Proximal Tubular Angiotensin II Levels and Renal Functional Responses to AT₁ Receptor Blockade in Nonclipped Kidneys of Goldblatt Hypertensive Rats

Ludek Cervenka, Chi-Tarng Wang, Kenneth D. Mitchell, L. Gabriel Navar

Abstract—Previous studies have shown that whereas the nonclipped kidney in two-kidney, one clip (2K1C) rats undergoes marked depletion of renin content and renin mRNA, intrarenal angiotensin II (Ang II) levels are not suppressed; however, the distribution and functional consequences of intrarenal Ang II remain unclear. The present study was performed to assess the plasma, kidney, and proximal tubular fluid levels of Ang II and the renal responses to intrarenal Ang II blockade in the nonclipped kidneys of rats clipped for 3 weeks. The Ang II concentrations in proximal tubular fluid averaged 9.19±1.06 pmol/mL, whereas plasma Ang II levels averaged 483±55 fmol/mL and kidney Ang II content averaged 650±66 fmol/g. Thus, as found in kidneys from normal rats with normal renin levels, proximal tubular fluid concentrations of Ang II are in the nanomolar range. To avoid the confounding effects of decreases in mean arterial pressure (MAP), we administered the nonsurmountable AT₁ receptor antagonist candesartan directly into the renal artery of nonclipped kidneys (n=10). The dose of candesartan (0.5 µg) did not significantly decrease MAP in 2K1C rats (152±3 versus 148±3 mm Hg), but effectively prevented the renal vasoconstriction elicited by an intra-arterial bolus of Ang II (2 ng). Candesartan elicited significant increases in glomerular filtration rate (GFR) (0.65±0.06 to 0.83±0.11 mL·min⁻¹·g⁻¹) and renal blood flow (6.3±0.7 to 7.3±0.9 mL·min⁻¹·g⁻¹), and proportionately greater increases in absolute sodium excretion (0.23±0.07 to 1.13±0.34 μmol·min⁻¹·g⁻¹) and fractional sodium excretion (0.38±0.1% to 1.22±0.35%) in 2K1C hypertensive rats. These results show that proximal tubular fluid concentrations of Ang II are in the nanomolar range and are much higher than can be explained on the basis of plasma levels. Further, the data show that the intratubular levels of Ang II in the nonclipped kidneys of 2K1C rats remain at levels found in kidneys with normal renin content and could be exerting effects to suppress renal hemodynamic and glomerular function and to enhance tubular reabsorption rate. (Hypertension. 1999;33:102-107.)

Key Words: renin-angiotensin system □ hypertension, renal □ angiotensin antagonist □ receptors, angiotensin ■ glomerular filtration rate ■ renal blood flow

Previous studies have shown the pivotal role of the renin-angiotensin system (RAS) in the development and maintenance of two-kidney, one clip (2K1C) hypertension.1–4 Recent studies have shown that the angiotensin II (Ang II) content of the nonclipped kidney is either normal or elevated even when plasma Ang II concentrations have returned toward normal and the renin content as well as the renin mRNA levels of the nonclipped kidney are reduced to barely detectable levels.5–7 In addition, it has been shown that tubuloglomerular feedback (TGF) responsiveness in the nonclipped kidney is either normal or elevated even may be slightly enhanced compared with sham-operated rats and, further, may be highly dependent on Ang II type 1 receptor activation.8 Such inappropriately maintained or elevated intrarenal Ang II levels and enhanced TGF responsiveness in the nonclipped kidney may contribute to a compromised ability of the kidney to maintain normal sodium excretion at normotensive arterial pressure and to a reduced natriuretic responsiveness to elevations of arterial pressure, thereby contributing to the hypertension in this model. The maintained or enhanced intrarenal levels of Ang II in the nonclipped kidneys of 2K1C hypertensive rats coupled with the Ang II–dependent influences on renal hemodynamics and sodium reabsorption suggest that the augmented intrarenal Ang II levels are functionally active. In view of the fact that these kidneys are renin depleted,5,7 however, it is possible that the distribution of intrarenal Ang II may differ from that seen in normal kidneys. Indeed, recent studies in normal rats9–11 have revealed that Ang II concentrations in proximal tubular fluid are in the nanomolar range and much greater than the coincident plasma and kidney levels. However, the proximal tubular concentrations of Ang II in 2K1C hypertensive rats...
have not been reported. Accordingly, one aim of the present study was to determine the proximal tubular fluid concentrations of Ang II in the nonclipped kidneys.

The functional responses of the nonclipped kidneys of 2K1C hypertensive rats to pharmacological blockade of RAS have not yielded consistent results. Indeed, the glomerular filtration rate (GFR) has been reported to be increased or unchanged; however, systemic receptor blockade also causes profound decreases in mean arterial pressure (MAP). Thus, it is possible that the compensatory mechanisms activated by systemic administration of angiotensin-converting enzyme inhibitors (ACEIs) or AT1 receptor antagonists could exert indirect effects to decrease renal function. Differences in responses to systemic and direct intrarenal blockade have been reported before. Siragy et al observed increases in renal plasma flow (RPF), GFR, sodium excretion, and urine flow when a combination of a renin inhibitor, an ACEI, and an Ang II receptor antagonist was infused directly into the renal artery of dogs. Similarly, Peng and Knox showed that renal interstitial infusion of an AT1 receptor antagonist increased sodium excretion in normotensive rats. However, these changes did not occur when the antagonist was infused systemically, eliciting a substantial decrease in MAP. In a recent study, we observed that renal responses to systemic administration of an AT1 receptor blocker were actually greater with lower doses that did not markedly lower arterial pressure than with higher doses that caused hypotension. Thus, rapid acute decreases in arterial pressure caused by systemic AT1 receptor blockade may elicit various indirect effects, including activation of the sympathetic nervous system, to increase peripheral vascular resistance and renal vascular resistance.

Previous studies attempting to evaluate the direct in vivo effects of intrarenal Ang II receptor blockade were compromised because the systemic infusion of receptor antagonists, even if given directly into the renal artery, leads to spillover into the systemic circulation and consequent reductions in systemic arterial pressure. Recent availability of highly potent, nonsurmountable AT1 receptor antagonists has enabled more complete characterization of the effects of direct intrarenal Ang II blockade on the nonclipped kidneys of 2K1C Goldblatt hypertensive rats in the absence of decreases in MAP. The nonsurmountable nature of candesartan is particularly advantageous since this allowed us to elicit almost complete intrarenal AT1 receptor blockade with a single bolus dose, thus obviating the need for continuous infusion that progressively increases the circulating concentration of the antagonist. Accordingly, the second major aim of this study was to delineate the magnitude of Ang II-dependent influences on renal hemodynamics and sodium excretory function under conditions in which the systemic arterial pressure was maintained.

Methods
The studies described here were performed in accordance with the guidelines and practices established by the Tulane University Animal Care and Use Committee.

Preparation of 2K1C Goldblatt Hypertensive Rats
Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 60 to 80 g were anesthetized with sodium pentobarbital (40 mg/kg IP). The right renal artery of each animal was isolated through a flank incision and, as described previously, a silver clip (0.25-mm internal gap) was placed on the renal artery. Sham-operated rats, which underwent the same surgical procedure except for placement of the renal artery clip, served as controls. All animals were fed standard rat chow and tap water ad libitum and were kept on a 12-hour light/dark cycle. The acute experiments were performed 3 weeks after placement of the clip.

Determinations of Plasma, Kidney, and Proximal Tubular Fluid Levels of Ang II
Experiments were performed to determine the Ang II levels in proximal tubular fluid, plasma, and kidneys of anesthetized 2K1C Goldblatt hypertensive rats (n=8). On the day of experiment, rats were anesthetized with pentobarbital sodium (50 mg/kg IP), placed on a thermoregulated table, and prepared for micropuncture experiments as described previously. After completion of surgery and a 60-minute equilibration period, two 30-minute clearance periods were performed to assess control renal function. By use of procedures previously described to collect samples of proximal tubular fluid, collection micropipettes were inserted into mid-to-late segments of proximal tubules, and complete free-flow tubular fluid samples were collected for 10 to 20 minutes. It has previously been shown that there is not significant in vitro generation of Ang II in the collection pipette. Immediately after collection, the tubular fluid sample volume was determined with a slide comparator (Gaertner Scientific) and the sample was transferred to a tube containing 1 mL of chilled methanol. Multiple free-flow proximal tubular fluid samples were obtained from each rat, and the samples were pooled. The pooled tubular fluid samples were stored in methanol at −20°C until the day of radioimmunoassay. Approximately 1 μL of pooled proximal tubular fluid was obtained from each animal. At the end of the experiment, the kidneys were excised, drained, weighed, and homogenized in chilled methanol. Immediately after removal of the kidney, a 1-mL arterial blood sample was collected into 9 mL of chilled methanol. Ang II levels in proximal tubular fluid, plasma, and kidney tissue were assessed by radioimmunoassays as described previously.

Renal Function Studies
For the studies evaluating responses to the AT1 receptor blockade, rats were prepared in a manner similar to that described above. In addition, a tapered PE-10 catheter was inserted into the left renal artery via the left femoral artery for selective intrarenal administration of agents by use of a slight modification of the technique described previously for renal blood flow (RBF) reactivity studies.22 The tip of a laser Doppler flow probe (Med Pacific) was placed near the surface of the kidney for measurement of relative changes in cortical renal blood flow (CRBF). Laser Doppler flow technology allows dynamic assessment of relative changes in cortical renal blood flow (CRBF). Laser Doppler flow technology allows dynamic assessment of relative changes in RBF. During surgery, an isotonic saline solution containing albumin (6%) was infused at a rate of 20 μL/min. After surgery, an isotonic saline solution containing albumin (1%), p-aminohippurate sodium (PAH) (1.5%), and polyfructosan (2%) was infused at the same infusion rate. After a 60-minute equilibration period, the response in CRBF to an intrarenal bolus of Ang II (2 ng) was tested. The experimental protocol consisted of three 30-minute clearance periods to assess control renal function. The rats then received a single injection of candesartan (0.5 μg) directly into the renal artery, which was followed by a 10-minute delay and three 30-minute experimental clearance periods. Pilot studies indicated that this dose of candesartan is sufficient to prevent the renal vasoconstrictor effects of an intrarenal bolus injection of 2 ng of Ang II for up to 3 hours yet not spill over to the systemic circulation in sufficient amounts to produce significant decreases in MAP or to attenuate systemic blood pressure responses to intravenous injections of 50 ng of Ang II. At the end of the experiment, the responses in CRBF to an intrarenal bolus of Ang
Intrarenal Ang II in 2K1C Rats

II (2 ng) were tested again. Two blood samples were collected at the midpoint of the second and fifth clearance periods to calculate GFR and PAH clearance. The following experimental groups were examined: group 1 (n=6), sham-operated+intrarenal vehicle control; group 2 (n=10), sham-operated+intrarenal candesartan (0.5 μg); group 3 (n=8), 2K1C rats+intrarenal vehicle control; group 4 (n=10), 2K1C rats+intrarenal candesartan (0.5 μg).

Analytical Procedures, Calculations, and Statistical Analyses
Urinary volume was measured gravimetrically. Inulin and PAH concentrations were measured colorimetrically.23 Sodium and potassium concentrations were determined by flame photometry. GFR, fractional sodium, and potassium excretion were calculated using standard formulas. PAH clearance was used as an index of PBF. RBF was estimated from the PAH clearance and hematocrit values but without correction for PAH extraction. Data are expressed as mean±SEM. Statistical comparisons within groups were conducted by use of ANOVA for repeated measures, followed by Newman-Keuls test. Student’s unpaired t test was used for comparisons between groups. Values exceeding the 95% probability limits (P<0.05) were considered statistically significant.

Results
Basal values of body weights, blood pressures, and renal function and electrolyte excretion from the left kidneys in sham-operated rats and the nonclipped kidneys in 2K1C rats are summarized in the Table. As expected, arterial pressures in 2K1C rats were significantly higher than in sham-operated rats. Although there is a suggestion that GFR values are lower in the 2K1C rats, these and other indices of renal function were not significantly different among the groups.

Plasma, Kidney, and Proximal Tubular Fluid Levels of Ang II
As shown in Figure 1, plasma Ang II concentrations were 483±53 fmol/mL, the Ang II contents in the nonclipped kidneys were 650±66 fmol/g, and proximal tubular fluid concentrations of Ang II were 9.19±1.06 pmol/mL. The values obtained from tubular fluid samples were, in all cases, substantially greater than the plasma and kidney Ang II levels. For comparison, the plasma and kidney tissue values are shown as pmol/mL and pmol/g of kidney tissue on the same graph as the tubular fluid Ang II concentrations.

Effects of Intrarenal Candesartan on MAP and on Pressor Responses to Intravenous Ang II
Administration of candesartan at a dose of 0.5 μg did not change MAP significantly either in 2K1C rats or in sham-operated rats (152±3 to 148±3 and 125±1 to 123±3 mm Hg, respectively); similarly, the saline vehicle alone did not elicit significant changes in arterial pressure (157±3 to 153±2 and 125±3 to 124±5 mm Hg, respectively). Intravenous administration of candesartan also did not alter the magnitude of pressor responses to intravenous bolus injections of 50 ng of Ang II in sham-operated rats (51±6 to 47±4 mm Hg, n=3) or in 2K1C rats (47±5 to 48±3 mm Hg, n=3). Collectively, these data indicate that the dose of candesartan did not spill over into the general circulation in amounts sufficient to elicit significant blockade of extrarenal AT₁ receptors.

<table>
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<tr>
<th>Parameters</th>
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<th>2K1C Series 2</th>
</tr>
</thead>
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<td>Mean arterial pressure, mm Hg</td>
<td>125±3</td>
<td>169±6*</td>
<td>155±3*</td>
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<td>Body weight, g</td>
<td>267±29</td>
<td>275±10</td>
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<td>Glomerular filtration rate, mL·min⁻¹·g⁻¹</td>
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<td>Renal blood flow, mL·min⁻¹·g⁻¹</td>
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<td>Sodium excretion, μmol·min⁻¹·g⁻¹</td>
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<td>Potassium excretion, μmol·min⁻¹·g⁻¹</td>
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<td>Fractional sodium excretion, %</td>
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<td>0.35±0.11</td>
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<td>Fractional potassium excretion, %</td>
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<td>20.1±3.1</td>
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<td>Urine flow, μL·min⁻¹·g⁻¹</td>
<td>6.8±0.5</td>
<td>7.4±0.5</td>
<td>6.9±1.7</td>
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</table>

*P<0.05 compared with sham-operated group.

Figure 1. Ang II levels in proximal tubular fluid (TF), plasma (PL), and nonclipped kidney (NK) of 2K1C Goldblatt hypertensive rats. Values are presented as pmol/mL of TF and PL and pmol/g of tissue in NK, respectively.
Effects of Intrarenal Candesartan on CRBF Responses to Intrarenal Bolus Injections of Ang II
As shown in Figure 2, decreases in CRBF in response to intrarenal Ang II (2 ng) before candesartan treatment averaged 39\%±5\% in 2K1C rats and 28\%±6\% in sham-operated rats. Intrarenal administration of candesartan completely blocked CRBF responses in both sham-operated and 2K1C rats. Interestingly, 2K1C rats treated with candesartan exhibited slight increases in CRBF in response to Ang II (7\%±6\%), suggesting a slight renal vasodilatory effect of Ang II under conditions of AT1 receptor blockade.

Effects of Intrarenal Candesartan on GFR and RBF
As shown in Figure 3 (top), candesartan elicited significant increases in GFR in both 2K1C and sham-operated rats (0.65\%±0.06 to 0.83\%±0.11 and 0.71\%±0.04 to 0.87\%±0.06 mL / min / g, respectively; \( P < 0.05 \) in both cases). Despite the differences in arterial pressure, the GFR responses to candesartan were similar in both groups. As shown in Figure 3 (bottom), candesartan elicited significant increases in RBF in 2K1C and in sham-operated rats as well (6.32\%±0.74 to 7.29\%±0.87 and 5.71\%±0.37 to 6.52\%±0.67 mL / min / g, respectively; \( P < 0.05 \) in both cases).

Effects of Intrarenal Candesartan on Urine Flow and Sodium Excretory Function
Intrarenal candesartan elicited significant increases in urine flow in 2K1C and sham-operated rats (6.1\%±0.9 to 9.4\%±1.6 and 6.5\%±0.5 to 10.2\%±1.1 \( \mu \)L / min / g, respectively; \( P < 0.05 \) in both cases). As shown in Figure 4, candesartan caused marked increases in sodium excretion in both 2K1C and sham-operated rats with increases from 0.23\%±0.07 to 1.13\%±0.34 and 0.21\%±0.04 to 1.19\%±0.26 \( \mu \)mol / min / g, respectively; \( P < 0.05 \) in both cases. Likewise, fractional sodium excretion increased about 3-fold (0.38\%±0.1\% to 1.22\% and 0.35\%±0.08\% to 1.07\%±0.18\%, respectively; \( P < 0.05 \) in both cases). No significant changes in potassium excretion were found in any of the groups.

Discussion
The current study was performed to determine the plasma, kidney, and proximal tubular fluid Ang II levels in the nonclipped kidneys of 2K1C Goldblatt hypertensive rats and to assess the renal functional responses of the nonclipped kidneys to direct intrarenal Ang II receptor blockade in the absence of the confounding indirect effects elicited by decreases in MAP. As previously reported,\(^5^-^7\) renal Ang II levels in the nonclipped kidneys of 2K1C hypertensive rats remained elevated even though the renal renin content and renin mRNA have been consistently shown to be suppressed. The novel finding reported in the present study is that proximal tubular fluid Ang II concentrations were also maintained in the nanomolar range and were similar to values recently reported for normal rats prepared in a similar manner.\(^3^-^11\) Thus, Ang II levels in the nonclipped kidneys of 2K1C rats are distributed to the proximal tubular fluid in a manner similar to that observed in normal rats. Importantly,
the tubular fluid Ang II concentrations are much higher than can be explained on the basis of filtration of circulating Ang II, supporting the concept that the Ang II in the proximal tubule fluid is primarily because of secretion of Ang II by the proximal tubule. This mechanism thus remains unabated in the renin-depleted kidney of the 2K1C rat, supporting the notion that there is a renin-independent mechanism responsible for maintenance not only of kidney tissue Ang II levels but also of proximal tubular Ang II concentrations in the nonclipped kidney. Such a mechanism may involve AT$_1$ receptor–mediated internalization of circulating Ang II, as has been shown to occur in Ang II–infused hypertensive rats.

The present data indicate that the nonclipped kidney has an impaired ability to appropriately suppress intrarenal levels of Ang II in response to sustained elevations of arterial pressure and despite the renin depletion. In view of the evidence that Ang II receptors are located on the luminal as well as on the basolateral membrane of proximal tubule cells and that the reabsorptive status of the proximal tubule is critically influenced by Ang II concentrations, it is likely that such inappropriately high intraluminal and intrarenal Ang II levels observed in nonclipped kidneys of 2K1C hypertensive rats would stimulate proximal tubular reabsorption rate. This effect combined with an enhancement of TGF responsiveness may contribute to the development and maintenance of hypertension by maintaining an inappropriately high sodium reabsorption rate even at elevated arterial pressures. It has been shown that vascular AT$_1$ receptor density is not decreased after 2 to 4 weeks of clipping and that tubular AT$_1$ receptor density may actually be increased by elevated Ang II levels. Thus, the Ang II dependency would apparently not be counteracted by reciprocal decreases in AT$_1$ receptor density.

The renal functional data provide further support for a high degree of Ang II dependency in the nonclipped kidney and show that selective intrarenal AT$_1$ receptor blockade elicits substantial increases in RBF, GFR, and proportionally even greater increases in sodium excretion. When Ang II blockade was restricted to the kidneys, avoiding confounding consequences of decreases in arterial pressure, we observed increases in GFR and RBF. These results are consistent with those reported by Imamura et al who reported that chronic treatment with losartan resulted in increases in GFR and RBF in nonclipped kidneys of 2K1C hypertensive rats. Increases in GFR could be caused not only by the vasodilatory actions on the renal microvasculature but also by increases in the glomerular filtration coefficient due to blockade of endogenous Ang II at the glomerulus. The specific mechanisms underlying the renal hemodynamic responses to intrarenal AT$_1$ receptor blockade require further investigation. The substantially greater increases in both absolute and fractional sodium excretion compared with RBF and GFR increases in response to intrarenal candesartan suggest that in addition to the natriuresis caused by renal hemodynamic changes, blockade of tubular AT$_1$ receptors contributed to increases in urinary sodium excretion.

In summary, the present data indicate that proximal tubular fluid of anesthetized 2K1C Goldblatt hypertensive rats contains nanomolar concentrations of Ang II. The maintained responsiveness of the nonclipped kidney to Ang II blockade and inappropriately high intraluminal and kidney tissue Ang II levels for hypertensive rats are consistent with the concept that functional derangements of the nonclipped kidneys of 2K1C Goldblatt hypertensive rats are strongly Ang II dependent and contribute to the development and maintenance of hypertension in this model. Further, when hypotension is prevented, renal responses in hypertensive rats as well as normal rats are consistently characterized by increases in RBF, GFR, and proportionally greater increases in sodium excretion.

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References

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