Upregulation of autocrine-paracrine renin-angiotensin systems in chronic renovascular hypertension

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Introduction: **The mechanism by which hypertension is maintained in renovascular hypertension remains poorly defined. Because plasma angiotensin II does not correlate with blood pressure in RVH, we postulated that activation of tissue-specific autocrine-paracrine renin-angiotensin systems may upregulate local production of angiotensin II and maintain hypertension in chronic RVH.**

Methods: **RVH was induced with a two-kidney one-clip (2K1C) rat model. Animals were killed at 1 or 12 weeks after surgery (acute or chronic RVH). Angiotensin II was quantitated with radioimmunoassay. Angiotensin II–type 1 (AT_1) receptor density was determined with immunoblotting and immunohistochemistry.**

Results: **Blood pressure was significantly elevated in 2K1C animals compared with sham animals at 1 week (141 ± 5 mm Hg versus 98 ± 3 mm Hg; $P < .0005$) and at 12 weeks (164 ± 14 mm Hg versus 110 ± 7 mm Hg; $P < .0005$) after surgery. No significant difference was seen in plasma angiotensin II levels between 2K1C and control animals during acute (38.2 ± 6.5 fmol/mL versus 27.6 ± 6.8 fmol/mL; P = not significant) or chronic (40.1 ± 17.4 fmol/mL versus 27.1 ± 6.5 fmol/mL; P = not significant) RVH. During acute RVH, intrarenal angiotensin II was significantly increased in both the clipped (126.0 ± 16.2 fmol/g versus 62.0 ± 6.2 fmol/g; $P < .005$) and unclipped (78.9 ± 6.3 fmol/g versus 39.9 ± 2.5 fmol/g; $P < .05$) kidneys of 2K1C animals compared with control animals. Increased intrarenal angiotensin II levels persisted in chronic RVH in the clipped (147.4 ± 37.7 fmol/g versus 59.2 ± 8.7 fmol/g; $P < .05$) and unclipped (130.8 ± 31.8 fmol/g versus 63.0 ± 11.0 fmol/g; $P < .05$) kidneys of 2K1C animals compared with controls. Adrenal angiotensin II content of 2K1C animals was unchanged in acute RVH (493.7 ± 51.4 fmol/g versus 522.6 ± 80.5 fmol/g; P = not significant) but increased nearly three-fold over control animals during chronic RVH (1129.0 ± 149.3 fmol/g versus 400.6 ± 59.1 fmol/g; $P < .0005$). No significant difference in AT_1 receptor density was noted in renal tubules of clipped and unclipped kidneys or in the adrenal glands of 2K1C animals during acute or chronic RVH compared with control animals.**

Conclusion: **Tissue angiotensin II production is upregulated in the kidneys and adrenal glands in chronic RVH, and AT_1 receptor density is maintained in these tissues, providing a potential mechanism for maintenance of hypertension in RVH. (J Vasc Surg 2002;36:386-92.)**

Renovascular hypertension (RVH) is postulated to be the consequence of pathologic activation of the renin-angiotensin system (RAS) induced by renal artery stenosis. This assertion is supported by the observation that angiotensin-converting enzyme (ACE) inhibitors have antihypertensive effects in both clinical and experimental models of RVH.[1-4] Despite evidence suggesting a central role for the RAS in RVH, plasma levels of the hormone angiotensin II are not always increased in RVH.[5,6] Brown et al[5] showed that patients with long-standing unilateral RVH may have normal levels of circulating angiotensin II and that angiotensin II levels do not correlate with blood pressure (BP). Similarly, the two-kidney one-clip (2K1C) rat model of experimental RVH is characterized by a chronic phase of hypertension in which circulating angiotensin II returns to normal.[5-8] This observation presents a paradox in which hypertension persists despite normal circulating levels of angiotensin II.

The observation that individual components of the RAS, such as renin, angiotensinogen, ACE, and the angiotensin II–type 1 (AT_1) receptor, are expressed in multiple tissues involved in BP regulation portends the possibility that angiotensin II may be generated locally by tissue-specific RAS. Upregulation of gene expression for elements of the RAS may ultimately permit increased local production of angiotensin II and its AT_1 receptor, without an increase in plasma angiotensin II. Increased tissue angiotensin II levels in the kidney and adrenal gland may aug-

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ment sodium reabsorption[9] and aldosterone release,[10] respectively, to increase BP and reconcile the discrepancy between BP and plasma angiotensin II in clinical RVH. In support of this notion, our laboratory and others have shown upregulation of components of the RAS in tissues involved in BP regulation in RVH.[8,11-17] This study examined the hypothesis that renal artery stenosis upregulates local angiotensin II levels in the kidneys and adrenal glands to maintain hypertension in chronic RVH.

METHODS

Animal protocol. The 2K1C model was used to induce experimental RVH. This model is described elsewhere.[2] The 2K1C model is characterized by three physiologic phases.[2] Phase I (0 to 4 weeks) is marked by increased plasma renin and angiotensin II content and hypertension. Phase II (5 to 8 weeks) is marked by decreasing plasma renin and angiotensin II, salt retention, and hypertension. In phase III (>9 weeks), hypertension becomes renin-independent because both plasma renin and angiotensin II content return to normal, simulating clinical RVH.[2]

Sprague-Dawley rats, weighing 200 to 250 g, were randomly allocated to two treatment groups: 2K1C and sham-operated control groups. Noninvasive BP was measured before surgery and again before death with a tail cuff photosensor (IITC, Inc, Woodland Hills, Calif). A midline laparotomy was used for placement of a 0.2-mm (internal diameter) partially occlusive clip on the left renal artery of 2K1C animals. Control animals underwent laparotomy without clip placement. Animals were killed at 1 or 12 weeks after surgery, corresponding to acute and chronic phases of the 2K1C model.[2] The study was approved by the Institutional Animal Care and Use Committee, and animal care complied with the Guide for the Care and Use of Laboratory Animals.[18]

Plasma and tissue angiotensin II assay. Angiotensin II assays were performed according to the technique of Fox et al.[19] Blood from the infrarenal aorta was collected into a chilled glass tube containing protease inhibitors (pepstatin A and 1,10-phenanthroline) and enalapril maleate (Sigma, St Louis, Mo). Tissues were homogenized in ice-cold methanol. After clarification with centrifugation, samples were applied to a phenyl-bonded solid phase extraction column (Varian, Harbor City, Calif). After elution from the column with 90% methanol, samples were dried and reconstituted for radioimmunoassay. The radioimmunoassay for angiotensin II was performed according to the technique of Fox et al,[19] with rabbit anti–angiotensin II antibody (Phoenix Pharmaceuticals, Inc, Belmont, Calif), with cross reactivity of less than 2% for angiotensin II precursors and degradation products. After incubation of the radioimmunoassay for 48 hours at 4° C, bound and free angiotensin II were separated with dextran-coated charcoal. The supernatant was counted with a γ-counter (ICN, Costa Mesa, Calif). The ratio B/B_o was corrected for nonspecific binding, expressed as a percentage of maximal binding, and read against a standard curve (log-logit transformation) with linear regression.

Kidney brush border membrane isolation. Kidney tubule brush border membranes (BBMs) were isolated for determination of AT_1 receptor density because this cell type is most relevant to the kidney's role in sodium reabsorption and BP regulation. AT_1 receptor expression is differentially regulated in glomeruli and tubules,[20,21] so isolation of tubule BBMs also circumvents this potential pitfall. BBMs were isolated with a standard magnesium precipitation protocol.[22] Briefly, superficial renal cortex was homogenized and magnesium precipitated twice with 0.54 mL of 1 mol/L $MgCl_2$ solution in 15 mL H_20. Ultracentrifugation at 39,800*g* for 36 minutes yielded a BBM pellet for immunoblotting.

Adrenal protein preparation for immunoblotting. Preparation of adrenal protein for immunoblotting was on the basis of the protocol of Harrison-Bernard et al.[20] Briefly, adrenals were homogenized, clarified with centrifugation, and treated with deoxycholate (0.05% weight/volume, 30 minutes). A pellet was obtained with centrifugation at 20,000*g* for 30 minutes at 4° C.

Immunoblotting. Multiple approaches were used for quantitation of AT_1 receptor density in renal tubules and adrenal glands. Radioligand binding is often used for determination of receptor density. However, binding assays with ^{125}I–angiotensin II consistently yielded high nonspecific binding that precluded reliable quantitation of AT_1 receptor density with this technique. Therefore, immunoblotting was used to quantitate AT_1 receptor density.

Twenty-microgram samples were boiled for 5 minutes and separated with gel electrophoresis on 10% polyacrylamide gels. Appropriate negative controls were included. Gels were transferred to nitrocellulose, blocked with blotto, and reacted with rabbit anti-AT_1 receptor primary antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) overnight at 4° C. Goat antirabbit secondary antibody (Research Diagnostics, Inc, Flanders, NJ) was added for 2 hours. Signal was visualized with enhanced chemiluminescence (Pierce, Rockford, Ill) and quantitated with densitometry (Biorad, Hercules, Calif).

Perfusion fixation and immunohistochemistry. Perfusion fixation of tissues was described previously.[23] After perfusion fixation with 3% paraformaldehyde, 0.1 mol/L cacodylic acid/sucrose buffer, 4% pentastarch, 3 mmol/L $MgCl_2$, and 0.05% picric acid, tissues were immersed in formalin for paraffin embedding. Sections were treated with target retrieval solution (DAKO, Carpenteria, Calif), quenched with 3% hydrogen peroxide, and blocked with avidin/biotin blocking solution (Vector, Burlingame, Calif) before staining with rabbit anti-AT_1 receptor primary antibody (Santa Cruz Biotechnology) overnight at 4° C. No cross reactivity of the anti- AT_1 receptor primary antibody with the AT_2 receptor was seen. Slides were treated with biotinylated goat antirabbit secondary antibody (Vector) for 1 hour and stained with streptavidin peroxidase (DAKO) before signal amplification with biotinylated tyrimide (TSA Kit, NEN Life Science, Boston, Mass).

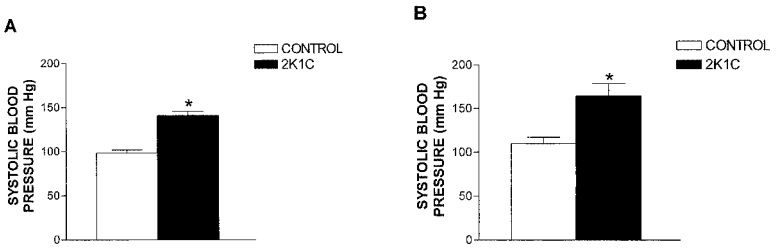

Fig 1. A, Mean systolic BP (mm Hg) 1 week after surgery for 2K1C (n = 12) and control (n = 12) animals (*$P <$ .0005). **B,** Mean systolic BP (mm Hg) 10 weeks after surgery for 2K1C (n = 12) and control (n = 12) animals (*$P <$ .0005).

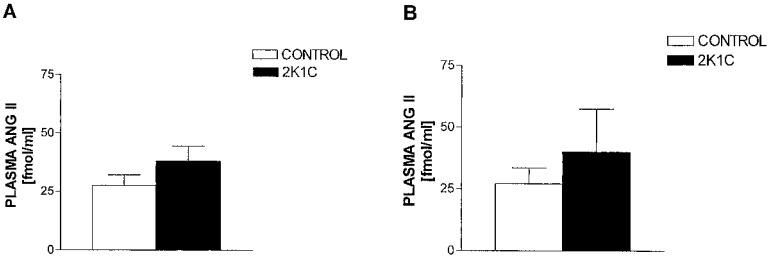

Fig 3. A, Mean plasma angiotensin II (fmol/mL) at 1 week after surgery for 2K1C (n = 11) and control (n = 10) animals (P = not significant). **B,** Mean plasma angiotensin II (fmol/mL) 12 weeks after surgery for 2K1C (n = 12) and control (n = 12) animals (P = not significant).

Staining was detected with diaminobenzidine (Vector). Appropriate negative control slides (no primary antibody) were included in each experiment. Antibody specificity was confirmed with blocking with a 100-fold excess of AT_1 receptor peptide. Slides were reviewed in a blinded manner by a renal pathologist (TER) who examined multiple high-power fields on each slide. Staining of the BBM was graded on a scale from 0 to 3+. A score of 0 was given for slides with no staining, 1+ for minimal staining, 2+ for moderate staining, and 3+ for intense staining at the BBM.

Statistical methods. Sample size was determined with power analysis. Ten animals per group were necesary to achieve a power of 0.8 for angiotensin II assays at a significance level of 0.05. Continuous data were expressed as the mean ± the standard error of the mean and compared between groups with an unpaired two-tailed Student *t* test. A *P* value of less than .05 defined statistical significance.

RESULTS

Morphometric and blood pressure data. Control and 2K1C animals showed no significant difference in body weight or BP before surgery or before death (data not shown). Hypertension developed in 2K1C animals by 1 week after surgery ($P <$ .0005; Fig 1, *A*) and persisted during chronic RVH ($P <$ .0005; Fig 1, *B*) compared with control animals. Weight of clipped and unclipped kidneys was not significantly different between 2K1C and control animals at 1 week after surgery (data not shown). By 12 weeks after surgery, a significant increase in renal mass was noted in the unclipped kidney of 2K1C animals compared with control animals ($P <$ .05; Fig 2, *B*, online only). No significant difference was seen in mean weight of the clipped kidney of 2K1C and control animalss at 12 weeks after surgery (P = not significant; Fig 2, *A*, online only). A significant increase was seen in the mean ratio of kidney weights (unclipped/clipped) in chronic 2K1C animals (1.23 ± 0.05) compared with control animals (0.99 ± 0.01; $P <$ .001).

Plasma and tissue angiotensin II. For determination of whether plasma angiotensin II is upregulated in either the acute or chronic RVH, animals were killed at 1 week or 12 weeks after surgery for quantitation of plasma angiotensin II. Despite significant hypertension in 2K1C animals, no significant difference was seen in plasma angiotensin II levels between 2K1C and control animals during acute RVH (Fig 3, *A*). A similar pattern of plasma angiotensin II was observed in chronic RVH (Fig 3, *B*); the plasma angiotensin II levels of 2K1C animals were not significantly different from control animals.

For determination of whether renal artery stenosis upregulates local RAS in tissues involved in BP regulation, angiotensin II was extracted from the kidneys of 2K1C animals. During acute RVH, intrarenal angiotensin II of the clipped kidney was increased two-fold in 2K1C animals compared with control animals ($P <$ .005; Fig 4, *A*). Interestingly, a similar pattern was observed in the contralateral unclipped kidney of 2K1C animals compared with

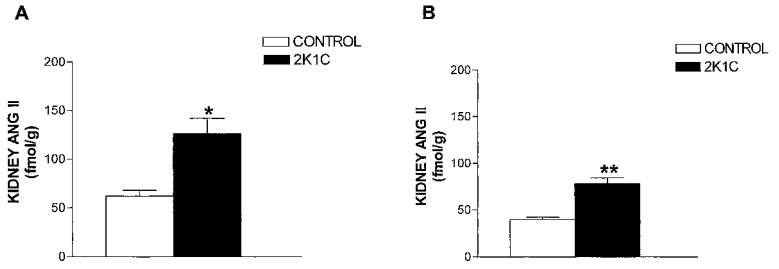

Fig 4. A, Mean intrarenal angiotensin II (fmol/g) in clipped kidney 1 week after surgery for 2K1C (n = 12) and control (n = 12) animals (*$P < .005$). **B,** Mean intrarenal angiotensin II (fmol/g) in unclipped kidney 1 week after surgery for 2K1C (n = 12) and control (n = 12) animals (**$P < .05$).

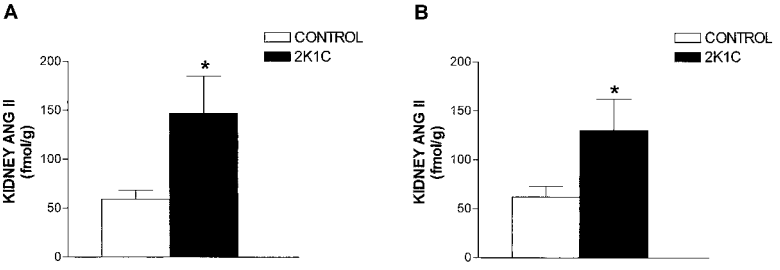

Fig 5. A, Mean intrarenal angiotensin II (fmol/g) in clipped kidney 12 weeks after surgery for 2K1C (n = 12) and control (n = 12) animals (*$P < .05$). **B,** Mean intrarenal angiotensin II (fmol/g) in unclipped kidney 12 weeks after surgery for 2K1C (n = 12) and control (n = 12) animals (*$P < .05$).

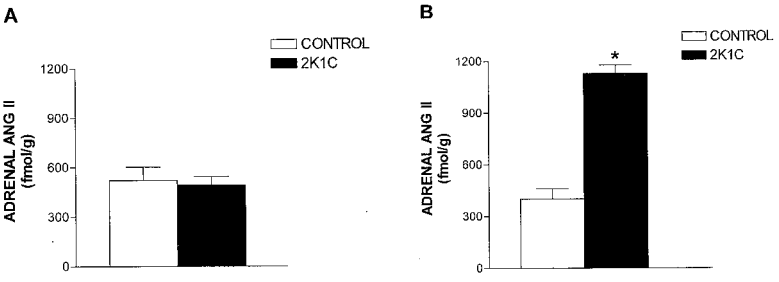

Fig 6. A, Mean adrenal angiotensin II (fmol/g) 1 week after surgery for 2K1C (n = 12) and control (n = 12) animals (P = not significant). **B,** Mean adrenal angiotensin II (fmol/g) 12 weeks after surgery for 2K1C (n = 12) and control (n = 12) animals (*$P < .0005$).

control animals ($P < .05$; Fig 4, *B*). The difference in intrarenal angiotensin II levels between 2K1C and control animals persisted in chronic RVH (Fig 5). At 12 weeks after surgery, intrarenal angiotensin II levels remained elevated in the clipped kidney of 2K1C animals ($P < .05$; Fig 5, *A*). The unclipped kidney of 2K1C animals showed a similar increase in intrarenal angiotensin II content compared with control animals ($P < .05$; Fig 5, *B*).

Because local angiotensin II levels in the adrenal gland are a known stimulant for induction of aldosterone production and secretion,[10] the angiotensin II content of the adrenal glands of 2K1C animals was examined (Fig 6). At 1 week after surgery, no difference was seen in adrenal angiotensin II content between 2K1C and control animals (Fig 6, *A*). However, adrenal angiotensin II content of 2K1C animals was increased nearly three-fold over control animals at 12 weeks ($P < .0005$; Fig 6, *B*). For determination of which of the adrenal glands was responsible for the observed upregulation of adrenal angiotensin II content in 2K1C animals, left and right adrenal glands of chronic RVH animals were processed separately and compared for angiotensin II content. The angiotensin II content of both adrenal glands of 2K1C animals was upregulated equally compared with control rats (data not shown).

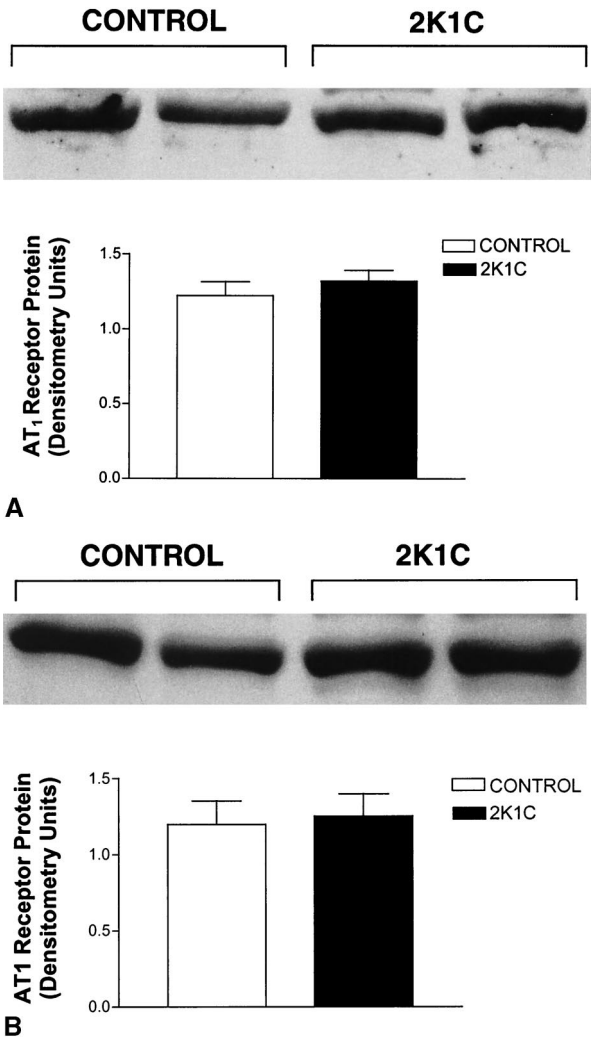

Fig 7. Immunoblot for AT_1 receptor protein (49 kDa) of BBMs from either unclipped **(A)** or clipped **(B)** kidneys of 2K1C and control animals at 12 weeks after surgery. Densitometry data for respective treatment groups (n = 12/group) are depicted (*P* = not significant).

Angiotensin II–type 1 receptor density. During acute RVH, no significant difference in AT_1 receptor protein was noted with immunoblotting of renal tubule BBMs from clipped and unclipped kidneys of 2K1C animals compared with control animals (data not shown). During chronic RVH, both clipped (Fig 7, *B*) and unclipped (Fig 7, *A*) kidneys showed no significant difference in AT_1 receptor density between 2K1C and control animals. A similar pattern of AT_1 receptor density was noted in the adrenal glands; no detectable difference in AT_1 receptor density was observed between 2K1C and control animals at 1 or 12 weeks after surgery (data not shown).

Immunohistochemistry for AT_1 receptor density corroborated the immunoblotting data. No difference in BBM staining for the AT_1 receptor was observed in clipped and unclipped kidneys of 2K1C (n = 4) and control (n = 4) animals during acute (data not shown) or chronic (Fig 8, online only) RVH. Furthermore, no difference in AT_1 receptor staining was noted in the adrenal glands of acute and chronic 2K1C animals (n = 4) compared with control animals (n = 4; data not shown).

DISCUSSION

This study showed marked upregulation of local RAS in tissues involved in BP regulation in a 2K1C model of chronic RVH. During both acute and chronic RVH, no significant increase was seen in plasma angiotensin II. In contrast, tissue angiotensin II content was markedly upregulated in both the clipped kidney and the unclipped kidney of both acute and chronic 2K1C animals. In addition, adrenal angiotensin II content was augmented during chronic RVH. These observations confirm that local RAS are upregulated in RVH, providing a potential explanation for the persistence of hypertension in RVH despite a lack of increased plasma angiotensin II.

The mechanism by which chronic hypertension is maintained in RVH is poorly understood. Several investigators have noted that activation of the systemic RAS cannot fully explain the maintenance of hypertension in chronic RVH.[5-7,13] In fact, plasma levels of renin and angiotensin II are not always increased in RVH.[5-7] Plasma angiotensin II may be unchanged or increased in the acute phase of the 2K1C model but is certainly not significantly elevated in the chronic phase of the 2K1C model or clinical RVH.[24-26] To reconcile the dissociation between plasma angiotensin II content and BP in chronic RVH, our laboratory hypothesized that local angiotensin II production is upregulated in tissues involved in BP regulation.[14-17] To date, most investigators have attempted to deduce the activity of local RAS by examining expression of its components, such as renin, ACE, and angiotensinogen.[11-17] This approach is predicated on the notion that differential expression of components of the RAS could provide a means of increasing local production of angiotensin II. However, examination of expression of components of the local RAS in RVH is a surrogate measure of angiotensin II content in the tissues examined. Posttranslational regulation of protein expression of RAS components may influence local biosynthesis of angiotensin II. In addition, receptor-mediated accumulation of circulating angiotensin II in tissues may augment local biosynthesis to increase local angiotensin II activity.[27] Hence, direct measurement of tissue angiotensin II content, as performed in this study, is essential to examination of the activity of the local RAS in RVH.

Only rarely have investigators attempted to directly measure tissue angiotensin II content in RVH, and even fewer studies have measured angiotensin II in the clinically relevant chronic phase of the 2K1C model. Guan et al[24] found that the angiotensin II content of the unclipped kidney is elevated during the acute phase of 2K1C hypertension, despite normal plasma angiotensin II content and depressed renin messenger RNA (mRNA). Before this

study, however, no studies had examined kidney angiotensin II content during chronic 2K1C hypertension, which most closely simulates clinical RVH. Morishita et al[17] found that brain and adrenal angiotensin II levels were unchanged in chronic RVH. This study found that adrenal angiotensin II levels were increased in chronic RVH. This discrepancy may be attributed to a difference in technique for isolation and quantitation of tissue angiotensin II content. This study used a protocol shown by Fox et al[19] to be least prone to artifactual changes in tissue angiotensin II levels. Thus, the findings of this study provide the most direct evidence to date of upregulation of local RAS in the kidneys and adrenal glands of 2K1C rats in chronic RVH.

This study also addressed the role of the AT_1 receptor in RVH. Without sufficient expression of the AT_1 receptor, increased tissue angiotensin II is of dubious consequence. Many cell types, such as vascular smooth muscle cells and adrenal fasciculata cells,[28,29] respond to prolonged agonist stimulation by downregulating AT_1 receptors. If normal or increased AT_1 receptor density is observed despite prolonged agonist exposure, pathologic activation of the RAS may ensue.[11,20] Consequently, expression of the AT_1 receptor may be a key determinant of whether homeostasis is maintained or hypertension persists.

For determination of the level of AT_1 receptor expression in RVH, prior studies quantitated AT_1 receptor mRNA levels in RVH.[11,14] Haefliger et al[14] noted a decrease in AT_1 mRNA in both kidneys during acute experimental RVH. In contrast, our laboratory found increased AT_1 receptor mRNA levels in both the clipped and unclipped kidneys during chronic RVH.[11] This study is the first to directly measure AT_1 receptor protein in the kidneys and adrenal glands of animals with RVH. AT_1 receptor protein abundance, as measured with immunoblotting and immunohistochemistry, was unchanged in both kidneys and adrenal glands of 2K1C animals during acute and chronic RVH. However, the semiquantitative techniques of immunoblotting and immunohistochemistry possibly may not detect minor changes in AT_1 receptor density. The discrepancy between mRNA and protein abundance may be a product of posttranslational regulation of AT_1 receptor protein expression or the result of the different fractions of the kidney examined (whole kidney homogenates versus BBMs in this study). Maintenance of AT_1 receptor expression, without downregulation of receptor numbers, is relevant to the pathogenesis of RVH. In the context of increased tissue angiotensin II content, this level of AT_1 receptor expression provides a mechanism for sustained signaling and physiologic effects to augment BP.

Upregulation of angiotensin II and maintenance of AT_1 receptor expression in the chronic phase of experimental RVH is consistent with the known pathophysiology of the 2K1C model.[2] As RVH evolves in this model, hypertension becomes progressively more renin-independent because both plasma renin and angiotensin II return to normal in the chronic phase of the model. Concurrently, 2K1C hypertension evolves into the volume hypertension that characterizes clinical RVH. Because intrarenal angiotensin II is known to augment tubular sodium reabsorption in normotensive and 2K1C rats,[9] the observation that chronic 2K1C rats have increased intrarenal angiotensin II levels in both kidneys is particularly relevant to the evolution of volume hypertension in RVH. Increased adrenal angiotensin II is known to stimulate aldosterone secretion,[10] so our demonstration of increased adrenal angiotensin II levels in 2K1C animals provides a mechanism for aldosterone release despite normal plasma angiotensin II. Taken together, our findings provide a mechanism for promoting volume retention and hypertension in RVH.

Although increased tissue angiotensin II in the kidneys and adrenal glands of 2K1C animals is presumed to lead to hypertension, a limitation of this study is its inability to directly establish a causal relationship between increased tissue angiotensin II and hypertension. Standard approaches to showing causality, such as blockade of the RAS during RVH, are problematic because ACE inhibitors affect both the systemic RAS and local RAS. Such a global effect on angiotensin II production would preclude any determination regarding the pathogenetic role of tissue-specific RAS in maintaining hypertension in chronic RVH. Future studies will likely require the use of alternative approaches, such as gene delivery of antisense DNA, to block the local RAS of specific organs to determine their pathogenetic role in RVH.

This study cannot exclude alternative scenarios involving interactions between the systemic RAS and local RAS. Plasma renin activity was not measured, and a previous report noted that plasma renin activity may be increased in rats during chronic RVH.[30] Thus, this study cannot exclude the possibility that renin may be synthesized in the ischemic kidney, released into the plasma, and transported to the nonischemic kidney and adrenal glands. After arriving in these tissues, renin could convert angiotensinogen to angiotensin I. This scenario would require activation of tissue RAS because plasma ACE activity is not increased in 2K1C hypertension and tissue ACE activity is upregulated in both the clipped and unclipped kidneys of 2K1C animals.

This study raises additional questions regarding activation of local RAS. How the local RAS of the unclipped kidney and adrenal glands are activated in RVH remains unclear from this study. Recent reports suggest that small incremental increases in plasma angiotensin II, such as that observed in RVH, may have profound affects on tissue angiotensin II levels in distant organs.[27,31,32] Such increases in tissue angiotensin II could not be explained with "trapping" of plasma in these organs because the concentration of angiotensin II in the kidney and adrenal glands are several-fold higher than plasma angiotensin II concentration. Instead, small incremental increases in plasma angiotensin II likely activate local RAS to induce angiotensin II production in the unclipped kidney and adrenal glands. Increased tissue angiotensin II could then act in an autocrine-paracrine fashion to increase BP. Careful deduction of the complex interactions between local and systemic

RAS will be critical to understanding the mechanisms by which hypertension is maintained in RVH.

In summary, this study confirmed that local RAS are upregulated in the kidneys and adrenals of chronic RVH animals. In addition, AT_1 receptor expression was maintained in proximal tubules and adrenal glands despite increased local angiotensin II content. These data provide a means by which increased tissue angiotensin II may augment BP. The mechanism by which such local RAS are activated remains unknown. Decreased perfusion pressure at the juxtaglomerular apparatus may activate the ischemic kidney's RAS, but how the local RAS of the nonischemic kidney and adrenal glands are subsequently activated is not known. Despite the questions that remain, this study offers further insight into the pathogenesis of RVH.

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