Increased renal vascular reactivity to ANG II after unilateral nephrectomy in the rat involves 20-HETE

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Increased renal vascular reactivity to ANG II after unilateral nephrectomy in the rat involves 20-HETE. Am J Physiol Regul Integr Comp Physiol 291: R977–R986, 2006. First published May 4, 2006; doi:10.1152/ajpregu.00401.2005—This study examined the role of intrarenal ANG II in the renal vascular reactivity changes occurring in the remaining kidney undergoing adaptation following contralateral nephrectomy. Renal blood flow responses to intrarenal injections of ANG II (0.25 to 5 ng) were measured in anesthetized euvolemic male Wistar rats 1, 4, 12, and 24 wk after uninephrectomy (UNX) or sham procedure (SHAM). At week 4, renal vasocostriction induced by 2 ng ANG II was greater in UNX (69 ± 5%) than in SHAM rats (50 ± 3%; P = 0.01). This response was inhibited, by 50 and 66%, and by 20 and 25%, in SHAM and UNX rats, after combined injections of ANG II and losartan, or PD-123319 (P < 0.05), respectively. Characteristics of ANG II receptor binding in isolated pregglomerular resistance vessels were similar in the two groups. After prostanoid inhibition with indomethacin, renal vasocostriction was enhanced by 42 ± 8% (P < 0.05), only in SHAM rats, whereas after 20-HETE inhibition with HET0016, it was reduced by 53 ± 16% (P < 0.05), only in UNX rats. These differences vanished after concomitant prostanoid and 20-HETE inhibition in the two groups. After UNX, renal cortical protein expression of cytochrome P-450 2c23 isoform (CYP2c23) and cyclooxygenase-1 (COX-1) was unaltered, but it was decreased for CYP4a and increased for COX-2. In conclusion, renal vascular reactivity to ANG II was significantly increased in the postuninephrectomy adapted kidney, independently of protein expression, but presumably involving interactions between 20-HETE and COX in the renal microvasculature and changes in the paracrine activity of ANG II and 20-HETE.

adapted kidney; renal blood flow; eicosanoids; indomethacin; HET0016

DURING THE LAST DECADE, ANG II has emerged as an important factor that produces hemodynamic, as well as nonhemodynamic, effects in the kidney. Indeed, ANG II affects the renal microvasculature, glomerular filtration rate (GFR), renal tubular salt and water reabsorption, and influences growth processes in a wide variety of renal cells (32, 43). In addition, ANG II stimulates the autocrine production of growth factors and can induce the expression of protooncogenes, which are activated in renal hypertrophic responses (14). Moreover, changes in the components of the renin-angiotensin system occur during the early phases of compensatory growth of the kidney (30, 39). Taken together, these findings suggest that the intrarenal renin-angiotensin system, which can operate indepen-dently of the systemic one (38), may be involved in the structural and functional compensatory adaptations occurring after a reduction in renal mass. Considering this possibility, the hypothesis was set forth that the intrarenal activity of ANG II could so interfere, either directly or indirectly, with the vasomotor tone of the renal microcirculation. In this regard, renal vascular reactivity to ANG II injected intrarenally was found to be exaggerated only in spontaneously hypertensive rats (9, 10, 23, 35, 40) and hypertensive ren-2 transgenic rats (20) or in rats submitted to an aortic coarctation or to a high-sodium diet (13, 36). On the other hand, it was attenuated in AT1A receptor null mice (37) and unchanged during acute renal failure in endotoxemic mice (3).

The aim of our study was to assess in vivo the renal vascular reactivity to ANG II in the adapted kidney at different time periods following unilateral nephrectomy (1, 4, 12, and 24 wk postnephrectomy). These time periods were selected because they corresponded to a continued enlargement of the adapted remaining kidney. Varying doses of ANG II were injected as a bolus into the renal artery (6) of anesthetized euvolemic male rats previously submitted to a sham-operated (SHAM group) or uninephrectomy procedure. The effect on renal blood flow (RBF), as well as the contribution of the angiotensin receptors (AT1 or AT2), was determined. The eventual interaction between ANG II and other vasoactive factors, such as eicosa-noids, was also studied in the adapted kidney, as interactions between ANG II and vasodilatory prostaglandins are known to influence renal hemodynamics (18). PGE2 and PGI2 can effectively buffer the renal vasoconstriction induced by an intrarenal injection of ANG II by attenuation of cytosolic calcium increases, as subsequently shown in pregglomerular vascular smooth muscle cells (7, 8, 33). Moreover, ANG II can increase the synthesis of 20-HETE in the kidney, as well as in the peripheral vasculature, whereas 20-HETE contributes to the pressor effects of ANG II (1, 12, 34). The impact of these potential interactions on the renal circulation was, therefore, also investigated in the adapted kidney using a similar experimental approach. To do so, the activity of cyclooxygenase (COX) was inhibited with indomethacin (25), whereas the synthesis of 20-HETE was inhibited with HET0016, a recently developed and highly specific inhibitor of 20-HETE synthesis (22, 31). Finally, the protein expression of cyclooxygenases (COX-1 and COX-2) and of cytochrome P-450 arachidonic acid pathways (CYP4a and CYP2c23) was studied in the postnephrectomy adapted kidney.

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METHODS

Animal preparation. This study conformed to the guiding principles of the American Physiological Society and was approved by the Comité d’Ethique Médicale of the Université de Mons-Hainaut. Experiments were performed on male Wistar rats (from Leuven, Belgium or Iffa Credo, L’Arbrésole, France, bred in our animal facility) maintained on a standard rat diet and with free access to tap water. Nephrectomy was undertaken when the rats weighed ~220 g. They were anesthetized with Nembutal (60 mg/kg body wt ip, Sanofi, Belgium) and submitted to a right flank laparotomy. The right kidney was rapidly removed after tying off the vessels and ureter. A sham procedure, that is, manipulation of the right renal pedicle, was undertaken in other rats. Some animals were not submitted to anesthesia or surgery and served as a further control. No marked functional differences were observed between these intact rats and those submitted to a sham procedure. Results were therefore combined.

Renal vascular reactivity was studied 1, 4, 12, or 24 wk after the unilateral nephrectomy (UNX) or SHAM. At each of these time periods, the rats were deprived of food, but not of water, the night before the experiment. Anesthesia was induced with Inactin (100 mg/kg body wt ip; Research Biochemicals International, Natick, MA). The animals were placed on a heated table to maintain rectal temperature between 37 and 38°C. The right femoral artery was catheterized to obtain blood samples and to monitor blood pressure (HSE Type 302; Hugo Sachs Elektronik, March, Germany). To avoid fluid shifts during further surgery, a 0.85% saline solution containing 2.5% albumin was infused immediately into the right femoral vein, as previously described in detail (25). After tracheostomy, the right jugular vein was cannulated for subsequent infusions. Midline and subcostal incisions were used to expose the left kidney, as previously described (24). The left renal artery was then carefully dissected from surrounding tissues. The left ureter or both ureters were cannulated for urine collection. A tapered and curved polyethylene (PE)-10 catheter was introduced into the left iliac artery and advanced through the abdominal aorta until its tip was positioned into the left renal artery. Lissamine green was then injected intrarrenally as a bolus to check the position of the catheter, as well as the uniform distribution of the dye on the entire surface of the kidney, as previously described (26). This test was repeated at the end of the experiment. The experiment was discarded when the lissamine green test failed. Throughout the experiment, isotonic saline was infused at a rate of 5 μl/min into the renal artery, except during the bolus injections of 10 μl of different test solutions that are described below. The infusion rate was then increased to 120 μl/min for 1 min, so that the bolus reached the kidney within 8 s (6). After completion of surgery, following a suitable prime, a 0.85% NaCl solution containing 3% inulin (Laevosan, Innsbruck, Austria) was infused at a rate of 50 μl/min to measure GFR. RBF was continuously measured using an electromagnetic flow transducer (2- or 2.5-mm circumference), which was fitted around the left renal artery and connected to a flowmeter (Carolina Medical Electronics, King, NC, or MDL 1401 compact, Skalar Medical, Delft, The Netherlands) and a recorder, as previously described in detail (25).

At the end of the experiment, the rats were killed with a lethal dose of Nembutal injected intravenously. Then, the kidneys were rapidly removed, decapsulated, blotted dry, and weighed.

Test drugs. Rat ANG II was synthesized by applying the solid-phase method of Merrifield (29) using a Beckman peptide synthesizer (model 990B), as previously described in detail (4). No differences were found with commercially available ANG II (Sigma, St. Louis, MO). Varying amounts of ANG II, 0.25, 0.5, 0.75, 1, 2, 3, 4 or 5 ng, were added to 10 μl of 0.85% NaCl solution, a fixed volume for all solutions injected into the renal artery. A solution containing 2 ng ANG II was considered as a reference since it reduced RBF by ~50% (6). Norepinephrine (NE), 40 or 80 ng, (Sigma, St. Louis, MO), was dissolved in a similar solution (20). Losartan, 1,000 ng (Merck, Rahway, NJ), was mixed to a solution containing 2 ng ANG II (9) or 80 ng NE. Varying doses of losartan, ranging from 1,000 ng to 100 μg, were added to a solution containing a dose of ANG II, varying from 3 to 5 ng, which induces a maximal renal vasconstrictor effect. PD-123319, 10 μg (Sigma, St. Louis, MO), was mixed to a solution containing 2 ng ANG II (9) or 80 ng NE. Losartan, 1,000 ng, and PD123319, 10 μg, were also added concomitantly to a solution containing 2 ng ANG II or 80 ng NE. Indomethacin sodium trihydrate, 3 or 5 mg/kg body wt (Merck, Rahway, NJ), was dissolved in a 0.85% NaCl solution. HET0016, 5 or 10 mg/kg body wt (Taisho Pharmaceutical, Tokyo, Japan), was dissolved in a 0.85% NaCl solution containing 10% lecithin. These drugs were injected intravenously, alone or coadministered, as previously described (5, 25).

Experimental protocol and groups. After 60- to 90-min equilibration, baseline measurements of mean arterial pressure (MAP) and RBF, as well as of renal function, were carried out during two control periods of 15 min duration each. Then, in each experiment, 10 μl of 0.85% NaCl solution, hereinafter referred to as vehicle solution, was injected intrarenally as a bolus to test the basal renal vascular reactivity. Thereafter, the different test solutions were injected as a bolus into the renal artery with a time interval of 15 min between each injection.

The following experimental groups were considered in rats that had undergone a unilateral nephrectomy or a sham procedure, 1, 4, 12, or 24 wk previously, as well as in some intact rats.

Group 1 included experiments with intrarenal injections of solutions containing varying doses of ANG II to evaluate their effects, as well as to test their reproducibility, on RBF.

Group 2 included experiments with intrarenal injections of solutions containing 1) 2 ng ANG II and losartan, and/or PD-123319, to determine the type of ANG II receptor involved; or 2) 3 to 5 ng of ANG II, representing a maximal renal vasconstrictor dose, and varying doses of losartan to establish a dose-response relationship for losartan.

Group 3 included experiments with intrarenal injections of solutions containing 1) varying doses of ANG II administered 1 h after COX inhibition with indomethacin to assess the eventual involvement of prostanooids in the ANG II-induced renal vasoconstriction, or 2) 2 ng ANG II administered repeatedly before (two injections) and 1 h after (5 injections) treatment with indomethacin, or its solvent, to further ascertain the effect of COX inhibition and to determine the reproducibility of the RBF response.

Group 4 included experiments with intrarenal injections of a solution containing 2 ng of ANG II administered repeatedly before (2 injections) and 1 h after (5 injections): 1) treatment with HET0016 to assess the interaction between ANG II and 20-HETE, as well as to ensure the reproducibility of the RBF response; or 2) coadministration of indomethacin and HET0016 to determine the effect of a simultaneous inhibition of cyclooxygenase and 20-HETE synthesis on the RBF responses to ANG II.

Group 5 included experiments with intrarenal injections of solutions containing 1) 40 or 80 ng NE, 2) 80 ng NE mixed with 1,000 ng losartan or 10 μg PD 123319, 3) 80 ng NE injected repeatedly before (two injections) and 1 h after (five injections) treatment with indomethacin or HET0016, or 4) 80 ng NE injected before and 2 h after coadministration of indomethacin and HET0016. These different experimental groups were undertaken to determine by comparison the specificity of the vascular effects of ANG II.

Group 6 included experiments to determine COX and CYP protein expression in intact and postnephrectomy adapted kidneys of rats prepared surgically, as described above.

Rats that underwent an uninephrectomy or a sham procedure are hereinafter referred to as UNX1, UNX4, UNX12, or UNX24, and SHAM1, SHAM4, SHAM12, or SHAM24. The numbering indicates the period of time, expressed in weeks, elapsed since the surgical procedure. Groups 3, 4, 5, and 6 included only UNX4 and SHAM4 rats.
Analytical methods. Urine was collected in preweighed cups, and the volume was estimated by gravimetry, assuming a water density of 1. To measure glomerular filtration rate, plasma and urine samples were analyzed for inulin by the anthrone method. In one series of experiments, urine was collected for the measurement of PGE_2 using an enzyme immunoassay according to the manufacturer’s instructions (Cayman Chemicals, Ann Arbor, MI). Hematocrit was determined on each blood sample.

Calculations. Renal hemodynamic data were obtained from the left kidney. Mean values of MAP and RBF were obtained by averaging hand-made measurements carried out every 5 min on the recordings, except during the intrarenal injections of test solutions. Renal vascular resistance was calculated as the ratio of MAP/RBF, where MAP refers to the pressure measured in the femoral artery. To determine the changes in renal blood flow and MAP occurring during the intrarenal injection of a test solution, the pressure monitor and the flowmeter were connected to a computer for real-time data acquisition. The data acquisition system (Lahtech notebook pro 10) consisted of an IBM PC-AT compatible computer and a 12-bit analog-to-digital converter (Analog Device). The outputs of the transducers monitoring arterial pressure and renal blood flow were sampled at 200 Hz for a period of 3 min, which was sufficient to allow renal blood flow to return to its baseline value after injection of the test solution. Each recording was started simultaneously with the increase in the intrarenal infusion rate to 120 µl/min. RBF values were normalized and expressed as a percent of baseline values. The magnitude of the inhibitory effect exerted by ANG II receptor antagonists on RBF responses induced by 2 ng ANG II was calculated as the ratio between the RBF response to ANG II injected alone or mixed with one of the receptor antagonists, or both. It was expressed in percent. After COX or 20-HETE inhibition, the change in the peak decrease of RBF induced by the intrarenal injection of 2 ng ANG II was evaluated by comparison with the peak renal vasoconstrictor response observed under baseline conditions. The latter was considered as no change and was expressed as 0%

Nonlinear least squares estimation was used to fit a smooth curve to each renal blood flow recording using Sigma Plot software (6). Kinetics of RBF responses to intrarenal bolus injections of test solutions were evaluated using the equation previously described (6). Area under the curve was calculated using GraphPad Prism software (San Diego, CA).

Receptor binding studies. The pregglomerular resistance vessels were isolated from kidneys of SHAM4 or UNX4 rats using the technique of iron oxide infusion (9). One SHAM rat was considered for an experiment while two UNX rats were pooled for each experiment. Modifications from the original method mainly concerned the separation of bound ligand from free ligand by filtration on GF/C filters 1.2 µm (Whatman, Maidstone, Kent, UK). After four washings (3 ml each time) with icid isotonic saline solution (NaCl 0.9%), filters were presoaked 60 min in a saline solution containing 0.3% polyethylenimine (Sigma, St. Louis, MO, USA). Nonspecific binding was evaluated in the presence of 500, 500, or 750 nM ANG II (Sigma, St. Louis, MO). Specific binding represented 90–95% of the total binding. Maximum specific binding (B_max) and dissociation constant (K_d) were estimated using Graph Pad prism software.

CYP and COX protein expression in kidney. The protein expression of CYP4A, CYP2C23, COX-1 and COX-2 in the kidney cortex was determined at 4 wk. Kidney cortex was processed as previously described (19). Samples were separated by electrophoresis on a 10% Tris-glycine gel, and proteins were transferred electrophoretically to a nitrocellulose membrane. The primary antibodies used were goat anti-rat CYP4A (1:2,000, Gentest), rabbit anti-rat CYP2C23 (1:5,000, Dr. Capdevila, Vanderbilt University), goat anti-mouse COX-1 (1:100, Santa Cruz) and rabbit anti-mouse COX-2 (1:1,000, Cayman Chemicals). The blots were then washed in PBS-0.1% Tween-20 and incubated with the secondary antibody conjugated to horseradish peroxidase for 1 h at room temperature and washed. Bands were detected, band intensity was measured densitometrically, and the values were factored for β-actin.

Statistics. One-way ANOVA was applied for multiple intergroup comparisons, and one-way ANOVA for repeated measurements (ANOVA RM) was applied for multiple intragroup comparisons, followed by a post hoc t-test (41). Two-way ANOVA was used to compare baseline variables between SHAM and UNX rats allowing for effects of time. Two-way ANOVA for repeated measurements was used to compare maximal variables between SHAM and UNX rats for allowing of different animal groups during injection of 2 ng ANG II. Two-way analysis was followed by a Newman-Keuls test for pairwise comparison. Differences between UNX rats and their corresponding SHAM were tested with the unpaired t-test. The paired t-test was used for a single comparison within a group. The regression equations were written as y = ax + b and were evaluated with the correlation coefficient (r). Differences between slopes were determined by the F test. All results are presented as means ± SE. P < 0.05 was considered to be statistically significant.

RESULTS

Time-dependent compensatory effect. As shown in Table 1, at each period of time, body weight was similar in SHAM and UNX rats, and increased progressively and similarly with age. The left kidney weight was always significantly higher in UNX than in corresponding SHAM rats. Hematocrit and MAP did not differ significantly between groups and remained stable until the end of the experiment, but hematocrit was slightly lower in the younger animals. After uninephrectomy, compensation in renal blood flow and glomerular filtration rate was appropriate at each postnephrectomy period considered. Also, renal vascular resistance was similar in SHAM and UNX rats and did not vary with time.

Effect of varying doses of ANG II on renal vascular reactivity. As illustrated in Fig. 1A, RBF decreased rapidly and significantly after intrarenal injections of 2 ng ANG II, the reference dose, to a maximum averaging 50 ± 3 and 69 ± 5% (P < 0.01) in SHAM4 and UNX4 rats, respectively. This difference was not related to the weight of the experimental kidney, as shown in Fig. 1B. These observations were reproducible at 2-h interval. Note that RBF was not modified by the intrarenal bolus injection of a vehicle solution and that the temporal profile of the RBF response to ANG II was similar in the two groups. As illustrated in Fig. 2, differences in maximal RBF response between SHAM4 and UNX4 rats were also significant after intrarenal injections of lower (from 0.5 to 1 ng) and higher (3 ng) doses of ANG II. At week 1, some significant differences in peak RBF decrease between SHAM and UNX rats were also induced after intrarenal injections of 0.25, 0.50, and 2 ng ANG II. In contrast, at week 12 and 24, no difference in maximal RBF decrease occurred between SHAM and UNX rats after intrarenal injections of the varying doses of ANG II tested. As shown in Table 2, the kinetic parameters characterizing the RBF responses induced by 2 ng ANG II neither differed between UNX rats nor from SHAM. Only the area under the curve was less in SHAM than in UNX rats but did not achieve statistical significance.

For the sake of brevity, further results will be focused on the 4-wk postnephrectomy period, unless otherwise stated.

Effect of AT_1 and/or AT_2 blockade on ANG II-induced renal vasoconstriction. In SHAM4, as well as UNX4 rats, the inhibitory effect of losartan on the renal vasoconstriction induced by 2 ng ANG II averaged 49 ± 6 (n = 6) and 66 ± 6%, [n = 6,
which averaged 25°F

P/H11005

R980 RENAL VASOREACTIVITY TO ANG II AFTER UNINEPHRECTOMY

P/H11021
greater (20 to 23°F, 71% NS), and 0.71 peak decrease in RBF averaged, respectively, between ANG II and eicosanoids were investigated in SHAM4 and UNX4 rats induced by a maximal vasoconstrictor dose of ANG II, from 41 ± 9

n (n = 4) and 45 ± 7% (n = 4, P = NS) in SHAM4 and UNX4, respectively. Kinetic parameters were not modified by losartan and/or PD-123319, except for a reduction in the area under the curve. As illustrated in Fig. 3, A and B, increasing doses of losartan reduced dose dependently the maximal peak decrease in RBF of SHAM4 and UNX4 rats induced by a maximal vasoconstrictor dose of ANG II, from 41 ± 7 to 18 ± 8%

y (y = 0.0314x + 4.34, r = 0.969, P < 0.0004), and from 36 ± 9 to 1 ± 5% (y = 0.0433x + 4.68, r = 0.924, P = 0.0022), respectively. In the absence of losartan, the maximal peak decrease in RBF averaged, respectively, 78 ± 11 and 71 ± 8%. A similar relationship was observed 1 or 12 wk after nephrectomy (data not illustrated). The maximal peak decrease in RBF was then reduced, respectively, from 35 ± 8 to 23 ± 4% (y = 0.0345x + 4.28, r = 0.986, P < 0.0001) and from 33 ± 19 to 32 ± 8% (y = 0.0265x + 4.01, r = 0.935, P = 0.0016). There was no statistical difference between the four regression lines (F test).

ANG II microvascular receptor binding. In preglomerular vessels isolated from kidneys of SHAM4 (6 experiments) and UNX4 rats (5 experiments), Bmax and Kd averaged, respectively, 1,500 ± 219 and 1,691 ± 208 fmol/mg protein (P = NS), and 0.71 ± 0.14 and 1.10 ± 0.06 nM (P = NS).

To further examine the difference in renal vascular reactivity to ANG II between intact and adapted kidneys, interactions between ANG II and eicosanoids were investigated in SHAM4 and UNX4 rats during inhibition of COX activity or 20-HETE synthesis.

Table 1. Baseline values of body and kidney weight, hematocrit, hemodynamic variables and glomerular filtration rate in SHAM and UNX rats

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>n</th>
<th>BW, g</th>
<th>LKW, g</th>
<th>Hct, %</th>
<th>MAP, mmHg</th>
<th>RBF, ml/min•g KW⁻¹</th>
<th>RVR, mmHg•ml⁻¹min•g KW⁻¹</th>
<th>GFR, ml/min•KW⁻¹</th>
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</thead>
<tbody>
<tr>
<td>SHAM 1</td>
<td>5</td>
<td>238 ± 4</td>
<td>0.94 ± 0.03</td>
<td>40.7 ± 1.4</td>
<td>110 ± 2</td>
<td>8.69 ± 1.27</td>
<td>13.96 ± 2.00</td>
<td>1.03 ± 0.14</td>
</tr>
<tr>
<td>UNX 1</td>
<td>7</td>
<td>238 ± 6</td>
<td>1.26 ± 0.04</td>
<td>42.1 ± 0.6</td>
<td>122 ± 5</td>
<td>8.19 ± 0.84</td>
<td>15.98 ± 1.92</td>
<td>1.09 ± 0.14</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. SHAM, sham rats; UNX, uninephrectomized rats; BW, body weight; LKW, left kidney weight; Hct, hematocrit; MAP, mean arterial pressure; RBF, renal blood flow; KW, kidney weight; RVR, renal vascular resistance; GFR, glomerular filtration rate. Statistical significance was evaluated by two-way ANOVA and Newman-Keuls test for pairwise comparisons. Statistical significance of differences in mean values after allowing for effects of nephrectomy: P < 0.001 for BW and Hct between SHAM and UNX at week 1 and each of the three other periods, and for GFR/gKW between SHAM and UNX at week 24 and at each of the three other periods; P < 0.01 for MAP between SHAM and UNX at week 24 and each of the three other periods. Statistical significance of differences in mean values after allowing for effects of nephrectomy and age: P < 0.05 between SHAM and UNX groups at each time period; P < 0.001 for LKW, between younger (week 1 or 4) and older animals (week 12 and 24).

Effect of acute COX inhibition on ANG II-induced renal vasoconstriction. Baseline values of hematocrit and of hemodynamic variables were in a similar range as those provided in Table 1 for SHAM4 and UNX4 rats. MAP decreased significantly, by ~10 to 15 mmHg, whereas RBF increased significantly by 10 to 15% after administration of the low dose of indomethacin to SHAM4 or UNX4 rats, as previously reported (25) (P < 0.05). In contrast, RBF did not change after administration of the high dose of indomethacin to SHAM4 rats whether treated with a low or a high dose of indomethacin. Recovery of RBF toward baseline was delayed compared with no indomethacin. After each dose of ANG II tested, except 0.25 ng, renal vasoconstriction was significantly more marked than in untreated SHAM rats. Findings were similar when the change in RBF was expressed in absolute values (data not shown). As shown in Fig. 4A, peak RBF decreased, respectively, 72 ± 6 and 73 ± 7% after the intrarenal injection of 2 ng ANG II in SHAM4 rats whether treated with a low or a high dose of indomethacin. Recovery of RBF toward baseline was delayed compared with no indomethacin.
Differences in renal vascular reactivity to repeated injections of 2 ng ANG II during an acute COX inhibition were further ascertained in terms of reproducibility and compared with the effects of 20-HETE inhibition, either alone or concomitant with COX inhibition, in another group of SHAM4 and UNX4 rats. Comparison between COX and 20-HETE inhibition on ANG II-induced renal vasoconstriction. Baseline values of hematocrit and of the hemodynamic variables were in the same range as shown in Table 1. Effects of indomethacin were as described above. HET0016 produced only transient and moderate effects on MAP and RBF, as recently reported (5). Effects of coadministration of the two drugs reduced MAP by 5 and 15 mmHg in SHAM4 and UNX4 rats, respectively, and enhanced RBF by 10% only in SHAM4 rats. As presented in Fig. 5, A and B, there were no time-related changes in RBF responses to 2 ng ANG II repeatedly injected in SHAM4 or UNX4 rats treated with vehicle. In SHAM4 rats, the peak renal vasoconstrictor response after each intrarenal injection of 2 ng ANG II was significantly and repeatedly increased by 40% during COX inhibition.

Fig. 1. Temporal changes in renal blood flow (RBF) induced by a vehicle or a test solution containing 2 ng ANG II injected in the renal artery of 4-wk sham rats (SHAM4; n = 11) and 4-wk uninephrectomized rats (UNX4; n = 6) rats, expressed as a percent of baseline (A), or in absolute values related to kidney weight (KW) (B). Mean values ± SE are illustrated; some SE are too small to be shown. Statistical significance between peak decreases in renal blood flow of SHAM4 and UNX4 rats: *P < 0.05.

Fig. 2. Maximal decrease in RBF, expressed as a percent of baseline, induced by varying doses of ANG II, from 0.25 to 3 ng, injected in the renal artery of SHAM (solid bars) and UNX (open bars) rats 1, 4, 12, and 24 wk after the surgical procedure. Mean values ± SE are illustrated. Statistical significance between peak decreases in renal blood flow of UNX and corresponding SHAM rats: *P < 0.05.
The change in maximal RBF response also was not marked during inhibition of 20-HETE synthesis, or COX and 20-HETE synthesis combined. At the end of the experiment, it averaged $-6 \pm 20$ ($n = 2$) and $5 \pm 18$ ($n = 4$), $0 \pm 9$ ($n = 2$) and $-12 \pm 7$ ($n = 3$), and $10 \pm 21$ ($n = 4$) and $8 \pm 10$% in SHAM4 and UNX4 rats, respectively.

*Indomethacin but not HET0016 decreases prostanoid metabolite production.* Urinary PGE2 excretion before treatment was significantly ($P < 0.05$) elevated in UNX4 ($97 \pm 21$ pg/min, $n = 9$) compared with SHAM4 ($35 \pm 10$ pg/min, $n = 9$) rats. Indomethacin treatment decreased PGE2 excretion by $96 \pm 2$% ($n = 6$) and combined indomethacin and HET0016 administration decreased PGE2 excretion by $97 \pm 2$% ($n = 5$). In contrast, PGE2 excretion was not significantly decreased ($10 \pm 26\%$, $n = 5$) by HET0016 treatment.

*CYP and COX expression following uninephrectomy.* The data for the CYP and COX kidney cortex protein expression in SHAM4 and UNX4 rats are presented in Fig. 6. Kidney CYP4a protein expression decreased by 71% in UNX4 compared with

### Table 2. Kinetic parameters of renal blood flow responses to intrarenal injection of 2 ng ANG II in SHAM and UNX rats at different postnephrectomy periods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SHAM4</th>
<th>UNX1</th>
<th>UNX4</th>
<th>UNX12</th>
<th>UNX24</th>
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<tbody>
<tr>
<td>$n$</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Time for the onset of the response, s</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Constriction half-time, s</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
<td>10 ± 1</td>
<td>12 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Time to maximum response, s</td>
<td>21 ± 1</td>
<td>20 ± 1</td>
<td>20 ± 2</td>
<td>22 ± 1</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>Recovery half-time, s</td>
<td>13 ± 1</td>
<td>13 ± 0</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>Area under the curve, %</td>
<td>1635 ± 177</td>
<td>2348 ± 185</td>
<td>2508 ± 195</td>
<td>2367 ± 433</td>
<td>2175 ± 373</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of rats. SHAM4, SHAM rats since 4 weeks; UNX1, 4, 12, 24, uninephrectomized rats since 1, 4, 12, or 24 weeks. No statistical difference between experimental groups (ANOVA). For the sake of clarity, comparison was only undertaken with one SHAM group corresponding to UNX4 in age since no kinetic differences between SHAM rats at different periods of time were noted.

![Fig. 3](http://ajpregu.physiology.org/)

**Fig. 3.** Relationship between the peak decrease in RBF and the increasing doses of losartan ($10^1$, $5.10^1$, $10^2$, $2.10^2$, $5.10^2$, $10^3$ ng) mixed to a maximal vasoconstrictor dose of ANG II injected in the renal artery of SHAM4 (A) and UNX4 rats (B). The peak decrease of RBF induced by a maximal vasoconstrictor dose of ANG II alone is also shown. Mean values ± SE are illustrated. Dose-dependent differences in peak decrease of RBF were evaluated by ANOVA for repeated measurements followed by a Newman-Keuls test. Statistical significance: *$P < 0.05$ from reference dose of losartan (1,000 ng). The relationship between the peak decrease in RBF and the dose of losartan established by best-fit linear regression corresponded to the following equations: $y = 0.0314x + 4.34$ ($r = 0.969$, $P = 0.0004$), and $y = 0.0433x + 4.68$ ($r = 0.924$, $P = 0.002$) in SHAM4 ($n = 5$) and UNX4 ($n = 5$) rats, respectively. There was no statistical difference between regression lines ($F$ test).
SHAM4 rats, whereas CYP2c23 expression was not altered. On the other hand, COX-1 expression was unaltered and kidney cortex COX-2 protein expression increased 2.2-fold in UNX4 compared with SHAM4 rats.

DISCUSSION

As expected, morphological and functional compensatory adaptations occurred in the remaining kidney at different time periods after ablation of the contralateral kidney (15). These adaptations were appropriate, as demonstrated by the increase in kidney weight and the adequate compensation of RBF and GFR in the adapted kidney of rats submitted to an uninephrectomy 1, 4, 12, or 24 wk previously. In both SHAM and UNX rats, ANG II injected into the renal artery as a bolus caused a transient, potent, and dose-dependent renal vasoconstriction without impairing blood pressure. Interestingly, renal vascular reactivity after the intrarenal injection of ANG II was significantly greater in the animals 1–4 wk after uninephrectomy.
compared with corresponding SHAM rats but was significantly attenuated in later postnephrectomy periods. It became then comparable to the response observed in SHAM rats 12–24 wk after the surgical procedure. The enhanced vasoreactivity to ANG II occurring in the residual kidney 4 wk after uninephrectomy was reproducible during the experiment and was specific for ANG II compared with the RBF responses induced by NE under different experimental conditions. The increased renal vasoreactivity to ANG II was not related to age because renal vasoreactivity to ANG II did not vary between the different groups of SHAM rats. These findings are in concordance with those of Zhang et al. (44) who did not observe differences in sensitivity to ANG II of afferent and efferent arterioles from young and old rats.

At week 4, the enhanced renal vascular reactivity to ANG II was not related to a change in density and/or affinity of the ANG II receptors localized in the renal microvasculature of the adapted kidney. As recently pointed out by Arendshorst et al. (2), AT1 receptors are mainly involved in the vasoconstrictor effect of ANG II. Therefore, their implication was further investigated in vivo in the adapted kidney by addition of 1,000 ng losartan, a specific AT1 receptor antagonist, to the intrarenal injectate containing 2 ng ANG II (9). Under these conditions, the renal vasoconstriction was reduced comparably, by 49% in SHAM, and by 66% in UNX rats. Also, the efficacy of losartan in the presence of a maximal vasoconstrictor dose of ANG II did not differ between SHAM and UNX rats. The vasoconstrictor effect could even be fully reversed in the two groups by increasing the dose of losartan. These findings suggest that AT1 receptors are also predominant in the renal microvasculature of the adapted kidney, the more so that the inhibitory effect of PD-123319, a putative AT2 receptor antagonist, on the renal blood flow response to ANG II was moderate and similar in SHAM and UNX rats. Our findings are in agreement with observations indicating that 80 to 85% of these receptors seemed to belong to the AT1 subtype in the rat (9). They are also in agreement with the proportion of AT1 receptor mRNA determined in the cortex and the inner stripe of the outer medulla of the residual kidney, 1, 4, and 12 wk after an unilateral nephrectomy, as recently reported by our group (21). All of these results suggest an involvement of (an)other factor(s) in the enhanced vascular reactivity to ANG II of the adapted kidney, at least during the first weeks after an uninephrectomy.

Eicosanoids are of interest in this regard since interactions between ANG II and prostaglandins have been reported in vivo using a similar experimental approach in the rat (6–8). During COX inhibition, the renal vasocostructor effect induced by varying doses of ANG II was markedly enhanced in the intact kidney but was not further affected in the adapted kidney. Thus differences in the renal vasoreactivity to ANG II exhibited by untreated SHAM and UNX rats were now abolished. By comparison, our results suggest that, at least under baseline conditions, some vasodilator prostanoid(s) attenuated the vasocostructor effect of ANG II in the intact kidney. In support of our findings, the renal blood flow responses to ANG II were also enhanced during cyclooxygenase inhibition in young normotensive Wistar-Kyoto rats (6). Subsequently, Chatziantoniou and Arendshorst (7, 8) showed the involvement of vasodilatory prostaglandins and found that the counteraction of PGE2 on the effect of ANG II was twofold more potent than the one exerted by PGI2 in the rat kidney. An important feature of our study concerned the temporal reproducibility of ANG II’s action on renal blood flow. It indicates that the sensitivity to ANG II of the renal vascular cells after repeated exposure to the peptide remained unchanged during control, as well as during COX inhibition. It is thus likely that the cellular processing of the ANG II receptor was not modified under our experimental conditions (16). Furthermore, our observations underlined the persistent difference in renal vasoconstrictor responses to ANG II between SHAM and UNX rats during cyclooxygenase inhibition. Nevertheless, some interference of a vasoactive prostaglandin cannot be excluded in UNX rats since the recovery half-time of RBF responses to ANG II was prolonged to a similar extent as in control rats, in concordance with observations in genetically hypertensive and normotensive control rats (7, 8).

On the other hand, renal vascular reactivity to ANG II was significantly reduced by 53% after inhibition of 20-HETE synthesis in UNX rats but only moderately so in SHAM rats. The marked countereffect induced by the inhibition of 20-HETE synthesis upon ANG II’s renal vasoconstrictor action was maintained throughout the time of the experiment in the adapted kidney. These observations thus suggest that 20-HETE was involved, at least to some extent, in the increased vasoconstrictor response to ANG II of the adapted kidney. Such an
interaction is not unexpected because ANG II enhances the synthesis of 20-HETE in the kidney and in the peripheral vasculature, and it also induces the release of 20-HETE from the preglomerular microvasculature (1, 12, 34). 20-HETE is the principal eicosanoid in the preglomerular microvessels and presents a potent vasoconstrictor action at low concentrations, at least in isolated arterioles of the rat kidney (12, 17, 27). It is therefore likely that changes in the paracrine environment of the adapted kidney involving ANG II and 20-HETE may affect transiently the sensitivity of its microvasculature. The stimuli and underlying mechanisms leading to these changes remain, however, to be determined. In this regard, it is of interest to point out that the differences in vasoconstrictor responses induced by ANG II in the intact and in the adapted kidney after inhibition of COX or 20-HETE synthesis alone vanished after the combined inhibition of these two pathways. The latter observation could be related to some extent to the suppression of 20-HETE metabolism by COX, an interaction first suggested by McGiff and Quilley (28). Such a possibility is further strengthened by the data of Cheng et al. (11) showing that COX inhibition enhanced the release of 20-HETE from preglomerular vessels of young rats fed a low-salt diet, which activates the renin-angiotensin system.

The changes in renal cortical CYP and COX protein expression in uninephrectomized rats did not explain the changes in ANG II vascular reactivity. In contrast to the angiotensin vascular reactivity responses that suggested an increased contribution of 20-HETE in uninephrectomized rats, the 4-wk uninephrectomized rats had decreased CYP4a protein expression. COX-2 protein expression and PGE2 urinary excretion were elevated in uninephrectomized rats, whereas COX inhibition had less of an effect on the renal vascular response to ANG II. This increase in renal cortical COX-2 and PGE2 in uninephrectomized rats is consistent with previous findings in the subtotal renal ablation model (42). One possible reason for these apparent conflicting data is that ANG II-stimulated production is independent of the protein expression or prevailing levels of the metabolites. This possibility is partially supported by the fact that norepinephrine vascular responses were not altered by 20-HETE or COX inhibition. Additionally, there could be an interaction between the 20-HETE and COX pathways. The vascular responses to ANG II in the presence of combined inhibition suggest that such an interaction is plausible. Last, uninephrectomy could alter the other aspects of arachidonic acid metabolism such as the prostaglandin receptors or other interacting vascular regulating systems. As emphasized by McGiff and Quilley (28), these apparently discrepant results could also be related to the multiple effects of ANG II exerted in an integrated system, such as ours, upon vascular and tubular structures of the kidney, and to the interactions between ANG II and other paracrine factors.

In summary, an enhanced renal vascular reactivity to ANG II was observed in the adapted kidney 4 wk after an unilateral nephrectomy, mainly involving AT1 receptors. The increased vascular responsiveness to ANG II was not related to a change in density or affinity of ANG II receptors in preglomerular vessels isolated from adapted kidneys. In contrast, inhibition of COX activity and of 20-HETE synthase suggested a differential interaction between ANG II and some eicosanoid(s) in the renal vascular responses to ANG II. COX inhibition revealed the involvement of vasodilator prostaglandin(s) in the intact kidney, whereas inhibition of 20-HETE synthesis indicated an involvement of 20-HETE in the adapted kidney. These findings vanished after combined inhibition of the two pathways. Differences in renal vascular responses to ANG II were, however, not related to the changes in COX and CYP protein expression after uninephrectomy. In conclusion, the in vivo results suggest that vasodilator prostaglandins modulate the vasoconstrictor effect of ANG II in the intact kidney but that their countereffect is diminished after unilateral nephrectomy. They further suggest that the enhanced sensitivity of the microvasculature to ANG II in the adapted kidney may be due to a change in the paracrine/autocrine activity of ANG II and 20-HETE, to interactions between COX and 20-HETE pathways in the renal microvasculature, and/or to some other aspects of arachidonic acid metabolism eventually induced by uninephrectomy.

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DISCLOSURE


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RENAL VASOREACTIVITY TO ANG II AFTER UNINEPHRECTOMY


