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Intrarenal Angiotensin-Converting Enzyme: the Old and the New

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Abstract

Purpose of Review—The intrarenal renin-angiotensin-aldosterone system (RAS) is an independent paracrine hormonal system with an increasingly prominent role in hypertension and renal disease. Two enzyme components of this system are angiotensin-converting enzyme (ACE) and more recently discovered ACE2. The purpose of this review is to describe recent discoveries regarding the roles of intrarenal ACE and ACE2 and their interaction.

Recent Findings—Renal tubular ACE contributes to salt-sensitive hypertension. Additionally, the relative expression and activity of intrarenal ACE and ACE2 are central to promoting or inhibiting different renal pathologies including renovascular hypertension, diabetic nephropathy, and renal fibrosis.

Summary—Renal ACE and ACE2 represent two opposing axes within the intrarenal RAS system whose interaction determines the progression of several common disease processes. While this relationship remains complex and incompletely understood, further investigations hold the potential for creating novel approaches to treating hypertension and kidney disease.

Keywords

ACE; CE2; RAS; Hypertension; Kidney

Introduction

The local intrarenal renin-angiotensin-aldosterone system (RAS) functions as a paracrine hormonal system, independent from the systemic RAS. For one, all elements of the classical RAS are present and expressed in the kidney. In the kidney, renin is produced not only by the juxtaglomerular apparatus but also by principle cells of the connecting tubules and collecting ducts [1, 2•]. While there is some evidence to suggest that much of renal angiotensinogen (Agt) is produced systemically by the liver, Agt is also generated by the proximal tubule of the nephron [3]. Angiotensin-converting enzyme I (ACE), the enzyme

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Compliance with Ethical Standards

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responsible for converting angiotensin I (Ang I) to angiotensin II (Ang II), is similarly present throughout the kidney [4]. Additionally, intrarenal levels of Ang II are 1000-fold higher than its circulating levels in plasma [5, 6•, 7], implying that the majority of intrarenal Ang II is actually generated from within the kidney as a paracrine hormone [8]. A number of studies have further demonstrated the independent effects of the local renal RAS. Transgenic mice that overexpress Agt in the proximal tubule develop marked hypertension [9], and renal cross-transplantation studies have shown that Ang II inducible hypertension occurs only in animals with intact renal angiotensin II receptor type 1 (AT1R) [6•, 10, 11•].

More recently, several new components of the RAS have been discovered, including the angiotensin-converting enzyme 2 (ACE2) and the corresponding ACE2/Ang(1–7)/Mas axis, which are also present in the kidney [6•]. These newer RAS pathways have differing and often counterregulatory functions to the classic RAS system. The regulation and respective roles of these different elements of the renal RAS therefore remains an area of ongoing investigation. The focus of this review is to provide an overview of recent advances in understanding the roles of ACE and ACE2 in various pathologic processes in the kidney including what is known regarding the relative interaction of intrarenal ACE and ACE2 in renal pathophysiology.

ACE and ACE 2

The central function of ACE, both systemically and in the kidney, is the conversion of Ang I to Ang II. ACE is encoded by a gene located on chromosome 17q23 [12]. Although traditionally the pulmonary epithelium has been considered the main source of ACE, local intrarenal ACE production is quite abundant in the human kidney, with at least five times more ACE there than what has been found in the human lung [8, 13]. The highest concentrations of renal ACE occur in the brush border of the proximal tubule but ACE expression has also been reported in glomerular endothelium, mesangial cells, podocytes, and distal nephron [4, 11•, 13, 14, 15••, 16]. Intrarenal ACE expression is increased in several models of renal injury and hypertension including Ang II-induced hypertension, Goldblatt hypertension, and diabetic nephropathy, suggesting that ACE plays a critical role in renal injury and hypertension [17, 18].

The ACE2 enzyme is a monocarboxypeptidase that was first reported in 2000 and consists of a signal peptide, potential transmembrane domain, and potential metalloproteinase zinc-binding site [19]. ACE2 generally counteracts many of the known functions of the conventional ACE/Ang II/AT1R axis. It degrades Ang II into the vasodilator and anti-proliferative Ang 1–7 and Ang I into the inactive Ang 1–9. Ang 1–7 in turn exerts anti-oxidant, anti-fibrotic, and anti-inflammatory properties through its receptor, known as Mas (MasR) [20, 21] (Fig. 1). ACE2 protein expression has been noted in the circulation as well as various tissues, including kidney, heart, liver, lung, and neurons [19, 22–25]. In the kidney specifically, ACE2 is highly expressed in tubular and glomerular epithelium, vascular smooth muscle cells, the endothelium of interlobular arteries, and glomerular mesangial cells [23, 26]. Similar to ACE, expression of ACE2 is altered in many renal disease states such as diabetic nephropathy [27•, 28•], hypertensive renal disease [29•, 30], Alport syndrome [31, 32], and renal fibrosis [33•, 34•].

Hypertension

RAS plays a central role in the pathogenesis of hypertension, and one area of recent interest regarding ACE has been its role in salt-sensitive hypertension. Salt sensitivity is one of the leading mechanisms behind hypertension. Renal injury, such as from increased inflammation or oxidative stress, increases RAS activity and impairs sodium excretion [35]. This in turn increases intravascular volume and causes an overall increase in blood pressure [11•]. The mechanistic role of ACE in this process was recently illustrated in an elegant series of experiments using strains of mice with minimal or no renal ACE expression. These mice notably have normal circulating ACE and Ang II levels as well as normal renal development, allowing researchers to determine the independent effects of intrarenal ACE [36•, 37]. In response to Ang II infusion, renal ACE-deficient mice did not develop hypertension and failed to increase renal Ang II or reduce urinary sodium excretion as seen in wild-type mice [37]. This effect was explained through differences in renal sodium handling, with ACE-deficient mice showing decreased activity of NCC and NKCC2 in response to Ang II compared to wild type [37]. To further study the role of intrarenal ACE in salt sensitivity, this model was given the nitric oxide synthase inhibitor L-NAME, an agent that activates renal RAS without increasing systemic RAS activity, followed by high-salt diet. L-NAME alone had previously been shown to cause hypertension in wild-type mice but not in renal ACE-deficient mice [38]. In the setting of L-NAME and high-salt diet, renal ACE-deficient mice did not develop salt-sensitive hypertension, failed to demonstrate an increase in renal RAS activity, and had an amplified natriuretic response to high salt when compared to wild-type mice. These effects also corresponded to a greater reduction in phosphorylation of the sodium chloride cotransporter and expression of α -ENaC than in wild-type controls [36•]. Notably, lack of renal ACE also allowed mice to maintain an elevated GFR in the face of high salt that was lost in wild-type mice treated with L-NAME [36•]. The precise intrarenal location of the ACE activity responsible for salt-sensitive hypertension was further investigated using a mouse lacking ACE only in the tubular epithelial brush border [15•]. This model was also resistant to L-NAME-induced salt sensitivity and did not increase renal Ang II while demonstrating increased natriuresis and similarly decreased renal sodium transporter activity compared to wild type. In contrast, a mouse model that expresses ACE only in renal tubular epithelium developed salt-sensitive hypertension with L-NAME exposure [15•]. Taken together, this series of experiments prove that renal epithelial ACE is a major player in generating salt-sensitive hypertension, independent of systemic RAS activity, by regulating changes in renal sodium transport.

With the discovery of newer RAS components including ACE2, an important area of ongoing research interest is directed toward the balance between ACE and ACE2 in hypertension. It has generally been understood that the ACE2/Ang(1–7)/Mas axis acts to counterregulate the vasoconstrictive and hypertensive activity of the ACE/Ang II/AT1R axis. Recent studies showed that high-salt diet in spontaneously hypertensive rats decreased ACE2 expression while increasing the ACE/ACE2 ratio, glomerular hypertrophy, loss of the podocytes foot processes, and proteinuria [29•]. These effects were attenuated by low salt and normal salt diets which also decreased the ratio of renal ACE/ACE2 expression [29•]. While these findings imply that changes in ACE/ACE2 ratio may be responsible for

hypertensive changes, this study unexpectedly found no difference in ACE or ACE2 activity during high salt intake despite an increase in ACE expression [29•]. In a different approach, continuous administration of losartan in spontaneously hypertensive rats decreased blood pressure and increased ACE2 expression, suggesting that increased ACE2/Mas activity could be responsible for reductions in blood pressure. Notably, though there was no increase in the renal ACE2 receptor Mas in this study [39]. Adding further uncertainty, another recent study showed that intrarenal alterations of the ACE2/Ang1–7 complex did not significantly modify the course of malignant hypertension in Cyp11a1-Ren-2 transgenic rats, which have abnormally increased activity of the ACE/Ang II/AT1 pathway [40]. Taken together, these studies not only generally support opposing roles for intrarenal ACE and ACE2 in hypertension but also make clear that further studies are needed to understand the specific interactions between these two axes in the kidney.

Another model that has been used for exploring temporal roles of intrarenal ACE and ACE2 in the development of hypertension has been the 2-kidney 1-clip (2K1C) rodent model of renovascular hypertension. In this model, ACE levels are increased in the clipped kidney. Early in the course of 2K1C hypertension, renal ACE levels are not changed while ACE2 levels decrease. However, at 5 weeks, ACE expression is increased while ACE2 remains suppressed [41•]. This study further revealed that AT1R was initially suppressed in the proximal tubule of the clipped kidney but then increased during the maintenance phase of hypertension [41•]. These data indicate that reductions in ACE2 may be important for initiating hypertension while ACE plays a central role in maintaining elevated blood pressure. At the same time, the simultaneous increase in ACE and AT1R despite known increases in Ang II may further work to sustain renovascular hypertension.

It is also worth noting that Collectrin, an ACE2 homolog that lacks the enzymatic activity of ACE2, is expressed in the proximal tubule and collecting duct and is downregulated in Ang II-induced hypertension via the AT1R [42••]. Collectrin knockout mice are hypertensive and have increased salt sensitivity as well as reduced renal blood flow and renal medullary neuronal nitric oxide synthase dimerization [42••, 43]. Furthermore, in the setting of high-salt diet, wild-type mice that received transplanted kidneys from collectrin knockout mice developed higher blood pressure than knockout mice given wild-type kidneys or wild type alone and these differences correspond to higher levels of the renal sodium-hydrogen antiporter 3 (NHE3) [42••]. Taken together, these results indicate that in addition to the evident roles for renal ACE/Ang II/AT1R and ACE2/Ang(1–7)/Mas in hypertension, modulations in other similar intrarenal RAS components are additional factors contributing to the increasingly complex picture of the intrarenal RAS and hypertension.

Diabetic Nephropathy

RAS is also known to play an important role in diabetic kidney disease and it is well established that ACE inhibition is protective against the development of diabetic nephropathy. Some reports suggested that different polymorphisms of the ACE gene affect renal as well as systemic ACE expression and confer varying degrees risk of diabetic nephropathy [12]. Further work is needed, however, to fully understand the implications of these ACE genetic variations.

Some previous studies have demonstrated that renal glomerular and tubular expression of ACE2 are significantly decreased in experimental and clinical diabetic nephropathy [44] while others have reported increased ACE2 expression in the renal cortex of non-obese mice with diabetes [45]. Nonetheless, subsequent investigation has established a protective role for ACE2 against diabetic nephropathy. ACE2 inhibition in the streptozotocin-induced diabetic mouse model showed increased urine albumin to creatinine ratio as well as expansion of the glomerular matrix relative to mice with diabetes alone [46]. More recently, ACE2 knockout in diabetic mice was associated with increased blood pressure, mesangial matrix expansion, podocytes loss, and renal fibrosis including increased α -SMA accumulation and collagen deposition when compared to wild-type diabetic mice [47, 48••]. Importantly, the protective effects of ACE2 expression have also been found to be specific to the kidney. Enhancement of circulating ACE2 alone in streptozotocin diabetic mice failed to improve glomerular filtration rate, albuminuria, or kidney histology [49]. Meanwhile, enhancement of ACE2 expression in renal tubular epithelial cells reversed high glucose-induced increases in reactive oxygen species (ROS) formation and apoptosis. In contrast, increasing ACE2 expression in the renal proximal tubules of diabetic mice decreased fibrosis and albuminuria [28•, 30].

ACE2 can be measured in the urine and there is good evidence that its levels may serve as a marker for monitoring disease progression and metabolic state [22]. In the setting of hyperglycemia, an ACE2 ectodomain is cleaved by “a disintegrin and metalloproteinase-17” (ADAM17) and PKC- δ in the apical membrane of the proximal tubule and shed in the urine [50, 51•]. Subsequent studies demonstrated that the urinary ACE2/Cr ratio positively correlated with metabolic parameters including fasting blood glucose, hemoglobin A1C, triglyceride, and total cholesterol [52]. Additionally, urinary ACE2 level is predictive of urinary nephrin level, a marker of podocyte health [53]. There is also some evidence to suggest that blocking renal shedding of ACE2 could be therapeutic. Studies giving paricalcitol to non-obese mice with type 1 diabetes demonstrated decreased ADAM17 expression and increased renal ACE2 expression compared to untreated diabetic mice [45]. These studies suggested that ACE2 levels may be increased by preventing its renal shedding. These findings raise the question as to whether urinary ACE2 could be a measure of disease progression as well as a marker of response to treatment.

Similar to hypertension, there is increasing evidence that the relative increase in renal activity of the classic ACE/Ang II/AT1R versus ACE2/Ang(1–7)/Mas pathway is key to the progression of diabetic nephropathy. The AT1R antagonist candesartan, a well-accepted therapy in diabetic nephropathy, has recently been shown to improve renal tubular damage and albuminuria, partially through upregulation of ACE2/AT2R/Mas axis activity in the kidney [27•]. Conversely, treatment of diabetic rats with calcitriol reduced renal ACE levels and increased renal ACE2 expression, thus decreasing the ACE/ACE2 ratio, and associated with a reduction in proteinuria [54•]. Interestingly, in this study, reductions in ACE were mediated by the p38MAPK pathway, while ACE2 was upregulated via the ERK signaling pathway [54•]. Systemic use of recombinant human ACE2 in diabetic mice similarly attenuated kidney injury while reducing blood pressure and decreasing NADPH oxidative activity [55]. In vitro investigations of this effect in high glucose or Ang II-treated renal mesangial cells demonstrated that the reduction in oxidative stress with ACE2 occurred, at

least in part, through a reduction in Ang II that was mediated by increasing its conversion to Ang(1–7) [55]. Further support for the cross talk between renal ACE and ACE2 was demonstrated by the finding of increased renal ACE expression during ACE2 inhibition [46]. However, at least one recent study using the ACE2 knockout mouse found that while ACE2 deletion increased systemic ACE expression and worsened nephropathy, renal cortical ACE was in fact decreased [48••]. These findings emphasize the importance of the ongoing investigations into interactions of ACE and ACE2 in diabetic kidney disease.

Chronic Kidney Disease and Renal Fibrosis

While the ACE/Ang II/AT1R axis is known to contribute to chronic renal injury, the protective effects of ACE2/Ang(1–7)/Mas are becoming evident as well. ACE2 deficiency enhances kidney inflammation and renal fibrosis and exacerbates the progression of chronic kidney disease [56]. Conversely, administration of Ang(1–7) significantly reduced the inflammatory markers in the plasma and kidney, including IL-6, TNF- α , CRP, and MCP-1 in high-fat diet-fed mice, modulated renal lipid metabolism through the LDLr-SREBP2-SCAP pathway, and as a result, reduced renal damage induced by lipid [57•]. Similarly, rACE2 supplementation in ApoE KO mice reduced expression of IL-1 β , IL-6, IL-17A, TNF- α , TGF- β and collagen I, and renal structural injury via modulation of the mTOR/ERK signaling pathway and renal Ang(1–7)/Ang II balance [33•, 56]. In a recent study, administration of the GLP-1 analog exendin-4, a renoprotective agent, in the setting of unilateral ureteral obstruction, reversed ACE upregulation and increased ACE2 expression while reducing renal fibrosis and expression of Ang II and profibrotic factors TGF- β /Smad3 [34•]. There was also a trend toward improved expression of the ACE2 product Ang(1–7) with exendin, but this did not reach statistical significance [34•]. These results nonetheless emphasize the importance of the relative activity of ACE and ACE2 in the progression of renal fibrosis.

Similar to findings in diabetes, there is also evidence that elevated urinary ACE2 levels may signal worsening kidney disease. Rats with subtotal nephrectomy not only had increased kidney cortical ACE and reduced cortical and medullary ACE2 activities but also showed increased urinary ACE2 levels which correlated positively with urinary protein excretion, and negatively with creatinine clearance [58]. In a similar manner, while circulating, ACE2 activity is significantly decreased in patients with stage 3–5 (CKD3–5) and on dialysis (CKD5D), urinary ACE2 is significantly higher in CKD3–5 patients and correlates with urine albumin/creatinine ratio [59, 60]. These data suggest that shedding of ACE2 in the urine may be a marker of decreased renal and systemic ACE2 activity and progression of chronic kidney disease.

Alport Syndrome

ACE2 may also represent a novel marker of Alport syndrome (AS)-associated kidney injury. In 7-week old type IV collagen $\alpha 3$ gene knockout (Col4A3 $^{-/-}$) mice, there was decreased expression and activity of renal ACE2 and the urinary excretion rate of ACE2 paralleled the decline in tissue expression [31]. Recombinant exogenous ACE2 treatment partially reversed these changes by decreasing expression of collagen I mRNA, TGF- β signaling, TNF- α -

converting enzyme, proinflammatory cytokine expression, and macrophage infiltration, leading to attenuation of the progression of Alport syndrome-related nephropathy [61].

Conclusion

While RAS has long been identified as a major player in renal pathophysiology, our conception of the intrarenal RAS and its role continues to evolve. As newer components of RAS are discovered, this picture continues to grow in complexity. The interplay between intrarenal ACE and ACE2 is now clearly central to any understanding of how RAS promotes or prevents various pathologic processes involving the kidney. At the same time, the precise ways in which these two important members of the RAS interact and regulate one another is far from resolved. Better characterization of this relationship will likely have significant implications for our future detection and treatment of hypertension and kidney disease.

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Fig. 1.

Diagram of the actions of intrarenal ACE and ACE2. ACE converts Ang I to Ang II which is the ligand for the AT1R and promotes pathologic processes including inflammation, fibrosis, and sodium retention. ACE2 converts Ang I to Ang(1-9) and Ang II to Ang(1-7) which is the ligand for the MasR and counteracts many of the actions of the AT1R