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Role of Stimulated Intrarenal Angiotensinogen in Hypertension

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Abstract

Experimental models of hypertension and patients with inappropriately increased renin formation due to a stenotic kidney, arteriosclerotic narrowing of the renal arterioles or a rare juxtaglomerular cell tumor have shown a progressive augmentation of the intrarenal/intratubular renin-angiotensin system (RAS). The increased intrarenal angiotensin II (Ang II) elicits renal vasoconstriction and enhanced tubular sodium reabsorption in proximal and distal nephron segments. The enhanced intrarenal Ang II levels are due to both increased AT1 receptor mediated Ang II uptake and AT1 receptor dependent stimulation of renal angiotensinogen (AGT) mRNA and augmented AGT production. The increased AGT formation and secretion into the proximal tubular lumen leads to local formation of Ang II, which stimulates proximal transporters such as the sodium/hydrogen exchanger. Enhanced AGT production also leads to spillover of AGT into the distal nephron segments as reflected by AGT in the urine, which provides an index of intrarenal RAS activity. There is also increased Ang II concentration in distal nephron with stimulation of distal sodium transport. Increased urinary excretion of AGT has been demonstrated in patients with hypertension, Type 1 and Type 2 diabetes mellitus, and several types of chronic kidney diseases indicating an upregulation of intrarenal RAS activity.

Keywords

Angiotensin II; AT1 Receptors; Proximal Tubule Cells; Sodium Reabsorption

INTRODUCTION

The intrarenal renin-angiotensin system (RAS) exerts pleiotropic regulatory actions on renal hemodynamic and transport processes which contribute to sodium balance and blood pressure homeostasis [Kobori *et al.*, 2007, Navar *et al.*, 2011]. When physiologically stimulated by reduction in salt intake, the increased renin release from juxtaglomerular cells leads to greater angiotensin II (Ang II) formation which stimulates tubular sodium reabsorption and thus helps maintain sodium balance and blood pressure [Ingert *et al.*, 2002, Shao *et al.*, 2013]. However, when the intrarenal RAS is inappropriately activated by arteriosclerotic narrowing of the renal arterioles, renal arterial stenosis or a rare juxtaglomerular tumor [Kobori *et al.*, 2007, Beevers *et al.*, 2008], an augmented renal RAS in a setting of inflammatory or oxidative stress conditions is a major contributor to excessive

sodium retention in the development of hypertension and progressive tissue injury. The increased Ang II levels lead to a stimulation of angiotensinogen (AGT) expression in proximal tubules cells which increases intrarenal AGT produced in renal proximal tubules. The AGT is secreted into the proximal tubular lumen where it gives rise to Ang I and Ang II formation at the level of the proximal tubule thereby stimulating proximal sodium reabsorption rate [Navar *et al.*, 1999]. Concomitant augmentation of intrarenal AGT mRNA and protein has been shown in various animal models of Ang II-dependent hypertension [Schunkert *et al.*, 1992, Kobori *et al.*, 2001, Kobori *et al.*, 2007, Gonzalez-Villalobos *et al.*, 2008]. The elevated intrarenal AGT levels are prevented by treatment with Ang II receptor blockers (ARBs) indicating that AT1 receptor activation exerts an augmentation effect which consequently accelerates the progression of hypertension. In rodents, there is a positive relationship between intrarenal Ang II levels and urinary AGT excretion rates indicating that urinary AGT can serve as an index of intrarenal RAS activity [Kobori *et al.*, 2002, Kobori *et al.*, 2003]. The urinary AGT levels in hypertensive patients are higher than in control subjects [Kobori *et al.*, 2009, Michel *et al.*, 2014] suggesting that urinary AGT may be a useful urinary biomarker of intrarenal RAS status in humans also.

In hepatocytes, Ang II directly increases AGT expression via activation of NF- κ B [Li *et al.*, 1996]. In contrast, direct treatment with Ang II alone has minor effects on AGT expression levels in cultured renal proximal tubular cells (PTC) [Satou *et al.*, 2008]. In addition, a preliminary study using a 2-kidney 1-clip Goldblatt hypertension model demonstrated that intrarenal AGT mRNA levels are elevated only in the non-clipped kidneys [Navar *et al.*, 2014] which has been shown to exhibit more renal injury [Kobayashi *et al.*, 1999] even though intrarenal Ang II levels are increased in both clipped and non-clipped kidneys. Furthermore, in response to a low sodium diet, intrarenal AGT is not stimulated even though the Ang II levels are markedly increased [Shao *et al.*, 2013]. These findings provide a basis for our hypothesis that kidneys have a unique system in which Ang II-stimulated pathogenic factors in addition to AT1 receptor activation are required for AGT augmentation. However, the mechanisms have not been clearly delineated.

Chronic elevations in systemic or renal Ang II levels stimulate pathogenic factors including pro-inflammatory cytokines produced by activated immune cells, growth factors, oxidative stress, and mechanical stress by high blood pressure [Ruiz-Ortega *et al.*, 2002, Ozawa *et al.*, 2007]. These factors synergize with the increased Ang II levels to augment AGT expression in the kidneys. Systemic and intrarenal Interleukin 6 (IL-6) has been identified as an abundantly expressed cytokine in Ang II-dependent hypertension which is markedly increased in conditions of inflammation and high oxidative stress associated with increased macrophage infiltration [Ruiz-Ortega *et al.*, 2002, Recinos *et al.*, 2007]. In addition, IL-6 levels in non-clipped kidneys of Goldblatt hypertensive rats are higher than in the clipped kidneys and sham-operated kidneys [Babu *et al.*, 2014]. IL-6 gene deletion also limits the progression of high blood pressure in Ang II-infused mice [Lee *et al.*, 2006, Brands *et al.*, 2010]. In a previous study [Satou *et al.*, 2008], it was demonstrated that IL-6 contributes to AGT augmentation via activation of the JAK-STAT pathway in PTC. Thus, IL-6 may be a major mediator of intrarenal AGT augmentation in hypertension. In this review, we will

discuss some of the critical findings that support the importance of intrarenal RAS activation as a major contribution to hypertension and renal injury.

Activation of AT1 receptors by elevated intrarenal Ang II levels in Ang II dependent hypertension

Ang II exerts its pathophysiological actions by binding to two major receptors, the type 1 (AT1) and type 2 (AT2) angiotensin receptors. The cardinal role of AT1 receptors in mediating Ang II dependent hypertension has been demonstrated in various studies. Experiments using AT1a receptor knockout mice demonstrated that unilateral renal arterial clipping failed to elicit any increases in systemic arterial pressure in the absence of AT1a receptors in contrast to the robust responses seen in wild type mice [Cervenka *et al.*, 2002, Cervenka *et al.*, 2008]. In addition the magnitude of the hypertension was not altered by treatment with AT2 receptor blockers or agonists. Similarly, the hypertension elicited with chronic Ang II infusions is also completely prevented or reversed by treatment with AT1 receptor antagonists [Zou *et al.*, 1996, Zou *et al.*, 1998]. The AT1 receptors in the kidney appear to be particularly important in maintaining the hypertension caused by chronic Ang II infusions [Crowley *et al.*, 2006]. This study used a kidney cross transplantation strategy to show that when AT1 receptors are eliminated from the kidneys, the pressor and cardiovascular responses to the chronic Ang II infusions were markedly attenuated.

Ang II activates both AT1 and AT2 receptors. However, the hypertensinogenic effects of Ang II are mediated primarily by the AT1 receptor which is expressed in almost all cell types within the kidneys [Harrison-Bernard *et al.*, 1997, Carey, 2005]. The AT1 receptor is a member of the G protein-coupled receptor family that physically associates with Gq/11, Gi, G12 and/or G13 [Higuchi *et al.*, 2007]. As shown in Figure 1, once Ang II binds to AT1 receptor, various second messengers are activated via G protein-dependent pathways, resulting in the induction of vasoconstriction, generation of reactive oxygen species (ROS), changes in gene transcription and the induction of cell growth and migration [Touyz *et al.*, 2002, Higuchi *et al.*, 2007]. In the G protein-dependent AT1 receptor signaling pathways, phospholipase C, A2 and D have been identified as initial mediators [Ushio-Fukai *et al.*, 1999]. The activated phospholipases stimulate other signal transducers including activation of Rho kinase in vascular smooth muscle cells [Higuchi *et al.*, 2007, Mehta *et al.*, 2007, Miyata *et al.*, 2014] leading to vasoconstriction. AT1 receptor activation also leads to substantial internalization of the ligand-receptor complex leading to increases in intracellular Ang II [Zou *et al.*, 1996, Zou *et al.*, 1998, Zhuo *et al.*, 2002]. Studies using infusions of Val⁵-Ang II have shown that approximately half of the increases in intrarenal Ang II levels are due to AT1 receptor mediated uptake of circulating Ang II [Shao *et al.*, 2009].

In the kidneys, AT1 receptor expression has been detected in microdissected glomeruli, proximal convoluted and straight tubules, medullary thick ascending limbs, medullary collecting ducts, cortical collecting ducts, vasa recta bundles and arcuate arteries as well as in vascular smooth muscle cells of afferent and efferent arterioles [Harrison-Bernard *et al.*, 1997, Navar *et al.*, 2000]. In particular, proximal tubules exhibit high levels of AT1 receptor expression [Burns *et al.*, 1993, Bouby *et al.*, 1997]. Activation of luminal AT1 receptors stimulates the sodium hydrogen exchanger and increases reabsorption rate [Navar *et al.*,

2000]. Furthermore, AT1 receptor blockade causes significant increases in glomerular filtration rate, renal blood flow and proportionately much greater increases in sodium excretion and fractional sodium excretion. Ang II blockade also leads to marked increases in the slope of the pressure natriuresis relationship [Navar *et al.*, 2000]. These findings demonstrate the important actions of activated intrarenal AT1 receptor in regulation of renal function, electrolyte and body fluid homeostasis and blood pressure. The role of activated intrarenal AT1 receptors in the development of hypertension has been also demonstrated using kidney-specific AT1 receptor knockout mice. Chronic Ang II infusions increased blood pressure only in the early stages, and the blood pressure was subsequently decreased and sustained within normotensive levels [Crowley *et al.*, 2006]. These results indicate that elevated Ang II contributes to blood pressure elevation by activation of systemic AT1 receptors with consequent vasoconstriction in the early stages but the activated intrarenal AT1 receptor maintains the high blood pressure by altering renal function and further stimulation of pro-hypertensive mechanisms.

Enhancement of intrarenal AGT in Ang II dependent hypertension

AGT expression is increased by Ang II treatments and accompanied by activation of NF- κ B in both liver tissue and cultured hepatocytes [Brasier *et al.*, 1996, Li *et al.*, 1996], leading to augmentation of circulating AGT levels. Intrarenal AGT levels are also increased in Ang II-infused rats and mice [Schunkert *et al.*, 1992, Kobori *et al.*, 2001, Gonzalez-Villalobos *et al.*, 2008] and in human renin/human AGT double transgenic mice producing extra Ang II only in kidneys [Kobori *et al.*, 2007]. The intrarenal Ang II-AGT amplification mechanism is a pivotal mechanism that facilitates the progression of hypertension. The intrarenal AGT augmentation is attenuated by treatment with AT1 receptor blockers in hypertensive patients and experimental models [Kobori *et al.*, 2004, Kobori *et al.*, 2009]. However, because physiological stimulation of the RAS in response to low sodium diets does not cause an upregulation of intrarenal AGT expression even though intrarenal Ang II levels are quite high [Shao *et al.*, 2013], additional factors are necessary to fully stimulate intrarenal AGT expression in pathological conditions.

During the chronic Ang II infusions, macrophage and T cell infiltration is enhanced in the kidneys [Ozawa *et al.*, 2007]. Administration of mycophenolate mofetil, an immunosuppressive drug, attenuates intrarenal Ang II elevation and mitigates the development of hypertension and renal injury [Bravo *et al.*, 2007] accompanied by suppression of the immune cell infiltration in the kidney [Crowley *et al.*, 2008]. The accumulation of immune cells in the kidneys contributes to intrarenal RAS activation and the development of hypertension and kidney injury. Activated immune cells produce many types of pro-inflammatory cytokines. The RAS and pro-inflammatory cytokines synergize to cause hypertension [Lee *et al.*, 2006], and there is substantial evidence of interactions between these factors. IL-6 production is strongly stimulated in activated macrophages and T-cells (Th2 cells) [Recinos *et al.*, 2007]. Indeed, IL-6 has been identified as the most abundantly expressed cytokine in isolated aorta and it exhibits the most augmented expression in response to Ang II, indicating that IL-6 is induced locally at increased levels in Ang II-infused mice [Recinos *et al.*, 2007]. Further, IL-6 levels are elevated in the kidneys of Ang II-infused animals [Ruiz-Ortega *et al.*, 2002].

As shown in Figure 1, IL-6 and the IL-6-activated JAK-STAT pathway are required for Ang II-induced AGT upregulation in renal PTC [Satou *et al.*, 2008]. This can explain previous findings that deletion of IL-6 gene mitigates increases in blood pressure and kidney injury in Ang II-infused animals [Lee *et al.*, 2006, Zhang *et al.*, 2012] and that AT1 receptor blockade attenuates intrarenal AGT augmentation accompanied by decreases in IL-6 levels in patients with diabetes mellitus [Ogawa *et al.*, 2009]. Enhanced interferon gamma (IFN- γ) formation in activated T cells, natural killer cells, and macrophages during the development of Ang II associated hypertension has also been demonstrated [Crowley *et al.*, 2008, Saha *et al.*, 2010]. In addition to the stimulating effect of IL-6 on proximal tubular AGT expression, IFN- γ increases AGT expression in PTC [Satou *et al.*, 2012] as well as in hepatocytes [Jain *et al.*, 2006]. These findings suggest that AGT synthesis in proximal tubules is stimulated via Ang II-induced pro-inflammatory factors, particularly IL-6 and IFN- γ , derived from immune cells, which may be primary mechanisms underlying elevated intrarenal AGT levels during Ang II-dependent hypertension. Thus, future studies will be needed to elucidate the cellular mechanisms underlying the complex interactions leading to proximal tubular AGT augmentation.

AGT mediated stimulation of endogenous intrarenal Ang II formation in Ang II dependent hypertension

Under normal conditions, AGT and Ang II concentrations in proximal tubules are greater than can be explained from the plasma concentrations [Navar *et al.*, 2002]. Thus, it has been proposed that the high levels of proximal tubular AGT contribute to establishment of the high concentration of tubular Ang II [Navar *et al.*, 2002]. In Ang II-dependent hypertension, elevated Ang II stimulates intrarenal AGT expression as described in the section above. In vivo experiments using chronic Val⁵-Ang II infusions have shown that there is increased formation of endogenous Ileu⁵-Ang II which is the native form in rodents in chronic Ang II-infused rats [Shao *et al.*, 2010]. Chronic Val⁵-Ang II infusion increased intrarenal and urinary endogenous Ileu⁵-Ang II levels although endogenous Ileu⁵-Ang II levels in plasma were lower in the Val⁵-Ang II infusion rats. These results indicate activation of intrarenal RAS leading to further Ang II production independent of systemic RAS activity in Ang II-dependent hypertension. Furthermore, animal models with proximal tubule-specific AGT overexpression exhibited increased intratubular Ang II levels and the development of hypertension [Sachetelli *et al.*, 2006, Ying *et al.*, 2012]. Accordingly, upregulation of proximal tubular AGT expression and secretion by elevated Ang II is of major importance in facilitating intrarenal Ang II production and consequent increases in blood pressure.

Importance of intrarenal ACE in the augmentation of intrarenal Ang II in Ang II dependent hypertension

Chronic Ang II infusion augments ACE levels in renal proximal tubules [Harrison-Bernard *et al.*, 2002]. This finding is consistent with increased kidney ACE activity reported in 2K1C and chronic ANG II-infused animals [Von Thun *et al.*, 1994, Prieto *et al.*, 2011]. Therefore, intrarenal ACE is also a key component dictating intrarenal RAS activity as well as proximal tubular AGT, renal tubular and arteriolar AT1 receptor, and collecting duct renin [Navar *et al.*, 2011]. Furthermore, systemic administration of an ACE inhibitor ameliorates the increases in blood pressure and intrarenal Ang II content caused by Ang II infusions

[Gonzalez-Villalobos *et al.*, 2009]. A study targeting intrarenal ACE-specific function revealed that chronic Ang I infusion into mice expressing ACE only in kidney resulted in elevation of intrarenal Ang II levels with the development of slowly progressive increases in arterial pressure compared to the responses in Ang I-infused wild type mice [Gonzalez-Villalobos *et al.*, 2011]. In further studies, mice with ACE deletion in kidneys, but expressing ACE elsewhere in body, failed to develop the same level of hypertension in response to Ang II infusions as wild type mice [Gonzalez-Villalobos *et al.*, 2013]. These results demonstrate that the presence of ACE in the kidneys is necessary to generate sufficiently high intrarenal Ang II concentrations to stimulate several sodium transport systems.

Translational studies of augmented urinary AGT in hypertension, diabetes and kidney diseases

Since intrarenal AGT expression is stimulated in hypertension and RAS associated diseases, urinary AGT levels have been proposed as an index of intrarenal RAS activation in hypertension, diabetic nephropathy and other cardiovascular and kidney diseases [Navar, 2013; 2013]. As shown in Figure 2, elevated urinary AGT levels were observed in hypertensive animal models including Ang II-infused rats with/without high salt diet [Kobori *et al.*, 2003, Lara *et al.*, 2012], spontaneous hypertensive rats with high salt diet [Susic *et al.*, 2011], Dahl salt rats [Kobori *et al.*, 2003], and mRenin2 transgenic rats [Milani *et al.*, 2010]. Although these animal models exhibited over 3-fold higher urinary AGT excretion levels compared to their respective controls, DOCA-induced hypertensive rats did not show higher urinary AGT levels [Kobori *et al.*, 2003], supporting the generally held concept that DOCA-induced hypertension is developed in a RAS-independent manner and that the urinary AGT levels can be dissociated from just elevated arterial pressure. Some animal models, including rats receiving the combination of chronic Ang II infusion plus high salt diet, exhibited over 100-fold higher urinary AGT levels compared to their respective controls (Fig. 2). These very high urinary AGT excretion rates are associated with an increased proteinuria indicating an increased glomerular permeability to macromolecules which allows increased filtration of circulating plasma AGT [Pohl *et al.*, 2010, Matsusaka *et al.*, 2014]. Under these conditions, it is likely that the very high levels of urinary AGT in these models reflects a contribution from filtered AGT due to glomerular dysfunction as well as elevated intrarenal AGT production. The relative contributions of elevated intrarenal AGT formation and filtered AGT in hypertension have not been determined and will be an important subject in future studies. A study using human subjects with hypertension established clinical relevance by showing that urinary AGT levels in the hypertensive patients are higher than in control subjects and that treatment with either AT1 receptor blockers or ACE inhibitors normalized the elevated urinary AGT levels [Kobori *et al.*, 2009].

Augmented urinary AGT levels have also been demonstrated in non-hypertensive diseases. Diabetic animal models and patients with diabetic mellitus exhibited greater urinary AGT levels [Saito *et al.*, 2009, Park *et al.*, 2010, Kamiyama *et al.*, 2012, Kim *et al.*, 2012, Lo *et al.*, 2012]. Importantly, urinary AGT augmentation was detected before the development of proteinuria in diabetic mice [Kamiyama *et al.*, 2012], suggesting that an elevated urinary

AGT may precede the increased proteinuria and may predict the development of diabetic nephropathy. AGT levels are increased in urine collected from patients with chronic kidney disease [Mills *et al.*, 2012], glomerulonephritis [Urushihara *et al.*, 2010], and IgA nephropathy [Nishiyama *et al.*, 2011]. Although these findings indicate that urinary AGT levels may serve as an index of augmented intrarenal RAS [Navar, 2013], larger clinical studies are needed in human subjects to determine if treatment of hypertensive and diabetic subjects with AT1 receptor blockers or ACE inhibitors can reduce urinary AGT levels, thus indicating successful reduction in intrarenal RAS activity which hopefully can reduce or stop the progression of renal injury [Navar, 2013].

CONCLUSIONS

The data obtained so far in animals indicate that in Ang II dependent hypertension, and other conditions such as diabetes, there is a progressive stimulation of intrarenal AGT augmentation which depends on synergistic effects of AT1 receptor activation with other pathologic factors including macrophage infiltration, increased IL-6 levels, and oxidative stress. The augmentation of intrarenal AGT expression leads to increased AGT protein which is secreted into the tubular fluid leading to additional endogenous formation of intratubular Ang I and Ang II along with other Ang peptides. These increases can then act on luminal AT1 receptors to stimulate transport in both proximal and distal nephron segments. In addition to stimulating transport, the combined increases in intrarenal Ang II and inflammatory factors can lead to progressive tissue injury and exacerbate the development and progression of hypertension.

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Figure 1. Proposed mechanism underlying vasoconstriction by Ang II and augmentation of proximal tubular AGT expression by Ang II and elevated cytokines during the development of hypertension

Activated AT_1 receptor induces vasoconstriction via elevation of intracellular calcium levels and activation of Rho kinase [Touyz *et al.*, 2002, Higuchi *et al.*, 2007, Mehta *et al.*, 2007, Guilluy *et al.*, 2010, Satou *et al.*, 2012]. As explained in the text, elevated Ang II induces infiltration of immune cells into kidneys, leading to increases in intrarenal cytokine levels. Ang II and the cytokines synergistically augment proximal tubular AGT expression and secretion in hypertension. PLC: phospholipase C, DAG: diacylglycerol, PKC: protein kinase C, ROS: reactive oxygen species, MAPK: mitogen-activated protein kinases, NF- κ B: nuclear factor κ B, CAM: calmodulin, JAK: Janus kinase, Arhgef: Rho guanine nucleotide exchange factor, c-Src: cellular Src kinase, Pyk2: Proline-rich tyrosine kinase 2, SHP-2: src homology 2 domain-containing tyrosine phosphatase 2, STAT: signal transducer and activator of transcription.

Figure 2. Urinary AGT levels in rat and mouse disease models

Reported urinary AGT levels are expressed as ratio to their respective controls. As explained in the text, emerging data show that hypertension, diabetes, and several kidney diseases are associated with increased urinary AGT excretion rates. Data are from refs [Kobori *et al.*, 2003, Kobori *et al.*, 2003, Milani *et al.*, 2010, Park *et al.*, 2010, Susic *et al.*, 2011, Kamiyama *et al.*, 2012, Lara *et al.*, 2012, Lo *et al.*, 2012, Rands *et al.*, 2012].