Angiotensin-converting Enzyme 2: Possible Role in Hypertension and Kidney Disease

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The discovery of angiotensin-converting enzyme (ACE) 2 adds a new level of complexity to the understanding of the renin-angiotensin system. The high catalytic efficiency of ACE2 for the generation of angiotensin (ANG)-1–7 from ANG II suggests an important role of ACE2 in preventing ANG II accumulation, while at the same time enhancing ANG-1–7 formation. ACE and ACE2 may have counterbalancing functions and a regulatory role in fine-tuning the rate at which ANG peptides are formed and degraded. By counterregulating the actions of ACE on ANG II formation, ACE2 may play a role in maintaining a balanced status of the renin-angiotensin system. This review focuses on the function of ACE2 and its possible roles in kidney disease and hypertension. Studies using models of ACE2 ablation and the pharmacologic administration of an ACE2 inhibitor suggest that decreased ACE2 activity alone or in combination with increased ACE activity may play a role in both diseases.

Introduction

The first human homologue of angiotensin-converting enzyme (ACE), termed *angiotensin-converting enzyme 2* (ACE2) was identified in 2000 by two separate groups using genomics-based strategies [1,2]. The gene encoding ACE2 is located on the X chromosome. Like ACE, ACE2 is a type 1 integral membrane protein; however, ACE2 contains only a single active site domain and consists of 805 amino acids [1,2]. ACE2 acts as a carboxypeptidase, removing single amino acids from the C-terminus of its substrates, whereas ACE acts predominantly as a peptidyl dipeptidase removing C-terminal dipeptides. The metalloprotease catalytic domains of ACE2 and ACE are 41% identical, and comparison of the genomic structures indicates that the two genes arose through duplication from a common ancestor [1,2]. The subsequent elucidation of the three-dimensional structure of the extracellular domain of ACE2 [3] revealed that the catalytic mechanism of ACE2 closely resembles that of ACE. However, the substrate-binding pockets differ significantly [3], explaining the differences in substrate specificity between the two enzymes and the failure of ACE inhibitors to bind to and inhibit ACE2 [4].

ACE2 is the only known enzymatically active homologue of ACE in the human genome. The carboxyl end of ACE2 is homologous to collectrin [5]. Collectrin, unlike ACE and ACE2, lacks carboxypeptidase catalytic properties and was initially localized in the collecting tubule [5]. More recently, however, it has been localized to the proximal tubule, where it is involved in regulating amino acid uptake [6•,7]. Collectrin also has been suggested to have a role in cystogenesis by interacting with cilia-specific membrane integral proteins [8•]. Another gene has been identified in the genomes of several mammalian species that encodes a novel, single-domain ACE-like protein that was named ACE3. In several species ACE3 seems to lack catalytic activity as a zinc metalloprotease [9]. Moreover, in humans, no evidence could be found that the ACE3 gene is expressed, and the presence of deletions and insertions in the sequence suggests that in humans ACE3 is a pseudogene [9].

ACE2 was initially found in heart, kidney, and testis with lesser amounts in colon, small intestine, and ovary [10]. ACE2 also has been found in the lungs where it plays an important role in angiotensin (ANG) II metabolism [11,12]. ACE2 protects mice from severe lung injury induced by acid aspiration, sepsis, and severe acute respiratory syndrome (SARS) virus infection [11,12]. We first suggested that ACE2 could be renoprotective, particularly in combination with low levels of ACE [13]. Recent
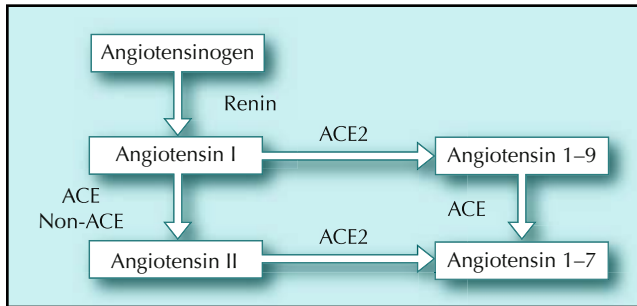
Figure 1. The *vertical arrows* show the classic angiotensin (ANG) II-forming pathway driven by angiotensin-converting enzyme (ACE) and non-ACE mechanisms. *Horizontal arrows* show the actions of the more recently discovered ACE2. Through the action of ACE2, less ANG I substrate is available for ACE to generate ANG II because ANG I is derived to ANG-(1–9) (*upper horizontal arrow*). ANG II is converted to ANG-(1–7) by ACE2 (*lower horizontal arrow*). The net effect of ACE2 is less ANG II accumulation and more ANG-(1–7) formation.

work by our laboratory is consistent with this hypothesis [14••,15••,16•]. In this review we discuss recent publications dealing with ACE2 as a pathway for ANG II metabolism and its possible role in hypertension and diabetic kidney disease.

ACE2 Substrates

The discovery of ACE2 adds a new level of complexity to the understanding of the renin-angiotensin system (RAS). ACE2 is a carboxypeptidase that facilitates the conversion of ANG II to ANG-(1–7) and the conversion of ANG I to ANG-(1–9) [1,2,17] (Fig. 1). ANG-(1–9) has no known effects on blood vessels but can be converted by ACE to a shorter peptide, ANG-(1–7), which is a blood-vessel dilator (Fig. 1). ANG I (with 10 amino acids) is an intermediate peptide without known biologic effects. ANG I is converted to ANG-(1–9) (with nine amino acids) by ACE2, but is converted to the eight-amino acid ANG II by ACE (Fig. 1). The actions of ACE2 should act to prevent the accumulation of ANG II, a vasoconstrictor, and lead to formation of ANG-(1–7), a vasodilator.

ACE and ACE2 may have counterbalancing functions and a regulatory role in fine-tuning the rate at which ANG peptides are formed and degraded. ACE2 has a high catalytic efficiency for the generation of ANG-(1–7) from ANG II [17]. This suggests an important role of ACE2 in preventing ANG II accumulation, while at the same time enhancing ANG-(1–7) formation. Recently, it was shown that ANG 1–7 inhibits ANG II–stimulated mitogen-activated protein kinase (MAPK) phosphorylation in proximal tubular cells [18•]. Generation of ANG-(1–7) by proximal tubular ACE2 could therefore also have a role in counteracting the effects of locally generated ANG II [18•].

ACE2-mediated degradation of ANG II to ANG-(1–7) has been documented in studies using kidney cortex preparations or isolated proximal tubules [19,20•,21•]. ACE2 affinity for ANG II conversion has been generally found

Table 1. Amino-acid sequences and cleavage sites of peptides hydrolyzed by angiotensin-converting enzyme 2

Peptide	Amino acid sequence[†]
Angiotensin I	DRVYIHPFH ↓ L
Angiotensin II	DRVYIHP ↓ F
Apelin-13	QRPRLSHKGPMP ↓ F
Apelin-36*	. . . QRPRLSHKGPMP ↓ F
des-Arg^9-bradykinin	RPPGFSP ↓ F
Lys-des-Arg^9-bradykinin	KRPPGFSP ↓ F
β-Casomorphin	YPFVEP ↓ I
Neocasomorphin	YPVEP ↓ I
Dynorphin A 1–13	YGGFLRRIRPKL ↓ K
Ghrelin*	. . . ESKKPPAKLQP ↓ R
Neurotensin 1–8	pE-LYENKP ↓ R

*C terminus shown.
†*Arrows* indicate cleavage sites.
(*Adapted from* Vickers et al. [17].)

higher than for ANG I to ANG-(1–9) [19,20•,21•]. In freshly dissected rat proximal tubular segments, however, Li et al. [22••] observed ACE2-dependent formation of ANG-(1–7) from ANG I, which depended on conversion of ANG I to ANG-(1–9), followed by ACE-mediated cleavage to ANG-(1–7). In another study in cardiac tissue, investigators suggested that ACE2 can act through ANG-(1–9) instead of ANG-(1–7), as a counterregulator of ACE [23].

The knowledge of ACE2 biologic peptide substrates is useful in providing an initial understanding of the substrate specificity of the protease and the physiologic role of ACE2. If ACE2 is a "converting enzyme," as ACE is, then its peptidase activity either produces or degrades (or both) a peptide with biologic activity [17]. Therefore, an understanding of the biologic activity of ACE2 substrates and products suggests putative physiologic roles for ACE2 [17]. Biologically active substrate peptides that are cleaved by ACE2 are shown in Table 1. Although the biologic peptides ANG I and ANG II are the main known ACE2 substrates, ACE2 can hydrolyze several other target peptides. ACE2 does not cleave bradykinin but inactivates both des-Arg^9-bradykinin and lys-des-Arg^9-bradykinin [17].

ACE2 can also remove the C-terminal residue from apelin and other vasoactive peptides such as neurotensin, kinetensin (a neurotensin-related peptide), and des-Arg bradykinin [10]. ACE2 has high catalytic efficiency to hydrolyze apelin-13 and apelin-36 peptides [10]. Apelin also induces an increase in myocardial contractility and a reduction of vasomotor tone [10]. Two opioid peptides, dynorphin A and β-casomorphin, are also cleaved by ACE2 [10,17]. These two ACE2 substrate peptides stimulate κ and δ opioid G-protein–coupled receptors, and may have negative effects on cardiomyocyte contractility [10].

Assays to Measure ACE2 Activity

Several assays to measure tissue ACE2 activity have been developed. The literature describes methods using different approaches, including high-performance liquid chromatography (HPLC) [24••], mass spectrometry (MS) [20•,22••], and fluorometry [16•,23,25–27]. HPLC and MS assay methodologies use the endogenous peptide substrate (ANG II) and provide information regarding the identity of the specific peptides formed in the hydrolytic reactions. Methods using fluorogenic peptide substrates offer the advantage of being simple, rapid, and convenient to quantitate tissue ACE2 activity in large numbers of samples simultaneously [16•].

Currently, most widely used methods for ACE2 activity measurements utilize fluorogenic peptide substrates Mca-YVADAPK(Dnp) or Mca-APK(Dnp) [16•,23,25–27]. In these assays, the substrate peptide contains a fluorescent 7-methoxycoumarin group (Mca), which is quenched by energy transfer to a 2,4-dinitrophenyl moiety (Dnp). This reaction is based on the cleavage of an amide bond between the fluorescent and quencher groups, resulting in an increase in fluorescence [28]. It can be used to measure the activity of ACE2 and of other peptidases [28].

The fluorescence signal of Mca-YVADAPK(Dnp) is partially quenched by specific inhibitors of both ACE and ACE2, but not by an inhibitor of another metalloprotease, carboxypeptidase A [16•]. We took advantage of this dual action to develop an assay to measure ACE and ACE2 activity concurrently [16•]. The combination of specific inhibitors for ACE and ACE2 quenched the signal almost completely and to the same degree as ethylenediaminetetraacetic acid (EDTA), a metal ion chelator, indicating that both metalloenzymes, ACE and ACE2, are involved in the degradation of the substrate. As noted previously, the dual cleavage of this substrate by ACE and ACE2 makes it possible to measure activity of these two carboxypeptidases concurrently in tissue samples [16•]. This is particularly important because ACE2 and ACE colocalize in numerous tissues where both enzymes have been shown to be enzymatically active [29]. The assessment of ACE2 activity, the only active homologue of ACE, in combination with ACE activity is useful in evaluating the RAS in the pathophysiology of different disease states [14••].

ACE2 activity has also been measured using a different fluorogenic substrate, Mca-APK(Dnp) [25–27]. This approach detected ACE2 activity in serum samples from mice overexpressing ACE2 (but not in wild-type mice) [26], and in rat heart tissue [25] and rat testes [27]. Using this substrate in mouse tissues, however, we could not quench the fluorescence with MLN-4760. Moreover, the Mca-APK(Dnp) substrate is highly selective for ACE2 [26]. Accordingly, it cannot be used for dual measurements of ACE and ACE2 activity.

Ferrario et al. [24••] used an HPLC-based method to measure ACE2 activity in cardiac membranes; this method was later extended to measurements in other tissues and body fluids [21•]. The assay is performed in two steps: 1) radiolabeled peptide precursors are incubated with ACE2; and 2) HPLC is used to separate the peptide products. The HPLC method utilizes the ability of ACE2 to hydrolyze ANG II to ANG-(1–7) [17]. The conversion rate of the exogenous radioactively labeled ANG II to ANG-(1–7) in the presence and absence of ACE2 inhibitor is the equivalent of the ACE2 enzymatic activity [24••]. Because this method requires HPLC measurements, the process involved is relatively time consuming, but it provides information on ANG-(1–7) formation directly.

Another newly developed assay also uses the natural substrate of ACE2, ANG II; this method utilizes surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS with ProteinChip Array technology (Ciphergen Biosystems, Fremont, CA) [20•]. In this assay, mouse kidney samples are mixed with ANG II and incubated for a specified time. ACE2 converts ANG II to ANG-(1–7), which both can be quantified by SELDI-TOF MS. Based on the observation of a linear relationship between recombinant ACE2 concentrations and the quotient of the formed ANG (1–7) to the hydrolyzed ANG II, the ratio of ANG (1–7)/ANG II was proposed as a measure of tissue ACE2 activity [20•].

ACE2 Activity Is Tissue Specific

The levels of ACE2 activity vary widely within tissues. For instance, using our fluorogenic assay the levels of ACE2 activity in mouse kidney cortex are about 10- to 20-fold higher than in the heart, whereas ACE2 activity is barely detectable in serum [16•]. Studies using other measurement methods found very low or undetectable ACE2 activity in plasma from rodents [20•,26]. Similarly, in healthy humans, the levels of circulating ACE2 are undetectable or very low [30]. Rice et al. [30] found that circulating ACE2 was 100-fold lower than ACE, and ACE2 was detectable in less than 10% of the study population. Low circulating ACE2 levels could result from less shedding of ACE2 as compared with ACE from the plasma membrane of endothelial cells [30]. The zinc metalloprotease ADAM17 is responsible for the regulated shedding of ACE2; there is little constitutive shedding of ACE2 as compared with ACE [31•]. ACE2 activity and protein have been detected in human urine from healthy subjects [32•], and ACE2 activity has been measured in urine and serum from sheep [21•]. Thus, ACE2, like ACE, appears to be secreted as a soluble protein in vivo. It would be important to determine whether circulating or urinary ACE2 increases in certain disease states, either due to increased expression, increased shedding from the membrane, or decreased clearance from the circulation [30]. The pattern of *ACE* and *ACE2* expression in the glomerulus of *db/db* mice is just the opposite of what we find in cortical tubules [13]. Consequently, the final urine may not reflect the site of the nephron where the alteration primarily resides.

In kidney cortex, a good correlation was found between ACE2 protein abundance and enzymatic activity [16•]. This indicates that the level of functional activity is highly dependent on the level of protein expression. In contrast, we found a lack of positive correlation between renal ACE2 activity and renal ACE2 mRNA levels in the *db/db* as well as streptozotocin (STZ)-induced diabetic mice. These findings illustrate the importance of not relying solely on mRNA levels when assessing whether ACE2 is altered. Measurements of ACE2 mRNA alone may not reflect existing differences occurring at a post-transcriptional level [16•]. Investigators must keep this in mind when interpreting data from studies reporting ACE2 gene polymorphisms in diseases such as hypertension or diabetes. This further underscores the importance of having reliable assays to measure ACE2 activity targeting the sites of possible ACE2 alterations in the kidney and other organs.

Kidney ACE2 Expression

Immunohistochemical analysis of ACE2 distribution within the kidney demonstrated that renal tubules clearly show the highest intensity of immunostaining [13,15••,33]. In both kidney sections and cultured polarized renal epithelial cells, ACE2 localizes predominantly to apical surface [15••,32•] where it can undergo regulated proteolytic shedding [31•]. ACE2 is also present in the glomerulus, although the level of expression in mouse kidneys is reduced as compared with renal tubules (see below). Li et al. [22••] have systematically examined the relative distribution of ACE2 in the rat kidney at the mRNA level. In microdissected rat nephron segments, semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) revealed that ACE2 mRNA was widely expressed, with relatively high levels in the proximal straight tubule. The high level of ACE2 expression in the proximal tubule suggests that it may regulate the local levels of ANG II, and generate ANG-(1–7). In rats, ACE2 has been localized by immunohistochemistry to the proximal tubule [34]. Interestingly, ACE2 colocalizes with ANG-(1–7), and the proximal tubular staining for ACE2 and ANG-(1–7) increases in normal pregnancy [34]. This correlated with increased urinary excretion of ANG-(1–7); however, ACE2 in urine was not measured [34].

Lely et al. [35] reported that in humans ACE2 is expressed in tubular and glomerular epithelium, as well as in vascular smooth muscle cells and the endothelium of interlobular arteries. In renal biopsies from patients with primary and secondary kidney diseases, these authors found neo-expression of ACE2 in glomerular and peritubular capillary endothelium [35].

Our laboratory recently showed that ACE2 colocalizes with glomerular epithelial cell (podocyte) markers, and its presence in the podocyte/slit diaphragm complex was confirmed by immunogold labeling [15••]. The presence of ACE2 was further demonstrated in immortalized podocytes grown in culture by Western blot and immunofluorescence [36]. Velez et al. [37] examined the processing of angiotensin substrates by cultured glomerular epithelial cells. They showed that podocytes express a functional intrinsic RAS characterized by neprilysin, aminopeptidase A, ACE2, and renin activities, which predominantly lead to ANG-(1–7) and ANG-(1–9) formation and ANG II degradation [37].

Our laboratory has characterized the pattern of glomerular staining for ACE2 and ACE in control and diabetic mice [15••]. In diabetic kidneys, glomerular immunostaining for ACE2 is attenuated in *db/db* mice of 8 weeks of age. In sharp contrast, ACE expression in glomeruli from diabetic *db/db* mice is increased as compared with glomeruli from their respective age-matched nondiabetic controls [15••]. Tikellis et al. [33] found the opposite pattern in rats made diabetic by STZ. However, glomerular staining for ACE2 in mice is weaker than in rats; thus, there may be a species difference. In humans, as in mice, glomerular staining is weaker than tubular staining [38].

Possible Significance of Altered ACE2 in Kidney Disease

What may be the significance of reduced ACE2 and increased ACE at the glomerular level? We reasoned that in diabetic mice such a combination could foster renal injury by resulting either in ANG II accumulation, decreased ANG-(1–7) formation, or both [15••]. To test this hypothesis, we used a specific ACE2 inhibitor, MLN-4760, to reduce ACE2 activity. The administration of MLN-4760 for 16 weeks exacerbated albuminuria in the *db/db* mice [15••]. This was associated with increased glomerular expression of fibronectin. In a different model of diabetes (STZ-treated mice), Soler et al. [14••] found both glomerular mesangial expansion and increased albumin excretion after MLN-4760 treatment. We further found that the glomerular expression of ACE was increased in STZ-treated mice and further increased after MLN-4760 administration [14••]. As outlined in Figure 1, changes in ACE and ACE2 can work in concert to regulate the level of angiotensin peptides. The scheme outlined in Figure 1 predicts that a pattern of low ACE and high ACE2 would lead to less ANG II and more ANG-(1–7) formation locally.

A combination of increased ACE and decreased ACE2 in the glomerulus is apt to favor increased glomerular ANG II accumulation and foster an increase in glomerular permeability manifested by albuminuria. This hypothesis does not necessarily conflict with prevailing views of enhanced formation of ANG II in the diabetic kidney. Rather, both enhanced ANG II formation and decreased ANG II degradation may occur in glomeruli of diabetic mice, thereby contributing to produce more damage.

Studies in animals with ACE2 genetic ablation have shown the development of renal lesions, particularly within the glomerulus, although glomerular injury was seen only in older animals. In male mice with genetic ACE2 ablation, early accumulation of fibrillar collagen in glomerular mesangium was followed by the development of glomerulosclerosis by 12 months of age. Female ACE2 mutant (*Ace2-/-*) mice were relatively protected. Urinary albumin excretion was increased as compared with age-matched control mice [39••]. These structural and functional changes in the glomeruli of male ACE2 mutant mice were prevented by treatment with the ANG II type 1 receptor antagonist irbesartan. The glomerular injury in male mice associated with the deletion of the *Ace2* gene were further accentuated by diabetes [40••]. In this more recent study, *Ace2* knockout mice were crossed with Akita mice, a model of type 1 diabetes mellitus. Diabetic *Ace2* knockout mice (*Ace2-/yIns2* WT/C96Y) exhibited a twofold increase in urinary albumin excretion compared with Akita mice not depleted in the *Ace2* gene. Increased mesangial matrix scores and glomerular basement membrane thicknesses in *Ace2-/yIns2* WT/C96Y mice were accompanied by increased fibronectin and α-smooth muscle actin staining in the glomeruli. There were no differences in blood pressure or heart function to account for the exacerbation of kidney injury. Although kidney levels of ANG II were not increased in the diabetic mice, treatment with an ANG II receptor blocker reduced urinary albumin excretion rate in *Ace2-/yIns2* WT/C96Y mice, suggesting that acceleration of glomerular injury in these mice is ANG II mediated [40••].

ACE2 and Hypertension

A seminal paper by Crackower et al. [41], primarily describing an *Ace2* knockout and its associated cardiac pathology, also reported that ACE2 was reduced at the gene and protein level in kidneys from three separate rat models of spontaneous and diet-induced hypertension. In various recombinant rat models, several quantitative trace loci (QTL) for hypertension have been identified [41]. *Ace2* maps to the X chromosome in humans and a QTL has been mapped to the X chromosome in several rat models of hypertension [41]. The finding by Crackower et al. [41] that the *Ace2* gene maps to a defined QTL on the X chromosome suggests *Ace2* as a candidate gene underlying the loci linked to hypertension.

More recently, Tikellis et al. [42•] showed that the developmental pattern of ACE2 expression in the spontaneously hypertensive rat (SHR) kidney was altered before the onset of hypertension. Specifically, the expression and activity of ACE2 were increased in tubules from the SHR, before the onset of hypertension. With the increase in blood pressure at 6 weeks of age, the tubular expression of ACE2 is reduced in SHR compared to Wistar-Kyoto rats (WKY). Over the course of renal development, ACE2 expression

does not significantly change in the SHR kidney, whereas ACE2 expression increases in the WKY kidney [42•]. This finding seems to be consistent with the concept that ACE2 is downregulated in kidneys from hypertensive rats.

In humans, few studies have been conducted to examine possible association of *Ace2* gene polymorphisms with hypertension. In three studies performed on Chinese cohorts, an association was found between single-nucleotide polymorphisms in *Ace2* gene and blood pressure in women with the metabolic syndrome [43] and in women with essential hypertension [44,45]. Moreover, one of the ACE2 alleles associated with high blood pressure seemed to confer a risk for reduced antihypertensive response to ACE inhibitors as well [45]. In another study, no link was reported between *Ace2* gene polymorphisms and essential hypertension in Australian individuals of white Anglo-Celtic origin [46]. Collectively, the role of the *Ace2* gene in conferring the predisposition to hypertension is far from clear; whether the discrepancies in genetic linkage analyses could be related to differences in ethnic origin of the cohorts studied needs further investigation. Polymorphisms in the *Ace2* gene were also sought in the general population regarding echocardiographically determined parameters of left ventricular mass, structure, or function [47]. This study provides some evidence that genetic variants in the *Ace2* gene may be associated with left ventricular mass and left ventricular hypertrophy in men, but no association with blood pressure was found in either men or women. In a study exploring human renal biopsy specimens, a significant correlation was found between the mRNA levels of ACE and ACE2 in a variety of renal conditions [48]. The correlation was highly significant ($P < 0.001$) but relatively weak ($r = 0.396$). Interestingly, the ACE to ACE2 ratio was significantly higher in subjects with hypertension than in subjects without hypertension. These findings are in keeping with the idea that ACE2 might play a role in maintaining a balanced status of local renal RAS by acting to counterregulate the actions of ACE.

Evidence is also emerging that ACE2 may be altered in pregnancy-related hypertension [49]. Joyner et al. [49] examined the questions of whether ANG-(1–7) and ACE2 colocalize and whether they change in parallel during normal and hypertensive pregnancies. The authors found that during normal pregnancies, concurrent changes of ACE2 and ANG-(1–7) occur, suggesting that ACE2 plays a role in regulating the renal levels of ANG-(1–7) at mid- to late gestation [49]. In contrast, in hypertensive pregnant rats, the ACE2 activity in cortex and medulla were unchanged, whereas ANG-(1–7) levels were reduced. It was therefore concluded that the decrease in renal ANG-(1–7) content in the absence of a concomitant decrease in ACE2 implicates the participation of other ANG-(1–7) forming or degrading enzymes during hypertensive pregnancy [49].

Genetic ablation of ACE2 may not result in spontaneous hypertension. Two different ACE2 knockouts

have been described, and hypertension is not an overt feature of either phenotype [41,50••]. In a study by Gurley et al. [50••] in mice on the C57BL/6 background, ACE2 deficiency was associated with a modest increase in blood pressure, whereas the absence of ACE2 had no effect on baseline blood pressures in 129/SvEv mice. However, this does not mean that ACE2 is not important in blood pressure regulation. After acute ANG II infusion, plasma concentrations of ANG II were almost threefold higher in ACE2-deficient mice than in controls; moreover, blood pressures were substantially higher in the ACE2-deficient mice than in wild-type mice [50••]. Severe hypertension in ACE2-deficient mice was associated with exaggerated accumulation of ANG II in the kidney, as determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [50••]. This study provided strong evidence that complete ACE2 insufficiency can increase blood pressure under conditions of ANG II excess. Based on this study, one cannot conclude whether relative ACE2 deficiency or surplus affects blood pressure. To more directly assess the role of ACE2 on blood pressure regulation, we recently used recombinant ACE2 (rACE2) administered over a period of 3 days via osmotic minipumps, with or without ANG II infusion [51]. The increase in blood pressure produced by ANG II alone was prevented by the concomitant administration of ACE2 [51]. Moreover, plasma ANG II levels were markedly reduced by rACE2 administration, thereby demonstrating the important role of this enzyme in the degradation of ANG II [51]. This suggests that the administration of rACE2 may have a role in the treatment of ANG II–dependent hypertension, and opens the way for the development of therapies based on ACE2 modulators capable of selectively increasing ACE2 activity.

Conclusions

ACE2 is the only enzymatically active homologue of ACE, and it plays a significant role in maintaining a balanced status of the RAS. ACE2 could act by either preventing ANG II accumulation or enhancing ANG-(1–7) formation, or both. There is also increasing evidence that alterations in ACE2 may be involved in disease states, such as experimental diabetic kidney disease and possibly hypertension. Investigating the role of ACE2 in those two prevalent diseases and whether its effects are mediated by ANG II or ANG-(1–7) and other biologically active peptides, which are also substrates of ACE2, opens the way for developing new therapeutic targets in hypertension.

Disclosures

No potential conflict of interest relevant to this article was reported.

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