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INTRATUBULAR RENIN-ANGIOTENSIN SYSTEM IN HYPERTENSION

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Introduction

The complexity of the intrarenal renin-angiotensin system (RAS) continues to reveal itself as evidence accumulates demonstrating its robust independent regulation in the interstitial and intratubular compartments within the kidney[1–3]. Early reports demonstrating the presence of angiotensin II (Ang II) receptors on the brush border of proximal tubules suggested physiological roles[4]. However, because of the abundance of degradating enzymes on the brush border, the concentrations of angiotensin peptides were considered to be relatively low. Nevertheless, the abundance of luminal Ang II receptors throughout proximal and distal nephron segments sustained interest in its luminal actions[5,6]. Tubular perfusion studies indicating that luminal Ang II alters tubular sodium and volume reabsorption rate [1,5,7,8] supported an important physiological role for luminal Ang II receptors[9].

A paradigm shift occurred when it was discovered that the proximal intratubular concentrations of Ang I and II were much greater than their corresponding plasma concentrations[7,10,11]. In addition, when proximal tubular fluid was incubated with excess renin, the resultant formation of Ang I indicated very high angiotensinogen (AGT) substrate availability in this segment[7,12]. Furthermore, tubular fluid collected from downstream segments of perfused tubules also had Ang II concentrations similar to those in non-perfused tubules thus supporting a local origin[11]. These findings, along with the demonstration that proximal tubule cells express AGT mRNA and protein[13,14], established the foundation for the existence of a robust physiologically important tubular RAS.

Intratubular Ang II Receptors and Ang II Concentrations

The principal AT receptor in adult kidneys is the AT_1 receptor[5], although AT_2 receptors are upregulated in certain conditions [15,16] and may also play a role in renin synthesis[17].

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Disclosures
None

Nevertheless overall renal AT_1 receptor abundance far exceeds AT_2 receptor levels [5,18] and AT_1 receptors are widely distributed on luminal membranes throughout the nephron segments including proximal tubule, thick ascending limb of Loop of Henle, macula densa, distal tubule and collecting ducts (CD)[5,19]. The regulation of intrarenal AT_1 receptors is complex as vascular AT_1 receptors are downregulated while tubular AT_1 receptors are either sustained or upregulated by elevated Ang II levels[1,6,20,21].

The presence of AT_1 receptors on luminal membranes of various nephron segments generated interest in the Ang II concentrations available to activate the receptors[7,22–24]. Proximal tubule fluid concentrations of Ang I and Ang II are in the range of 5–10 pmol/ml [2,7] which are similar to renal interstitial fluid concentrations25. The tubular Ang II concentrations remain elevated in hypertension models including Ang II infused hypertension26, Goldblatt hypertension [27] and TGR(mRen2) rats [28] suggesting their sustained actions on proximal reabsorption rate. The critical importance of kidney AT_1 receptors in the regulation of normal blood pressure and development of hypertension has been demonstrated by studies showing that AT1a receptors in the kidneys are essential for normal blood pressure regulation and for mediating the hypertensive response to Ang II infusions[29,30]. Furthermore, AT_{1a} knockout mice fail to develop hypertension in response to unilateral renal arterial constriction[31,32].

The tubular fluid Ang II concentrations in other nephron segments have not been measured due to difficulty in collecting sufficient fluid for analysis. Measurements made from urine samples collected under conditions where the major distal nephron transport systems were pharmacologically blocked, suggest CD concentrations in the range of 0.5 pmol/ml for control mice with about a two fold increase in Ang II infused hypertensive mice[33]. Increased urinary excretion rates of Ang II also occur in chronic Ang II infused rats [34,35] and these were decreased during treatment with AT_1 receptor blockers even though the circulating Ang II concentrations were increased35. These recent studies indicate that distal nephron Ang II is formed locally in the tubules at concentrations that are sufficiently high to influence distal nephron transport function which has been shown to respond to Ang I and Ang II[7,22,23]. Distal nephron Ang II was recently shown to enhance the sensitivity of the "connecting tubule glomerular feedback mechanism" that communicates signals between the connecting tubule (CNT) and the afferent arteriole[36]. In contrast to the macula densa tubular glomerular feedback mechanism where Ang II augments its vasoconstriction capability[5,37], the effect of Ang II on the CNT feedback mechanism is afferent vasodilatation36.

AT_1 receptors are also responsible for internalizing Ang II and the presence of substantial Ang II in endosones in both control and Ang II infused hypertensive rats supports their internalization into a protected compartment that prevents degradation of some of the internalized Ang II[20]. AT_1 receptor blockade prevents the internalization of the Ang II. Intracellular Ang II may activate various signaling pathways and also contribute to fibrogenic proliferative responses and microthrombosis[38–40]. Internalized Ang II may also migrate to the nucleus to exert transcriptional effects[38,41]. Ang II binding sites have been shown on nuclear membranes [41,42] and co-localization with nuclear markers suggests migration of the receptor complex to the nucleus[38,43].

Augmentation of Intrarenal AGT in Hypertension

The seminal findings that AGT mRNA and protein are present in proximal tubule cells generated a great deal of interest regarding its intrarenal function[13,44–46]. Chronic Ang II infusions augment intrarenal AGT mRNA and protein in proximal tubule cells in rats and mice[13,14,47,48]. This effect is mediated via activation of AT_1 receptors as it is prevented by treatment with ARBs[48,49]. Ang II also stimulates AGT production in proximal tubule cell

cultures[50]. Thus, chronic infusions of Ang II in rats and mice lead to an augmentation of AGT expression leading to greater generation and intrarenal production of Ang II (Figure 1). Importantly, this process appears to be self limiting as higher Ang II infusions do not stimulate AGT mRNA [48] and complex signaling mechanisms are activated to prevent uncontrolled positive feedback[51,52].

Because the level of AGT is close to the Michaelis-Menten constant for renin, AGT levels can also control RAS activity; thus, upregulation of AGT levels may lead to elevated angiotensin peptide levels[53]. Studies on rat and mouse models of hypertension have documented the effect of augmented AGT in the activation of the RAS[54–58]. Genetic manipulations that lead to overexpression of the AGT gene cause hypertension[55,59]. In human genetic studies, a linkage has been established between the AGT gene and hypertension[60–63]. Enhanced intrarenal AGT mRNA and/or protein levels occur in experimental models of hypertension and diabetes including Ang II-dependent hypertensive rats [13,14,47,49] and mice[48,56,64], Dahl salt-sensitive hypertensive rats[65], and spontaneously hypertensive rats[66], as well as in kidney diseases including diabetic nephropathy[67–69], IgA nephropathy[70–72], and radiation nephropathy[73]. Thus, increased intrarenal AGT contributes to the development and progression of hypertension and may be useful as a predictor of developing kidney disease[1,74]. While clearly related to activation of AT_1 receptors[49], the mechanism by which Ang II stimulates AGT mRNA and protein is complex and appears to require interactions with inflammatory factors including interleukin 6, and increased oxidative stress[75–77].

Urinary excretion rates of AGT provide an index of intratubular RAS status and are correlated with kidney Ang II levels in Ang II-dependent hypertensive rats[78,79]. Furthermore, mice overexpressing AGT only in proximal tubules have increased urinary Ang II excretion[77]. Because of its potential importance in identifying Ang II dependent hypertension in human subjects, direct quantitative methods to measure urinary AGT using human/mouse/rat AGT ELISA were recently developed[80,81]. Using this system, urinary excretion rates of AGT have been used as an index of intrarenal RAS status in patients with chronic kidney disease[74,82,83], diabetes mellitus[84,85], and hypertension[86–88]. In a cross-sectional study, we reported that urinary AGT levels are significantly greater in hypertensive patients not treated with RAS blockers compared with normotensive subjects (Figure 2). Moreover, patients treated with RAS blockers showed reduced urinary AGT levels[87]. In a population study, we showed that urinary AGT levels are correlated with high blood pressure in humans[88]. Urinary AGT levels were significantly correlated with systolic and diastolic blood pressures and high correlations between urinary AGT and blood pressure were shown in male subjects, especially in male African-American subjects[88]. These recent translational studies strengthen the hypothesis that intratubular AGT exerts a crucial role in the development and progression of hypertension and kidney disease. The augmentation of proximal tubule AGT leads to spillover into the distal nephron segments providing substrate for additional generation of Ang I and subsequent formation of Ang II (Figure 3).

Renin and (Pro)renin Receptor in the Collecting Duct During Ang II-dependent Hypertension

Renin is also produced by the principal cells of CNT and cortical and medullary CD of mouse, rat, and human kidneys[89–91]. Renin co-localizes with aquaporin 2[91]. In response to chronic Ang II infusions, renin mRNA and protein levels increase in CNT and CD[91]. This effect contrasts with the effect of Ang II to suppress JG renin[92], but is also an AT_1 receptor-mediated process[93]. As shown in Figure 4, the stimulation of CD renin during Ang II-dependent hypertension occurs independently of blood pressure since both non-clipped and clipped kidneys of Goldblatt hypertensive rats exhibit augmentation of renin synthesis and

renin activity in the renal medulla, which is devoid of JG cells94. Thus, CD renin is increased by Ang II in association with increased AGT spillover from the proximal tubules95. In hypertensive models, the increased renin is primarily active renin [94] while in diabetic models, the increased CD renin is primarily (pro)renin[90]. There is also an enhancement of ACE and inhibition of ACE2 gene expression associated with decreases in intrarenal Ang 1–7 levels 96،97 suggesting that suppression of ACE2 activity contributes to augmentation of intrarenal Ang II.

The (pro)renin receptor, (P)RR, a 350-amino acid protein with a single transmembrane domain which binds renin or (pro)renin, increases the catalytic activity of renin and fully activates (pro)renin98. (P)RR activation also elicits intracellular signals via extracellular signal-regulated kinase (ERK)1 and ERK2 mitogen-activated protein (MAP) kinase. (P)RR has been localized in glomerular mesangial cells, subendothelium of renal arteries, podocytes, macula densa cells, distal tubules and collecting ducts98،99. (P)RR is predominantly expressed at the apex of the intercalated cells100. An example of this localization is depicted in Figure 5. Recent findings have also shown that the full length form of (P)RR can be processed intracellularly by cleavage leading to a soluble form (s(P)RR) that can be secreted into the plasma and consequently bind renin101. While the function of (P)RR or s(P)RR in hypertensive conditions has not been established102, (P)RR data from various models suggest its contribution to hypertension, diabetes and associated cardiovascular and renal diseases90،103،104. These observations are of relevance in light of CD renin upregulation in Ang II-dependent hypertensive rats91،93،[94], and renin and/or (pro)renin secretion by CD cells[89],90،94.

Intrarenal ACE-derived Ang II formation in hypertension

ACE is responsible for most conversion of Ang I to Ang II and is expressed on endothelial cells of the vasculature, on brush border of proximal tubule cells, glomeruli and distal nephron segments including inner medullary CD[6,23,105–107]. ACE knockout mice display very low arterial pressures coupled with an impaired capacity to generate Ang II, that is reflected as low levels of circulating and intrarenal Ang II, high levels of circulating Ang I[108], and failure to show increases in blood pressure in response to Ang I infusions[109].

As shown in Figure 6, mice treated chronically with an ACE inhibitor show markedly attenuated responses in arterial pressure and lower intrarenal Ang II levels with low dose infusions of Ang II htat elicit a slow pressor response[64]. Thus, endogenous ACE-derived Ang II formation contributes to the development of high local Ang II levels and hypertension induced by chronic Ang II infusions. To further determine the ability of kidney-specific ACE to augment intrarenal Ang II content and blood pressure, mice expressing ACE exclusively in the kidneys were infused chronically with Ang I[110]. Kidney specific ACE-derived Ang II formation increased Ang II content and led to the progressive development of hypertension, indicating that intrarenal ACE is a major contributor to the development of hypertension and increased intrarenal Ang II levels. Indeed, ACE expression is sustained or even augmented during Ang II-dependent hypertension [6,106] and other models of kidney injury[111].

Perspective

The results obtained to date indicate that increases in circulating or local Ang II concentrations elicit a positive augmentation of intrarenal AGT mRNA and protein leading to increased secretion of AGT into the tubular fluid. Together with the sustained or increased tubular ACE levels, the augmented AGT increases intratubular Ang II which further augments sodium transport via stimulation of AT_1 receptors. The augmented AGT production and secretion increase AGT delivered to the distal nephron segments which can

interact with renin and ACE produced by principal cells of CNT and CD cells to form more Ang II and stimulate distal transport activity. Nevertheless, in a pathophysiologic environment, inappropriate stimulation of the intratubular RAS may be an important contributor to the development and maintenance of hypertension and associated renal injury[112]. While this positive augmentation of intrarenal angiotensin by Ang II appears to be counter-intuitive to normal feedback regulation, the process is primarily a local amplification mechanism to increase intratubular Ang II thus effecting rapid homeostatic regulation of sodium reabsorption without equivalent increases in circulating Ang II. Furthermroe, there are “brakes” in the system as described earlier to prevent uncontrolled positive feedback[51].

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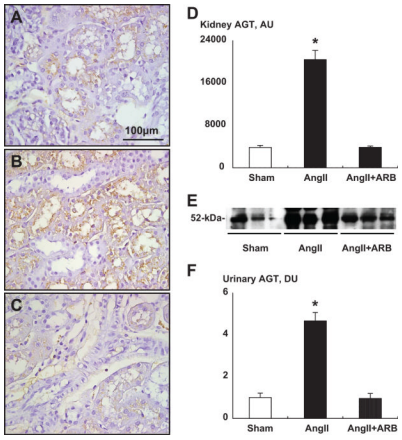

Figure 1. A–C, Representative immunohistochemical analysis of rat kidney AGT from Sham, Ang II infused and Ang II infused plus AT_1 receptor blockade groups

The immunoreactive areas were restricted only to proximal tubular cells. Vascular structures were negative. D, Kidney AGT immunostaining showed a significant enhancement in Ang II group (B) compared with sham group (A). ARB treatment prevented this augmentation (C). E, Representative Western blot analysis of urinary AGT levels among groups showing the stimulation in Ang II-infused group. F, Urinary excretion rates of AGT were enhanced 4.7-fold in Ang II-infused animals. ARB treatment prevented this augmentation. Ang II indicates angiotensin II; ARB, angiotensin II type1 receptor blocker, olmesartan; AGT, angiotensinogen. * $P < 0.05$ compared with the sham group. Data from Kobori et al. Hypertension 43:1126–1132, 2004[49].

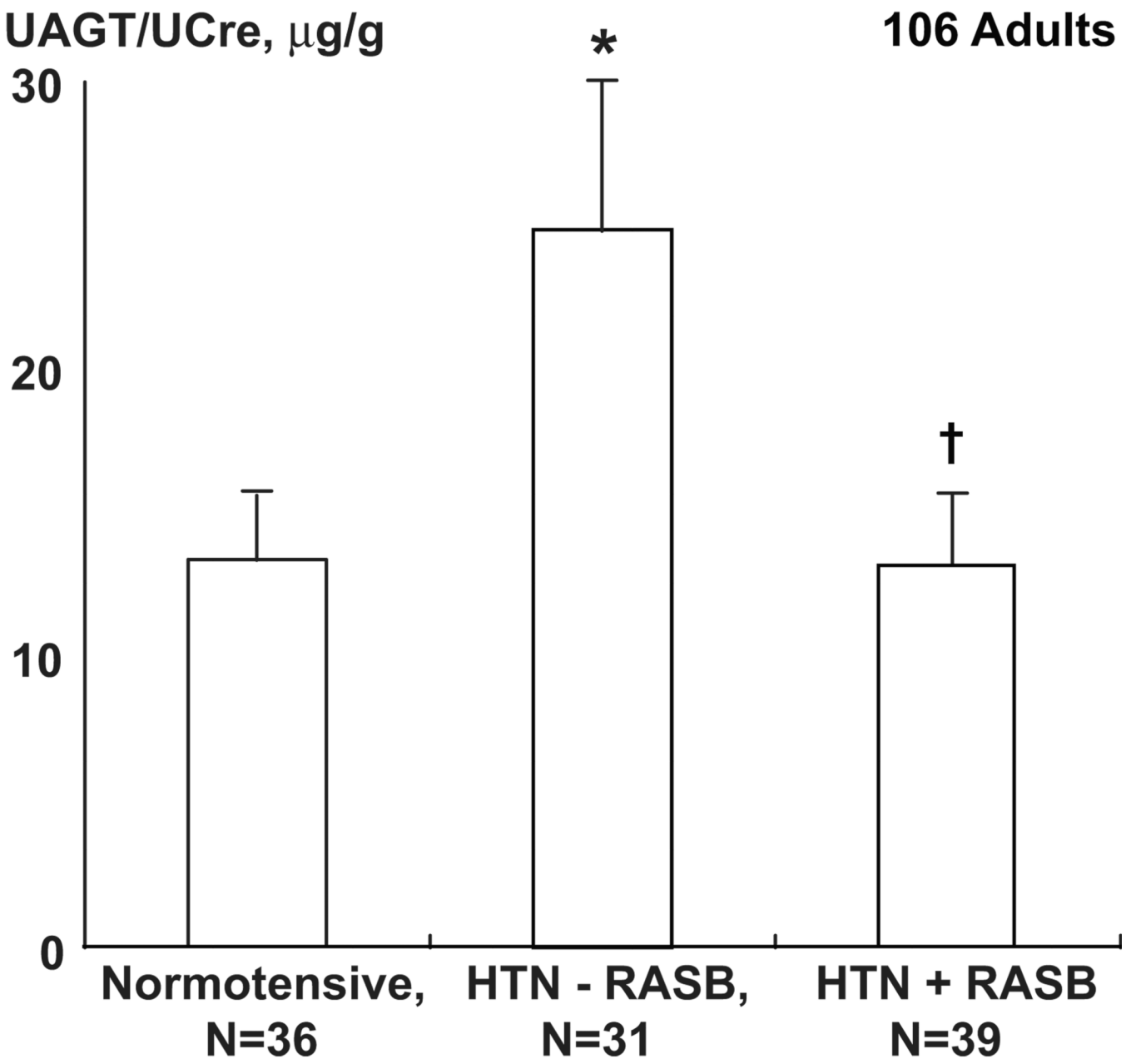

Figure 2.
Uriinary AGT (uAGT), expressed as ratio of uAGT/uCreatine, in normotensive, and in hypertensive patients (HTN) treated with renin-angiotensin system blockers (RASB) and compared with those treated with other drugs. **P*<0.05 vs normotensive; P<0.05 vs HTN–RASB. Data from Kobori et al. Hypertension 53[Part 2]:344–350, 2009[87].

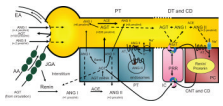

Figure 3. Cascade of Intratubular RAS in Ang II Dependent Hypertension

In Ang II dependent hypertension, the kidney maintains *de novo* intrarenal Ang II formation enhanced proximal tubule AGT formation and spillover into distal nephron segments coupled with enhancement of CD renin and stimulation of tubular ACE. (Refer to text for relevant references).

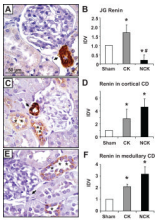

Figure 4. Renin immunoreactivity in juxtaglomerular (JG) cells and cortical collecting duct cells

Renin immunoreactivity by immunoperoxidase technique (A,C, and E) in paraffin embedded kidney sections (3 μm) from sham rats (A), and clipped (C) and non-clipped kidney (E) of Goldblatt rats. Specific JG renin immunoreactivity (arrows; DAB chromogen) in a sham (A) and in the clipped kidney (C) of a Goldblatt rat. Higher renin immunoreactivity (asterisks) are shown in the collecting ducts of the renal cortexes of both, clipped (C) and non-clipped (E) kidneys relative to the sham kidney (A). Densitometry the renin immunoreactivity in JG cells (B) and cortical (D) and medullary (F) collecting duct cells of sham, and clipped (CK) and non-clipped (NCK) kidneys of Goldblatt rats were performed using four kidney sections/animal (10 microscopic fields/kidney sections at the renal cortex and medulla regions) and compared to sham kidneys. Sham rats (n= 5). Goldblatt rats (n= 6). Glom: Glomerulus. Values are mean ± S.E. **P*<0.0001 *versus* sham. Renin antibody dilution 1:4,000. **P*<0.05 *versus* sham. #*P*< 0.05 clipped kidney *versus* non-clipped. CD: collecting duct; JG: juxtaglomerular; CK: clipped kidney; NCK: non-clipped kidney; IDV: integrated densitometric values. *Modified from Prieto-Carrasquero et al. Hypertension 51:1590–1596, 2008*[94].

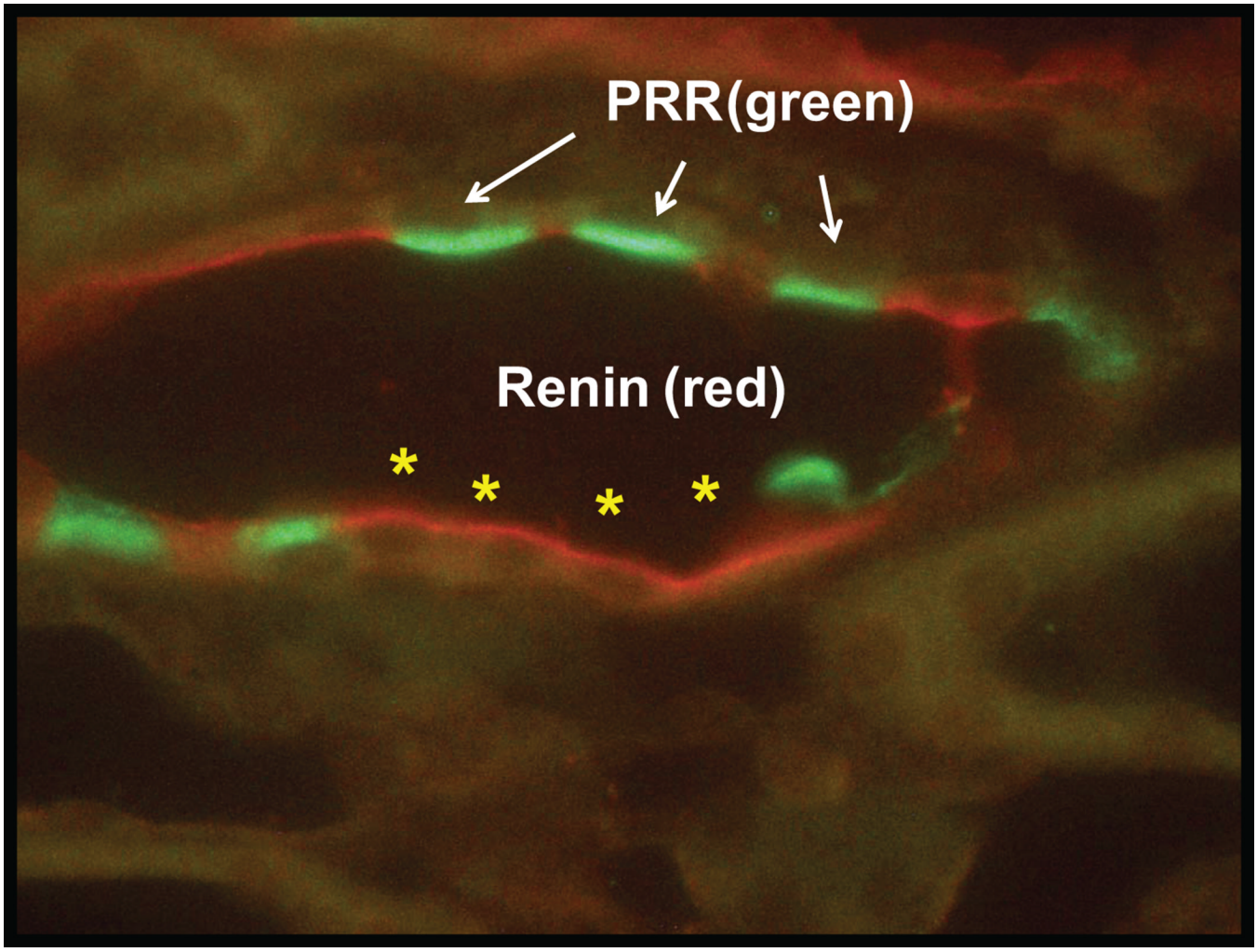

Figure 5. Double immunolabeling for renin and (pro)renin receptor ((P)RR) in the chronic Ang II-infused rat kidney

Double immunolabeling for renin (red) and (P)RR (green) was performed to confirm that renin is localized in principal cells (asterisks) while (P)RR is expressed in intercalated cells (arrows). A rabbit polyclonal anti-renin antibody (T. Inagami, Vanderbilt University) at a 1:4,000 dilution detected by a fluorescent secondary antibody (Alexa Fluor 594, red; Invitrogen) chicken anti-rabbit, was followed by a goat anti-(P)RR antibody (Abcam 5959, Cambridge, MA) at 1:400 dilution detected with a fluorescent secondary antibody donkey anti-goat (Alexa Fluor 488, green; Invitrogen). (Unpublished data)

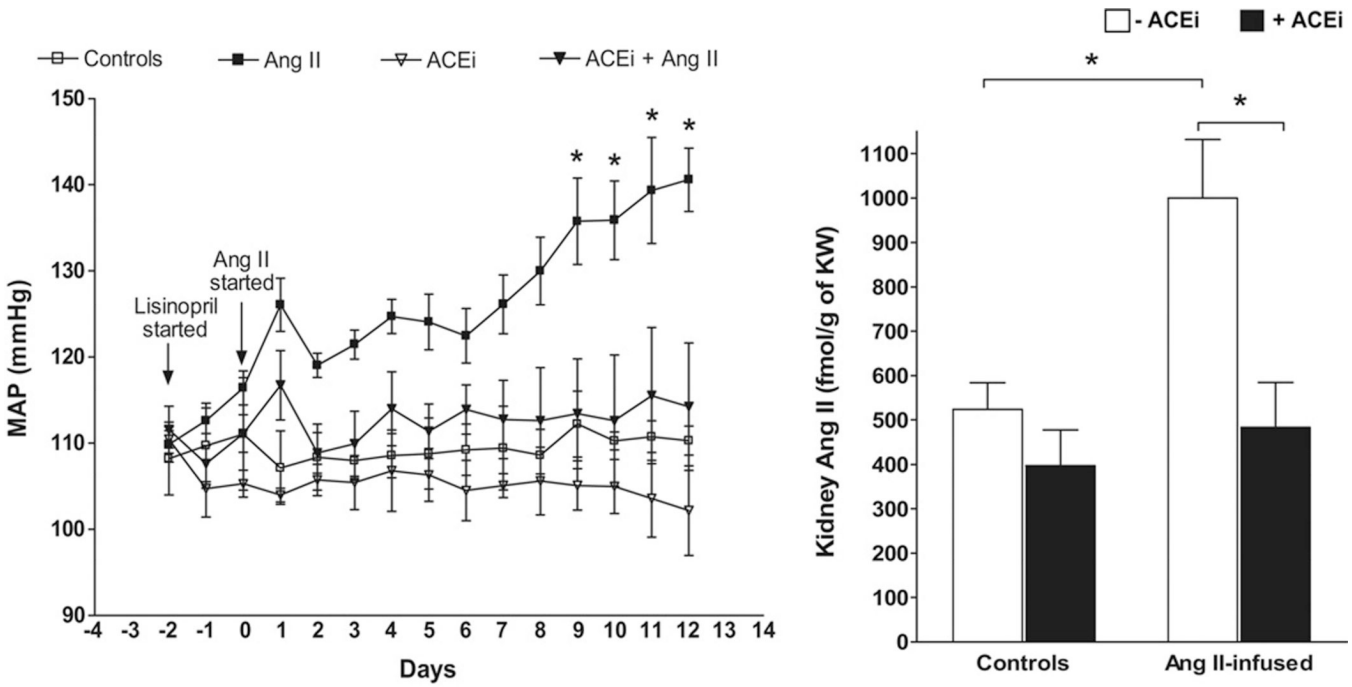

Figure 6. Changes in mean arterial blood pressure and intrarenal Ang II in Ang II-infused mice with or without an ACE inhibitor

Blood pressure and Ang II concentrations were determined by telemetry and radioimmunoanalysis respectively. Ang II = Angiotensin II (400 ng/kg/min), ACEi = Lisinopril (100 mg/L in the drinking water). *$p < 0.05$ vs. controls by TWO-WAY ANOVA for MAP changes and ONE-WAY ANOVA for Ang II changes. (From Gonzalez-Villalobos et al. Hypertension 53:351–355, 2009)[64].