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Proximal tubule angiotensinogen modulation of arterial pressure

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Abstract

Purpose of review—Although the existence of a complete intrarenal renin–angiotensin system is now well established, its role in modulating tubule sodium transport and blood pressure is incompletely understood. Several recent studies have shed light on one component of the system, proximal tubule-derived angiotensinogen (AGT). This review discusses the synthesis, regulation and function of AGT in the proximal tubule.

Recent findings—Under normal sodium intake, AGT within the S1 and S2 segments of the proximal tubule may derive from the systemic circulation, whereas the S3 segment synthesizes AGT. Urinary AGT likely primarily reflects proximal tubule-derived AGT. Proximal tubule AGT synthesis is regulated by high Na intake, angiotensin-II and inflammatory cytokines. Transgenic expression of mouse AGT in the proximal tubule causes hypertension. Overexpression of rat AGT in the proximal tubule leads to hypertension, enhanced reactive oxygen species generation via NADPH oxidase, tubular apoptosis and tubulointerstitial fibrosis; these effects can be mitigated by catalase overexpression.

Summary—Proximal tubule-derived AGT has the potential to modulate blood pressure and sodium balance, and promote renal injury. Interactions with the systemic renin–angiotensin system may influence the role of proximal tubule-derived AGT in the kidney.

Keywords

angiotensinogen; hypertension; proximal tubule

INTRODUCTION

The renin–angiotensin system (RAS) plays a key role in regulating blood pressure (BP) and sodium (Na) and water homeostasis. As our understanding of this complex system evolved, there has been a shift from focusing on the systemic RAS to that of a local RAS, particularly in brain, heart, adrenal glands, vasculature and kidney. Of these systems, the nephron is notable for containing all the components of the RAS: angiotensinogen (AGT) is synthesized in the proximal tubule, renin is produced in the juxtaglomerular apparatus and distal nephron, and angiotensin-converting enzyme (ACE) and angiotensin II (Ang-II)

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Conflicts of interest

There are no conflicts of interest.

receptors are found throughout the nephron. In fact, intrarenal Ang-II levels are reportedly 50-fold higher than those in plasma.

Although all components of the RAS are found in the nephron, this review will focus on intrarenal AGT biology. Because the first description of AGT synthesis is within the kidney [1], substantial work has been done on characterizing the function and regulation of intrarenal AGT. This review will summarize the evidence for synthesis of AGT in the kidney, its regulation and biologic effects.

ANGIOTENSINOGEN SYNTHESIS IN PROXIMAL TUBULE

Several studies indicate that the primary source of AGT production in the kidney is the proximal tubule [2-4]. Mice transgenic for human AGT under control of the endogenous mouse promoter expressed high levels of human AGT in the proximal tubule (as well as in the liver) [5]. It is likely that proximal tubule-derived AGT is released into the tubule lumen and can reach all distal nephron segments, including appearing in the urine [6-8]. In support of this, mice with proximal tubule-specific expression of human AGT excreted human AGT in the urine, whereas human AGT was undetectable in plasma [9,10].

A recent study suggests that most of the intrarenal AGT is not synthesized locally, but derives from the liver [11■■]. Using tissue-specific Cre recombinase-expressing mice, this group disrupted the AGT gene in the liver, proximal tubule [using the proximal tubule-specific kidney androgen protein (KAP) promoter driving Cre], or both sites. Despite reducing proximal tubule AGT mRNA levels by approximately 86% in proximal tubule AGT knockout mice, no change in renal AGT protein was observed. Further, no change in proximal tubule AGT immunostaining was found between control and proximal tubule AGT knockout mice; AGT was detected in S1 and S2 segments of the proximal tubule, but not in the S3 segment where AGT mRNA has been localized. The proximal tubule AGT knockout mice also had a 50% reduction in urinary AGT excretion, indicating that proximal tubule-derived AGT is secreted into the tubule lumen. Liver AGT knockout mice had a marked reduction in intrarenal AGT protein content and AGT immunostaining in the S1 and S2 segments of the proximal tubule, suggesting that proximal tubule AGT in these segments primarily derives from filtered AGT. In accordance with this, proximal tubule from megalin knockout mice did not have immune-detectable AGT. Liver AGT knockout mice had almost a 90% reduction in intrarenal Ang-II levels (whereas proximal tubule AGT knockout mice had no significant reduction in renal Ang-II levels), indicating that local renal Ang-II generation is largely dependent upon circulating AGT. Notably, urinary AGT excretion was not significantly different in liver AGT knockout mice as compared to controls, indicating that urinary AGT derives in large part from AGT synthesized in the proximal tubule. Taken together, these elegant studies by Matsusaka *et al*. [11■■] suggest that the bulk of proxi- mal tubule AGT, particularly in the S1 and S2 seg- ments, derives from the liver and is primarily responsible for intrarenal Ang-II generation. In con- trast, AGT synthesized in the proximal tubule is mainly released into the tubule lumen and is the primary determinant of urinary AGT. In accordance with this, Kobori *et al*. [12] observed negligible amounts of human AGT protein in the urine when rats were infused with human AGT protein, further supporting the notion that urinary AGT primarily reflects renal AGT production. Finally, it

should be noted that the above transgenic mouse studies were performed on a normal Na diet without disease induction, hence, it remains to be seen what the relative roles of systemic and proximal tubule AGT are under altered Na intake and in disease conditions.

REGULATION OF PROXIMAL TUBULE ANGIOTENSINOGEN SYNTHESIS

Ang-II has been established as an important stimulant of intrarenal AGT synthesis. Ang-II infusion, via AT1 receptors, increases renal AGT mRNA and protein in rats [13,14] and mice [15,16]. Mice transgenic for both KAP-driven proximal tubule human AGT and for systemic human renin expression have increased intrarenal endogenous (i.e., mouse) AGT mRNA and protein expression [17]. In addition, Ang-II increases AGT mRNA levels in cultured rat proximal tubule cells [2]. Thus, Ang-II exerts positive feedback on proximal tubule AGT production.

Cytokines, including tumor necrosis factor (TNF)-a and interleukin (IL)-6 can modulate proxi- mal tubule AGT expression. IL-6 alone or synergistically with Ang-II can stimulate AGT production by cultured proximal tubule cells [18,19]. In contrast, TNF-α suppressed AGT production in HK-2 cells via activation of an NF-κB-p50/p50 homodimer-dependent pathway [20]. These data suggest that inflammatory cytokines can exert complex effects on proximal tubule AGT synthesis. At this juncture, it is not possible to predict how a particular inflammatory state would affect proximal tubule AGT production; clearly, more studies are needed.

FUNCTIONAL SIGNIFICANCE OF PROXIMAL TUBULE ANGIOTENSINOGEN

A key question is whether proximal tubule-derived AGT is converted into Ang-II and whether such Ang-II can in turn exert biologic effects. Numerous studies indicate that Ang-II stimulates Na reabsorption in the nephron, including the proximal tubule [21] and collecting duct [22]. In addition, Ang-II may stimulate collecting duct water transport [23]. In the proximal tubule, luminal AGT may be acted upon by filtered renin and the resultant luminal Ang-II can stimulate Na/H exchanger activity [24,25]. Proximal tubule-derived AGT may also act distally due to downstream effects of proximal tubule luminal Ang-II synthesis or to collecting duct-derived renin conversion of luminal AGT [26■]; luminal Ang-II can enhanced collecting duct epithelial Na channel activity [22].

In support of a potential functional role for proximal tubule-derived AGT, mice doubly transgenic for the KAP promoter driving proximal tubule-specific human AGT expression along with systemic human renin expression had high renal Ang-II content, no changes in plasma Ang-II concentration, and were hypertensive [27]. In an effort to differentiate the effects of intrarenal RAS activation from systemic RAS involvement, Sachetelli *et al*. [28] established a transgenic mouse line with proximal tubule-specific expression of rat AGT (under control of the KAP promoter). Although baseline SBPs were similar in transgenic and nontransgenic controls, transgenic mice treated with testosterone (which induces the KAP promoter) developed hypertension and proteinuria in both sexes compared to nontransgenic controls. Further, treatment with an ACE inhibitor or an angiotensin receptor blocker prevented development of hypertension and proteinuria. One concern with these rat AGT studies is that rat AGT has up to 64-fold greater affinity for mouse renin as compared

to the homospecific reaction [29], hence, the RAS may have been markedly overactive. To help address this issue, we developed a mouse model with overexpression of mouse AGT in the proximal tubule under control of the KAP promoter [30■■]. These mice were hypertensive despite having normal plasma AGT levels and plasma renin activity. Notably, urinary AGT and Ang-II levels were elevated. Thus, transgenic mice with proximal tubule-specific AGT overexpression, despite the endogenous RAS being able to normally compensate, manifest increased BP. Importantly, the finding that urinary Ang-II is increased in these mice supports the notion that proximal tubule-derived AGT can modulate tubular fluid Ang-II levels.

Ang-II has been shown to be a potent stimulator of reactive oxygen species via increased NADPH oxidase in renal proximal tubule cells [31]. Transgenic mice with proximal tubule rat AGT overexpression demonstrated hypertension, and enhanced generation of reactive oxygen species (ROS), NADPH oxidase activity, tubular apoptosis and tubulointerstitial fibrosis compared to nontrans- genic controls [32]. Further, mice with proximal tubule rat AGT overexpression and proximal tubule catalase overexpression showed normalization of BP and albuminuria and attenuation of ROS generation and tubulointerstitial fibrosis compared to proximal tubule rat AGT overexpression mice [33]. Thus, specifically targeting proximal tubule catalase negated Ang-II-mediated fibrosis and hypertension, suggesting that proximal tubule-derived Ang-II can act in an autocrine manner to modulate BP and exert additional pathologic effects.

SALT-SENSITIVE HYPERTENSION AND DIABETES

Studies by Matsusaka *et al*. [11■■] found that mice with liver, but not kidney, AGT knockout were hypotensive and polyuric. These findings clearly support the concept that under normal dietary conditions, liver-derived AGT plays a greater role than kidney-derived AGT in maintenance of BP. These findings should not be construed, however, to indicate that kidney-derived AGT does not have the potential to influence BP.

Evidence for dietary salt intake modification of tubular AGT secretion comes from a study by Lantelme *et al*. [6] in which two mouse strains, a salt-sensitive inbred strain (C57BL/6) and an outbred strain (CD19), were evaluated. C57BL/6 mice had elevated urinary total AGT levels and unaltered plasma AGT levels with high Na intake compared to low Na intake. Our group also observed salt-sensitive hypertension in transgenic mice with proximal tubule-specific mouse AGT overexpression [30■■]. Further, high salt intake increased proximal tubule luminal Ang-II concentrations and proximal tubule reabsorption, while decreasing plasma and total kidney Ang-II in rats [34]. Although urinary or proximal tubule AGT levels were not directly measured in this study, it is likely that proximal tubule luminal Ang-II derives from proximal tubule AGT synthesis. Compared to rats maintained on a high salt di*et al*one, rats on a high salt diet with Ang-II infusion demonstrated hypertension, suppression of plasma renin activity, elevated kidney Ang-II levels, and increased urinary AGT excretion rate [14]. In contrast, there were no differences in urine AGT excretion rate or plasma and kidney Ang-II levels between rats on a high salt diet versus normal salt diet without infusion of Ang-II. In preliminary studies, our group has compared mice with overexpression of mouse AGT in liver alone, proximal tubule alone, or in both organs [35].

Mice with overexpression of AGT in kidney alone or in both liver and kidney were comparably hypertensive on a normal Na diet, whereas liver AGT overexpression alone did not affect BP on a normal Na diet. All lines of transgenic mice were hypertensive on a high Na diet. Notably, there was a direct correlation between urinary AGT excretion and BP, whereas high Na intake increased urinary AGT excretion in wild-type and liver AGT transgenic mice. Taken together, the above data suggest that high salt intake enhances proximal tubule AGT synthesis that in turn is involved in modulation of renal Na excretion and BP. That high salt intake increases proximal tubule AGT production seems counterintuitive in so far as one envisions its role in BP regulation. Further, as Ang-II *per se* increases proximal tubule AGT, there are multiple factors that sustain proximal tubule AGT production regardless of salt intake. These observations beg the question as to what biologic effects such sustained proximal tubule-derived AGT exerts; clearly, additional studies are required in this regard.

A comparative study of type 1 diabetic mice (Akita mice), mice with proximal tubule rat AGT overexpression alone, and Akita mice with proximal tubule rat AGT overexpression was performed to determine if RAS activation and diabetes can interact to modulate BP [36■]. Akita mice with rat AGT overexpression had higher SBP, albuminuria, renal structural damage and tubulointerstitial fibrosis as compared to Akita mice or proximal tubule rat AGT overexpression mice. Hence, activation of intrarenal AGT production in disease states such as diabetes may amplify hypertension and renal injury.

CONCLUSION

In summary, studies support the notion that proximal tubule AGT regulates BP and Na and water homeostasis. Transgenic mouse models of proximal tubule AGT overexpression suggest that proximal tubule AGT mediates a profibrotic, Na-retentive state that is more pronounced with high salt intake and in disease states such as diabetes. Some of the key remaining questions concern whether proximal tubule AGT works in concert with systemic AGT; the relative role of proximal tubule AGT in regulating renal Ang-II production; the effect of inflammatory conditions and disease states on proximal tubule AGT synthesis; and the role of distal nephron renin in converting AGT as well as the biologic consequences of this system.

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KEY POINTS

- Overexpression of AGT in the proximal tubule causes hypertension, tubular apoptosis and tubulointerstitial fibrosis.
- AGT in the S1 and S2 segments of the proximal tubule may derive primarily from systemic AGT and contribute primarily to the generation of intrarenal Ang-II.
- Urinary AGT primarily reflects proximal tubule synthesis of AGT, the latter occurring in the S3 segment of the proximal tubule.
- The relative contributions of systemic and renal AGT in regulating BP are unclear but an interaction between the two systems is likely.