNa⁺V) and potassium excretion rate (UK⁺V) and kidney weight (KW, [per kidney]) in experimental groups are presented in Table 1. UNx rats in Groups 2A had significantly increased baseline GFR, RPF and KW as compared with control values in Group 1 animals. GFR increased 64 and 65% in Groups 2A and 3A, respectively, and RPF increased 66 and 69% in Groups 2A and 3A, respectively. There was no significant between Groups 2A and 3A in GFR and RPF values. UNa⁺V, UK⁺V, and urine flow rate in Groups 2A and 3A were also significantly higher than those of Group 1 rats. In addition, the KW of Groups 2A and 3A rats were 66 and 80% higher, respectively, than those of
TABLE 1. Summary of baseline values for systemic and renal hemodynamics and excretory parameters in Groups 1, 2A, and 3A

<table>
<thead>
<tr>
<th></th>
<th>Hct (vol%)</th>
<th>MAP (mm Hg)</th>
<th>GFR (mL/min)</th>
<th>RPF (mL/min)</th>
<th>FF</th>
<th>V (µEq/min)</th>
<th>UNa⁺V (µEq/min)</th>
<th>UK⁺V (µEq/min)</th>
<th>KW (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>48 ± 1</td>
<td>104 ± 2</td>
<td>0.94 ± 0.12</td>
<td>3.83 ± 0.17</td>
<td>0.25 ± 0.02</td>
<td>0.012 ± 0.004</td>
<td>1.61 ± 0.21</td>
<td>1.26 ± 0.14</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>Group 2A</td>
<td>46 ± 1</td>
<td>120 ± 3</td>
<td>1.54 ± 0.16b</td>
<td>6.36 ± 0.23³</td>
<td>0.24 ± 0.02</td>
<td>0.027 ± 0.004</td>
<td>4.83 ± 0.40³</td>
<td>2.89 ± 0.27</td>
<td>1.51 ± 0.05³</td>
</tr>
<tr>
<td>Group 3A</td>
<td>46 ± 1</td>
<td>121 ± 8</td>
<td>1.56 ± 0.10b</td>
<td>6.47 ± 0.35³</td>
<td>0.25 ± 0.02</td>
<td>0.028 ± 0.006³</td>
<td>5.26 ± 0.37³</td>
<td>2.88 ± 0.36³</td>
<td>1.64 ± 0.15³</td>
</tr>
</tbody>
</table>

FF, filtration fraction; V, urine flow rate. Values are mean ± SE.

b P < 0.01 versus Group 1 controls.

Group 1 animals, whereas again no significant difference existed between this parameter of Group 2A and 3A rats.

Effects of cP-450 Inhibition on Physiologic Parameters. The changes in GFR and RPF after the inhibition of cP-450 enzyme systems by ketoconazole in Groups 1, 2A (1WK), and 3A (2WKS) are depicted in Figure 1. As seen, the treatment of rats in Group 1 had no effect on GFR (0.94 ± 0.12 to 0.92 ± 0.10 mL/min) or RPF (3.83 ± 0.17 to 3.81 ± 0.22 mL/min). Ketoconazole administration to UNx rats in Group 2A animals, however, increased GFR (1.54 ± 0.16 to 1.70 ± 0.20 mL/min) and RPF (6.36 ± 0.23 to 6.99 ± 0.44 mL/min), neither of which gained statistical significance, and in Group 3A, increased GFR (1.56 ± 0.10 to 1.82 ± 0.18 mL/min; P < 0.05) and RPF (6.47 ± 0.35 to 7.54 ± 0.37 mL/min; P < 0.05), both of which attained statistical significance. Filtration fraction was unchanged in both groups (0.24 ± 0.02 to 0.26 ± 0.02 in Group 2A, not significant [NS]; 0.25 ± 0.02 to 0.25 ± 0.02 in Group 3A, NS). Hct and MAP were also unchanged (Hct, 47 ± 1 to 46 ± 1 in Group 2A, NS; 46 ± 1 to 45 ± 1 in Group 3A, NS; MAP, 121 ± 3 to 118 ± 5 mm Hg in Group 2A, NS; 122 ± 8 to 123 ± 7 mm Hg in Group 3A, NS). Contrary to the effect on GFR and RPF, the inhibition of the cP-450–linked enzyme system had no effect on UNa⁺V and UK⁺V in control rats in Groups 1, as well as in UNx rats in Groups 2A and 3A. UNa⁺V changed from 1.61 ± 0.21 to 1.74 ± 0.43 µEq/min (NS) in Group 1; 4.83 ± 0.40 to 5.01 ± 0.51 µEq/min (NS) in Group 2A; and 5.26 ± 0.37 to 5.33 ± 0.44 µEq/min (NS) in Group 3A. UK⁺V changed from 1.45 ± 0.22 to 1.41 ± 0.10 µEq/min (NS) in Group 1; 2.89 ± 0.27 to 3.10 ± 0.37 µEq/min (NS) in Group 2A; 2.88 ± 0.36 to 2.68 ± 0.32 µEq/min (NS) in Group 3A.

Biochemical Parameters

The Renal cP-450–Linked Arachidonate Activities. The microsomal cP-450–linked arachidonate epoxygenase/monooxygenase activities in the remnant kidneys in experimental groups are depicted in Figure 2. The specific activity of the total cP-450/AA enzyme system in Group 2B (1WK) increased by 100% as compared with the control value in Group 1 (CON), (0.31 ± 0.01 in Group 2B versus 0.14 ± 0.03 nmol/min per mg of protein; P < 0.005) and in Group 3B (2WKS), the value (0.40 ± 0.04 nmol/min per mg of protein) was even higher than that of Group 2B (P < 0.025 versus Group 2B). The activities of cP-450/AA enzymes in Groups 2A and 3A were suppressed to 0.07 and 0.10 nmol/min per
mg of protein, respectively, with the treatment of ketoconazole, which corresponded to 75% inhibition in Groups 2B and 3B.

Renal Alkaline Phosphatase and Na⁺-K⁺-ATPase Activities. In sharp contrast to cP-450 activity, the specific activities of renal alkaline phosphatase (0.445 ± 0.020 in Group 1 [CON] versus 0.441 ± 0.044 μmol of p-NPP/mg of protein per min in Group 3B [2WKS]; NS) and Na⁺-K⁺-ATPase (0.0017 ± 0.0001 in Group 1 [CON] versus 0.0015 ± 0.0002 μmol of p-NPP/mg of protein per min in Group 3B [2WKS]; NS) were unchanged (Figure 3).

DISCUSSION

The remaining kidneys of UNx animals in this study were characterized by increased GFR, RPF, and urinary sodium and potassium excretions, which is in accordance with previous studies (14–16). Importantly, these altered renal functions were associated with dramatic and time-dependent increases in the synthetic activity of total cP-450/AA in remnant kidneys. The increased activity of cP-450/AA was not a nonspecific consequence of the increased renal mass, because the specific activities of renal alkaline phosphatase and Na⁺-K⁺-ATPase were unchanged. This observation is consistent with the fact that the activity of the hexose monophosphate shunt pathway, which is capable of furnishing the cell with NADPH, an essential cofactor for the cP-450 system, was enhanced in the hypertrophying kidney after contralateral UNx in the rat (7).

In addition to the demonstration of an increased activity of the cP-450/AA enzyme system in this model, the inhibition of this system in the remnant kidney of rats 2 wk after UNx resulted in significant augmentation of vasodilation, indicated as the further increases in GFR and RPF. Because EET, major renal vasoactive AA metabolites through cP-450, possess renal constrictor effects in vivo (1,3), these observations suggested that the enhanced production of EET may subserve a counterregulatory function in the face of the hyperfiltration and hyperperfusion observed in this model.

In addition, in view of the fact that the inhibition of significantly increased cP-450 activity at 1 wk after UNx was not accompanied by a significant change in renal hemodynamics, as observed at the later stage, it is unlikely that cP-450/AA products play important roles in mediating the induction of the functional abnormalities. Alternatively, significant effects of ketoconazole on renal hemodynamics only at the later stage might suggest the possible action of ketoconazole through a non–cP-450 enzyme system, which could be induced at a later stage. However, in view of the high specificity of ketoconazole for cP-450, it is more likely that the importance of the role of cP-450 metabolites in this model increases over the period after UNx. The mechanism underlying this putative adaptation, however, remains to be addressed.

Interestingly, consistent with our data, the augmentation of vasorelaxation by cP-450 inhibition was previously reported in lamb ductus arteriosus (17), implicating a cP-450–linked mechanism in sustaining the vascular tone in this model.

As indicated earlier by Jacobson et al. (4) and reviewed recently by others (5,6), sodium balance is an important factor in the regulation of the renal cP-450/AA enzyme system. Hence, in view of the fact that each nephron in the remnant kidneys of UNx animals must increase the excretion of water and ions (15) and of our previous data demonstrating natriuretic action of 20-OH-AA (1), it is reasonable to postulate functional roles for the increased production of cP-450–linked AA metabolites in regulating sodium handling in the remnant kidney. In our experiment, however, UNaV from remnant kidneys was unchanged by the treatment with ketoconazole either at 1 or 2 wk after UNx. It remains possible, though, for the augmentation of GFR and RPF by the inhibition of the cP-450 system to have masked the decrease of UNaV, which might have occurred simultaneously. Another potential reason might relate to the evidence that cP-450/AA metabolites mediate the effects of other control systems of salt and water handling such as vasopressin (18,19).

In summary, our experiments provide evidence to suggest a role for the cP-450–linked AA metabolites in mediating the change in renal vascular and excretory functions of UNx rats. First, the kidneys in UNx rats were capable of generating cP-450/AA metabolites analyzed by HPLC in higher amounts than those of normal rats. Second, the inhibition of cP-450/AA in the remnant kidneys of rats at 2 wk after UNx resulted in significant augmentation of vasodilation. These data, together with our previous reports (1,3), suggested strongly that cP-450/AA metabolites may play important roles in mediating the renal hemodynamic responses to renal mass reduction.
REFERENCES


