Angiotensin and Angiotensin Converting Enzyme Tissue Levels in Two-Kidney, One Clip Hypertensive Rats

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Renal tissue angiotensin I (Ang I) and II (Ang II) content and angiotensin converting enzyme activity were assessed in both kidneys during initial (7 days) and maintenance (25 days) phases of two-kidney, one clip hypertension in rats. At 7 and 25 days, systolic arterial pressure was 146±2 and 170±7 mm Hg, respectively. After 7 days, Ang I content of clipped kidneys was 64% and 70% higher (p<0.001) than in nonclipped and sham-operated kidneys, respectively. When compared with levels in kidneys from sham-operated rats, Ang II content in clipped kidneys was increased 102% and 24% (p<0.01), respectively. Ang II content was also 32% higher in nonclipped kidneys. Angiotensin converting enzyme activity in nonclipped kidneys was greater (p<0.05) than in either clipped (46% higher) or sham-operated kidneys (57% higher). Plasma Ang I and Ang II levels were elevated at 7 days but were not different at 25 days in clipped rats. These results demonstrate a dissociation between intrarenal and circulating levels of Ang I and Ang II and suggest that qualitatively different mechanisms may be responsible for the elevated intrarenal Ang II levels during the initial and maintenance phases of renal hypertension. (Hypertension 1992;20:763–767)

Key Words • renin-angiotensin system • hypertension, renal • hypertension, renovascular • angiotensin I • angiotensin II • kininase II

There is now considerable evidence demonstrating a close relation between the renin-angiotensin system (RAS) and the development of hypertension in the two-kidney, one clip (2K1C) Goldblatt hypertensive rat model.1,2 After a constrictor clip is placed on one renal artery, there is an increase in renin secretion from and an increase in renin content of the clipped kidney, and both plasma renin activity (PRA) and angiotensin II (Ang II) concentrations are elevated.1,2 It is generally recognized that the contralateral kidney is also functionally impaired,2,4,5 suggesting that the unique circumstances of altered renin-angiotensin activity in this model may contribute to the observed abnormal hemodynamic and tubular reabsorptive function. Supporting this possibility is the finding that pharmacological blockade of the RAS results in increased renal blood flow and glomerular filtration rate and increased urine flow and sodium excretion in the contralateral kidney.2,5,7 These findings indicate that Ang II–dependent alterations in renal hemodynamics and tubular reabsorptive function act to impair the ability of the nonclipped kidney to achieve normal rates of sodium excretion at normotensive pressures and thereby contribute to the development of hypertension in the 2K1C Goldblatt model.

The initial phase of the hypertension that develops after clipping one renal artery appears to be dependent on the increase in PRA and a renin-induced elevation of Ang II concentration in plasma.9–10 The increase in blood pressure can be prevented by treatment with antagonists of the RAS from the time of clipping, and normotension can also be restored by acute intervention with one of these agents during the initial days after clipping.10–13 The maintenance phase of the hypertension was initially thought to be less dependent on the RAS since PRA and plasma Ang II returned toward normal levels.2 Even in the maintenance phase, however, the nonclipped kidney exhibits natriuretic and diuretic responses to angiotensin converting enzyme (ACE) inhibition.6,8,11–13 These findings indicate that the nonclipped kidney is still under the influence of Ang II even at a time when circulating Ang II levels have returned toward normal levels. Although circulating Ang II concentrations diminish and renin content of the nonclipped kidney is markedly reduced, it has been reported that the Ang II content of the nonclipped kidney is either normal14 or slightly elevated1 during the maintenance phase of 2K1C hypertension. Such appropriately maintained Ang II levels may contribute to the compromised ability of the nonclipped kidney to regulate its sodium excretion at normotensive pressures.

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thus requiring sustained elevations in arterial pressure. Because renal renin content and renin messenger RNA levels are decreased in the nonclipped kidney, it is likely that other factors such as increased ACE activity may be responsible for the maintained or elevated Ang II content of the nonclipped kidney. The present study was designed to investigate changes in intrarenal angiotensin peptides and ACE activities during both the initial and maintenance phases of 2K1C hypertension in rats.

Methods
Preparation of Two-Kidney, One Clip Goldblatt Hypertensive Rats
To study the changes in the RAS during the maintenance phase (25 days) of 2K1C hypertension, male Sprague-Dawley rats (Charles River, Wilmington, Mass.) initially weighing 125-150 g were anesthetized with pentobarbital sodium (50 mg/kg i.p.). The left renal artery of each animal was isolated through a flank incision, and as described previously, a silver clip (0.25 mm i.d.) was placed on the renal artery. Sham-operated rats served as controls. All rats were housed in hanging wire cages and were fed Purina rodent chow and tap water ad libitum for 18 days. One week before the study, the animals were transferred to a semisynthetic diet (Teklad, Madison, Wis.) containing 0.14 meq sodium per milligram protein. Protein levels were determined by refractometry.

To study changes in the RAS during the initial phase (7 days) of 2K1C hypertension, rats weighing 225-250 g were prepared as described above. Larger rats were used in this protocol so that body weights in both groups would be similar at the time of study. The 7-day rats were fed the semisynthetic diet described above from the time of clip placement to eliminate the effects of differences in dietary sodium intake on angiotensin peptide levels. Thus, both 7- and 25-day groups were fed the diet for the same length of time before analysis. In the 7-day clipping groups, systolic arterial pressures were measured using a tail cuff (Harvard Apparatus, South Natick, Mass.) in conscious rats 1 day before and 3 and 7 days after clip placement. In the 25-day groups, blood pressure was measured 14 days after clipping and thereafter every 3-4 days until study. All experimental procedures were approved by the Tulane University Animal Care and Use Committee.

Collection, Processing, and Purification of Samples
The rats were anesthetized with pentobarbital sodium (50 mg/kg i.p.), and a polyethylene catheter was placed in the carotid artery for blood sampling. The collection of samples and extraction of angiotensin peptides and ACE were performed using methods described previously. Briefly, both renal arteries were clamped through a midline incision, and the kidneys were rapidly removed and bisected. One half of each kidney was placed into cold methanol and rapidly homogenized (10%, wt/vol) with a glass homogenizer. The other half of each kidney was bisected again, and one quarter of each kidney was homogenized in Tris buffer (0.1 M, pH 7.4) for ACE measurement. Immediately after removal of the kidneys, blood was collected via the carotid artery into a prechilled syringe containing (final concentrations) EDTA (5 mM), pepstatin (10 μM), 1,10-phenanthroline (1.25 mM), and enalaprilat (20 μM). After centrifugation at 4°C for 10 minutes, angiotensin peptides were extracted immediately from the plasma samples by adsorption to and elution from a phenyl-bonded, solid-phase extraction column (Bond-Elut, Analytchem, Harbor City, Calif.). The supernatants from the kidney homogenates were dried overnight in a vacuum centrifuge. The dried residue was reconstituted in 4 ml of 50 mM sodium phosphate buffer, pH 7.4, containing 0.1 mg human serum albumin per milliliter and was purified as described for plasma. Samples were kept on ice throughout all the procedures. The eluants were collected and stored at -20°C. Before radioimmunoassay, the eluants were evaporated to dryness under vacuum and reconstituted in assay diluent. We previously demonstrated that recoveries of 125I-angiotensin I (Ang I) and II are >80%.

Analyses
Ang I and II levels were quantitated by radioimmunoassays as recently improved and validated in our laboratory. The interassay and intra-assay coefficients of variation for appropriate internal reference standards in the Ang I and II assays averaged <15%. PRA was measured by generation of Ang I using a kit (Baxter Scientific Products, Stillwater, Minn.). ACE activities in the kidney supernatant and plasma were determined by fluorimetric measurement of the enzymatic cleavage of hippurate from hippuryl-histidyl-leucine. Renal ACE activity was expressed as nanomoles per minute per milligram protein. Protein levels were determined by refractometry.

All data are presented as mean±SEM. The significance of differences between groups was determined when appropriate by paired and unpaired t tests or by analysis of variance and Tukey's test.

Results
The body weights of the rats subjected to sham operation or clipping were 286±6 and 285±6 g, respectively, after 7 days, and 315±16 and 302±10 g, respectively, after 25 days. In the rats clipped for 7 days, the systolic arterial pressure increased by 28% (from 114±2 to 146±2 mm Hg; p<0.001). No changes in systolic pressure occurred in the sham-operated rats (Figure 1). In the rats clipped for 25 days, systolic pressure was significantly higher in 2K1C than in sham-operated rats by 2 weeks and continued to increase. At 25 days, systolic pressure averaged 170±7 mm Hg. In the sham-operated group, systolic blood pressure did not change significantly from 14 to 25 days after surgery (Figure 1).

Plasma levels of Ang I and II and PRA were elevated in 2K1C rats 7 days after clipping, but plasma ACE activity did not change (Figure 2). However, in the rats clipped for 25 days, plasma levels of Ang I and II, ACE activity, and PRA were not significantly different in 2K1C versus sham-operated rats (Figure 2). In 2K1C rats, Ang I content of the clipped kidney increased significantly versus that of the sham-operated and nonclipped kidneys after both 7 and 25 days. Ang I content of nonclipped kidneys was not different from sham-operated kidneys at either time point (Figure 3). In contrast, Ang II contents were significantly elevated both in clipped and nonclipped kidneys versus sham-operated kidneys at 7 and 25 days after clipping, al-
FIGURE 1. Line graphs show systolic blood pressures of sham-operated (○) and two-kidney, one clip (●) rats clipped for 7 days (upper panel) and 25 days (lower panel). Values are mean±SEM, n=5–9 per group. *p<0.05, **p<0.001 when compared with sham-operated controls (analysis of variance for repeated measures and Tukey's test).

though the magnitude of the increase in the nonclipped kidney was reduced in the 25-day group (Figure 3). In the rats clipped for 7 days, ACE activity was significantly elevated in both clipped and nonclipped versus sham-operated kidneys. In contrast, in the rats clipped for 25 days, ACE activity was significantly elevated only in the nonclipped kidney (Figure 4). As an indirect assessment of ACE activity in plasma and kidney, the ratio of Ang II to Ang I was calculated. The Ang II/Ang I ratio in plasma was unchanged at both 7 and 25 days after clipping. However, the ratio was significantly higher both in the clipped and nonclipped kidneys after 7 days, the ratio being significantly greater in nonclipped versus clipped kidneys. In contrast, after 25 days, the Ang II/Ang I ratio was significantly higher only in the nonclipped kidneys (Figure 5). In the sham-operated rats, there were no differences in the tissue contents of Ang I and II, the Ang II/Ang I ratios, and ACE activity between the sham-operated kidney and the intact kidney at either 7 or 25 days (data not shown).

Discussion

By using an improved methodology for measurement of renal angiotensin peptide contents, we extended previous preliminary findings that Ang II levels are increased in the contralateral kidneys as well as in the clipped kidneys of 2K1C hypertensive rats. The present study demonstrates that the intrarenal Ang II contents are elevated during both the initial and maintenance phases of 2K1C hypertension in rats despite a return of PRA and plasma Ang II levels to normal by 25 days. The latter findings are consistent with previous reports. The Ang I levels were elevated in the clipped kidneys at both 7 and 25 days. These findings indicate that the elevated renal Ang II contents in the clipped kidney results from the elevated or maintained renin and Ang I levels that persist during both initial and maintenance phases. The present findings also demonstrate that ACE activity was increased in the clipped kidneys on day 7 after clipping, suggesting that activation of the enzyme contributed to the increased production of Ang II in the clipped kidney during the initial phase. Even though the ACE activity in the clipped kidneys was similar to that in the nonclipped kidneys, the Ang II/Ang I ratio in the clipped kidneys was significantly less than that of the nonclipped kidneys (Figure 5), suggesting combined effects of the enzyme and substrate on the resultant high Ang II levels in the clipped kidney.
In the nonclipped kidneys, the Ang I levels were not suppressed on either day 7 or 25, although a small decrease (not significant) was seen in the 25-day group. Furthermore, the Ang II levels in the nonclipped kidneys were not reduced at either 7 or 25 days after clipping; rather, the Ang II content of the nonclipped kidneys was actually significantly increased at both these times. It is probable that the previous failure to demonstrate significant increases of Ang II levels in the nonclipped kidney was due to the variability created by the protracted extraction techniques used that may result in substantial Ang II generation in vitro. The mechanisms responsible for the maintained Ang I levels and the elevated Ang II levels observed in the present study remain unclear since it has been shown consistently that the nonclipped kidney undergoes a marked reduction in renin content. The responsible mechanism for the maintained Ang I levels and the elevated Ang II levels observed in the present study remains unclear since it has been shown consistently that the nonclipped kidney undergoes a marked reduction in renin content.11,16 The mechanisms responsible for the maintained Ang I levels and the elevated Ang II levels observed in the present study remain unclear since it has been shown consistently that the nonclipped kidney undergoes a marked reduction in renin content.11,16 The mechanisms responsible for this differential regulation of ACE activity remains to be determined. Similarly, the mechanism responsible for maintained or increased substrate availability in the nonclipped kidney remains unresolved. However, the low renin content that has been shown to exist raises the possibility that there is an alternate non-renin-dependent mechanism that is responsible for intrarenal Ang II enzymatic degradation of hippuryl-histidyl-leucine and by radioligand binding. Nevertheless, there must also be sustained availability of Ang I, which suggests that substrate formation is also maintained or increased.

It appears that the acute phase of hypertension in the 2KIC model is dependent on increased circulating activity of the RAS caused by renin release from the clipped kidney. In the maintenance phase, it is evident that elevated Ang II in the nonclipped kidney continues to exert an important role even though intrarenal renin content is reduced to barely detectable levels. It was initially thought that the Ang II, or its decapeptide precursor, was delivered to the renin-poor nonclipped kidney by the systemic circulation. This issue remains controversial since the initially elevated circulating levels of angiotensin peptides decrease toward normal levels, as shown by previous studies as well as the present one. The present study suggests that the elevated Ang II levels are generated intrarenally as a result of enhanced ACE activity. The mechanism responsible for this differential regulation of ACE activity remains to be determined. Similarly, the mechanism responsible for maintained or increased substrate availability in the nonclipped kidney remains unresolved. However, the low renin content that has been shown to exist raises the possibility that there is an alternate non-renin-dependent mechanism that is responsible for intrarenal Ang II
formation in the nonclipped kidney. Whatever the mechanism, it is clear from the present data that Ang II levels in the nonclipped kidney are significantly elevated during both the development and maintenance phases of 2K1C Goldblatt hypertension. Since an increase in intrarenal Ang II levels would exert a substantial sodium-retaining influence on the nonclipped kidney, it is likely that such actions of the elevated Ang II levels within the nonclipped kidney would contribute importantly not only to the development of 2K1C Goldblatt hypertension but also to the maintenance of the hypertension after the increased circulating Ang II levels subside.

In summary, the present study demonstrates a dissociation between intrarenal and circulating levels of Ang I and II in 2K1C Goldblatt rats. A similar dissociation has been reported recently in rats receiving ACE inhibitors. Thus, these data provide further evidence supporting differential regulation of intrarenal Ang II levels.

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