Depletion of Tissue Angiotensin-Converting Enzyme Differentially Influences the Intrarenal and Urinary Expression of Angiotensin Peptides

J. Gregory Modrall, Javid Sadjadi, K. Bridget Brosnihan, Patricia E. Gallagher, Chun-hua Yu, Gerald L. Kramer, Kenneth E. Bernstein, Mark C. Chappell

Abstract—The relative contribution of circulating versus tissue renin-angiotensin systems to the tissue expression of angiotensin peptides in the kidney remains unresolved. To address this issue, intrarenal and urinary levels of the peptide products of the renin-angiotensin system were assessed in a tissue angiotensin-converting enzyme knockout (tisACE−/−) mouse model. Systolic blood pressure was significantly lower (64.6±3.6 versus 81.4±4.5 mm Hg; *P<0.02) and urinary volume was increased (7.25±0.86 versus 2.86±0.48 mL/d; *P<0.001) in tisACE−/− mice compared with wild-type mice. Intrarenal angiotensin II was 80% lower in tisACE−/− mice compared with wild-type mice (5.17±0.60 versus 25.5±2.4 fmol/mg protein; *P<0.001). Intrarenal angiotensin I levels also declined by a comparable extent (73%) in the tisACE−/− mice (*P<0.01). Intrarenal angiotensin-(1–7) concentrations were similar between the strains, but the ratio of intrarenal angiotensin-(1–7) to angiotensin II and angiotensin I in tisACE−/− mice increased 470% and 355%, respectively, compared with wild-type mice. Urinary excretion of angiotensin II and angiotensin-(1–7) were not different, but the excretion of angiotensin I increased 270% in tisACE−/− mice (*P<0.01). These studies suggest 2 potential mechanisms for the reduction of intrarenal angiotensin II in tisACE−/− mice: (1) an attenuated capacity to form angiotensin II by renal angiotensin-converting enzyme and (2) significant depletion of its direct precursor angiotensin I in renal tissue. Sustained intrarenal levels of angiotensin-(1–7) may contribute to chronic hypertension and polyuria in tisACE−/− mice, particularly in the context of depleted angiotensin II in the kidney. (Hypertension. 2004;43:849-853.)

Key Words: angiotensin II ■ angiotensin I ■ renin-angiotensin system ■ mice

The renin-angiotensin system (RAS) plays a central role in blood pressure (BP) regulation and electrolyte homeostasis. The RAS was initially described as a circulating endocrine system with angiotensin II (Ang II) as its only biologically active product. With cloning of the genes of the RAS, it became apparent that each of the components of the RAS is critically active product. With cloning of the genes of the RAS, it became apparent that each of the components of the RAS is critically active product. With cloning of the genes of the RAS, it became apparent that each of the components of the RAS is critically active product. With cloning of the genes of the RAS, it became apparent that each of the components of the RAS is critically active product. With cloning of the genes of the RAS, it became apparent that each of the components of the RAS is critically active product. With cloning of the genes of the RAS, it became apparent that each of the components of the RAS is critically active product. With cloning of the genes of the RAS, it became apparent that each of the components of the RAS is critically active product.
active but entirely secreted from cells. Mice expressing the transgene have no tissue ACE and possess ~40% of normal circulating levels of ACE. Unlike the profound phenotypic abnormalities observed in ACE null mice, tisACE⁻/⁻ mice demonstrate only mild hypertension, urine-concentrating defects, and male infertility. As such, this knockout mouse model permitted a careful analysis of the relative contributions of local and endocrine RAS to steady-state levels of angiotensin peptides in the kidney.

Methods
An extended method section can be found in an online supplement available at http://www.hypertensionaha.org.

tisACE⁻/⁻ Knockout Mice
The tisACE⁻/⁻ mice were generated as previously described. Wild-type (ACE⁻⁻) littermates were used as controls. Noninvasive BP was measured using a tail-cuff photosensor (ITC, Woodland Hills, Calif). The study was approved by the Institutional Animal Care and Use Committee.

Angiotensin Peptide Assays
Urine was collected in 1 N HCl to prevent ex vivo metabolism of angiotensin peptides and extracted on Sep-Pak C₁₈ cartridges (200 mg; Waters Corporation, Milford, Mass) as described previously. Blood was collected via cardiac puncture into chilled tubes containing 3.8% sodium citrate (Lonza, Walkersville, Md). Plasma was separated from the blood by centrifugation at 3,000 × g for 10 minutes at 4°C.

Angiotensin Peptide Assays
To determine if there is differential expression of angiotensin peptides in the plasma and kidneys of wild-type and tisACE⁻/⁻ mice. Intrarenal Ang II levels were quantified in the kidneys of wild-type and tisACE⁻/⁻ mice (Figure 1A). To assess the levels of alternative peptide products of the RAS, intrarenal levels of the vasoactive peptide Ang-(1–7) were quantified in contrast to Ang II, the intrarenal Ang-(1–7) concentrations were similar between the strains (Figure 1A), but the ratio of intrarenal Ang-(1–7) to Ang II in the kidneys of tisACE⁻/⁻ mice increased 470% and 355%, respectively, compared with wild-type mice (Figure 1B). As shown in Figure 2, the HPLC/RIA analysis of pooled tissue samples from the wild-type (Figure 2A) and tisACE⁻/⁻ (Figure 2B) mice confirmed the reduction in Ang II and the sustained levels of Ang-(1–7) in ACE-depleted kidneys.

Plasma Angiotensin Peptides
To determine if there is differential expression of angiotensin peptides in the plasma and kidneys of wild-type and tisACE⁻/⁻ mice. Intrarenal Ang II levels were decreased 80% in tisACE⁻/⁻ mice compared with wild-type mice (Figure 1A). This finding suggests that tissue RAS plays a pivotal role in the maintenance of intrarenal Ang II levels. The intrarenal levels of its precursor Ang I were also decreased by 80% in tisACE⁻/⁻ mice (Figure 1A). To assess the levels of alternative peptide products of the RAS, intrarenal levels of the vasoactive peptide Ang-(1–7) were quantified in contrast to Ang II, the intrarenal Ang-(1–7) concentrations were similar between the strains (Figure 1A), but the ratio of intrarenal Ang-(1–7) to Ang II in the kidneys of tisACE⁻/⁻ mice increased 470% and 355%, respectively, compared with wild-type mice (Figure 1B). As shown in Figure 2, the HPLC/RIA analysis of pooled tissue samples from the wild-type (Figure 2A) and tisACE⁻/⁻ (Figure 2B) mice confirmed the reduction in Ang II and the sustained levels of Ang-(1–7) in ACE-depleted kidneys.

Blood Pressure and Renal Function
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tisACE−/− mice, angiotensin peptides were also quantified in the plasma. As expected, plasma Ang II levels were decreased 50% in tisACE−/− mice (Figure 3). In contrast to intrarenal expression of Ang I (Figure 1A), plasma Ang I was increased 3-fold in the plasma of tisACE−/− mice (Figure 3). Plasma levels of Ang-(1–7) were also increased in tisACE−/− mice compared with wild-type mice (Figure 3). The ratios of angiotensin peptides in the plasma of tisACE−/− and wild-type mice are shown in online Figure I (http://www.hypertensionaha.org). These data suggest that there is differential regulation of angiotensin peptide expression in the plasma and kidneys of tisACE−/− mice.

Urinary Angiotensin Peptides

Urinary peptide levels were assessed to determine if urinary excretion of these peptides parallels intrarenal expression. The 24-hour urinary excretions of Ang II and Ang-(1–7) were not different between the strains (Figure 4). It is notable, though, that urinary excretion of Ang I increased 270% in the tisACE−/− mice (Figure 4). The concentration (pmol/mL urine) of urinary Ang I was unchanged in tisACE−/− mice, compared with wild-type mice (tisACE−/− = 1.27 ± 0.035; ACE+/+ = 1.63 ± 0.21; P = 0.393), because of the increased urinary volume of the knockout mice. The ratios of angiotensin peptides in the urine of tisACE−/− and wild-type mice are shown in online Figure II (http://www.hypertensionaha.org).

Renal Renin, Angiotensinogen, and ACE2 mRNA Levels

The mRNA levels (in relative absorbance units) for renin in the kidney were markedly elevated (>600%) in the tisACE−/− mice (5.38 ± 0.43 versus 0.65 ± 0.09; online Figure III, http://www.hypertensionaha.org). In contrast, the mRNA levels for angiotensinogen in the kidney were not changed in tisACE−/− mice (0.70 ± 0.07 versus 0.92 ± 0.17; Figure III).
tissue ACE activity in the kidney by permitting complete blockade of ACE
terminated in wild-type (n=8) and tisACE−/− mice (n=8). *P<0.01.

**Discussion**

Recognition of tissue-specific local RAS has prompted questions regarding the primacy of the intrarenal versus the systemic RAS in determining steady-state levels of Ang II in the kidney. The tisACE−/− model provided a unique opportunity to experimentally dissect the relative contributions of the local and systemic RAS to steady-state Ang II levels in the kidney by permitting complete blockade of ACE-dependent Ang II production by tissue RAS. Using this approach, the absence of tissue ACE activity in tisACE−/− mice induced a profound (∼80%) decrement in intrarenal Ang II levels. These data confirm the critical role of tissue RAS in the maintenance of steady-state Ang II levels in the kidney. The physiological relevance of this finding is underscored by the relative hypotension and polyuria in tisACE−/− mice that is presumed to be derived from decreased intrarenal Ang II in these mice.

These data further suggest that tissue ACE activity per se is critical for maintenance of steady-state Ang II levels in the kidney. This finding appears to contradict a previous study that used an alternate transgenic mouse model and noted that deletion of intrarenal ACE did not reduce intrarenal Ang II levels.10 This discrepancy may be explained by subtle differences in the transgenic mouse models used. Wei et al used a transgenic model in which intrarenal ACE was reduced to 20% of wild-type mice, while no tissue ACE activity is detectable in tisACE−/− mice.11 Taken together, these data indicate that even small amounts of residual tissue ACE activity are sufficient to maintain intrarenal Ang II levels. This observation corroborates previous studies that noted that incomplete ACE inhibition does not decrease intrarenal Ang II levels.10,22

An additional explanation for the marked reduction of intrarenal Ang II in tisACE−/− mice is the significant depletion of its immediate precursor Ang I in renal tissue (Figure 1). Depletion of intrarenal Ang I in ACE-depleted mice was unexpected because previous studies reported an increase in plasma Ang I with ACE inhibition.22 This observation per-
hydrolysis of Ang-(1–7) by ACE is an important determinant of steady-state levels of Ang-(1–7) in the kidney.

Perspectives
This study provided direct evidence of the relative contribution of the local RAS in the kidney in maintaining steady-state Ang II levels in the kidney, compared with the systemic endocrine RAS. Interruption of the tissue RAS in in vivo ACE knockout mice induced a profound decrement in intrarenal Ang II levels, producing chronic hypotension and polyuria. In addition to augmenting our understanding of the RAS, this finding has direct clinical physiological and clinical relevance. These data explain the dissociation between plasma ACE activity and tissue renin-angiotensin system (RAS) levels.21 These data suggest that future pharmacological strategies for modulation of the RAS should focus on impacting tissue RAS, recognizing that the circulating endocrine RAS represents only a small fraction of the functional RAS.

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