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Review

Membrane-associated zinc peptidase families: comparing ACE and ACE2

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Abstract

In contrast to the relatively ubiquitous angiotensin-converting enzyme (ACE), expression of the mammalian ACE homologue, ACE2, was initially described in the heart, kidney and testis. ACE2 is a type I integral membrane protein with its active site domain exposed to the extracellular surface of endothelial cells and the renal tubular epithelium. Here ACE2 is poised to metabolise circulating peptides which may include angiotensin II, a potent vasoconstrictor and the product of angiotensin I cleavage by ACE. To this end, ACE2 may counterbalance the effects of ACE within the renin–angiotensin system (RAS). Indeed, ACE2 has been implicated in the regulation of heart and renal function where it is proposed to control the levels of angiotensin II relative to its hypotensive metabolite, angiotensin-(1–7). The recent solution of the structure of ACE2, and ACE, has provided new insight into the substrate and inhibitor profiles of these two key regulators of the RAS. As the complexity of this crucial pathway is unravelled, there is a growing interest in the therapeutic potential of agents that modulate the activity of ACE2.

Keywords: Metallopeptidase; Angiotensin; SARS; Coronavirus; Structure

1. Introduction

The cell surface, particularly of some cell types, is endowed with proteolytic activity able to modulate the activity of membrane proteins or circulating regulatory peptides. These proteases or peptidases, mostly metalloenzymes using zinc as cofactor, are structurally organised as ectoenzymes with their catalytic sites facing into the extracellular space. Examples include neutral endopeptidase (neprilysin, NEP), aminopeptidases and carboxypeptidases of various specificities and the subjects of this article, the angiotensin-converting enzymes (ACE and ACE2). These ectopeptidases mainly exist as single membrane-spanning proteins of type I (C-terminus intracellular), type II (N-terminus intracellular) topology, or glycosyl-phosphatidylinositol-linked proteins. Undoubtedly the best studied of these ectopeptidases by far is ACE, largely because of its physiological and pathological roles. For almost 50 years, ACE was a unique mammalian enzyme distinct from other growing peptidase families. The discovery of a human homologue of ACE (ACEH or ACE2) has led to the resurgence of studies on ACE biology coupled with detailed structural information on the enzyme itself. The pace of knowledge concerning ACE2 has been breathtaking in comparison to the relatively leisurely developments in understanding ACE structure and function over several decades [1]. In a period of 4 years, ACE2 has been cloned, purified, knocked-out, knocked-in, inhibitors have been developed, its 3D structure determined and new functions have emerged. ACE2 is implicated in cardiovascular and renal biology, diabetes, and obesity, and most remarkably,

Abbreviations: ACE, angiotensin-converting enzyme; CP-A, carboxypeptidase-A; CoV, coronavirus; NEP, neutral endopeptidase/neprilysin; QTL, quantitative trait locus; RAS, renin–angiotensin system; SARS, severe acute respiratory syndrome; SNP, single nucleotide polymorphism; SHR, spontaneous hypertensive rat

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ACE2 may serve as a receptor for the severe acute respiratory syndrome (SARS) virus.

2. ACE, the renin–angiotensin system (RAS) and the kallikrein–kinin system

Cardiovascular diseases continue to be one of the leading causes of death in developed countries, most commonly resulting from hypertension and congestive heart failure. A major revolution in the treatment of hypertension came with the development of inhibitors of ACE. ACE inhibitors (e.g., captopril and lisinopril) have proved to be highly effective and safe drugs, which are used clinically to treat not only hypertension, but also congestive heart failure and renal disorders. Inhibition of ACE imposes its effects primarily via the RAS. The RAS mediates numerous effects in the cardiovascular system including blood pressure homeostasis and maintenance of fluid and salt balance [2]. ACE is a crucial regulator of the RAS, converting the inactive decapeptide angiotensin I to the potent vasoconstrictor angiotensin II [3] whilst also metabolising the hypotensive peptide, bradykinin [4].

3. The discovery of ACE2: a type I transmembrane glycoprotein

In 2000, a novel mammalian homologue of ACE, originally termed ACEH and subsequently ACE2, was identified by two distinct molecular strategies [5,6]. The *ace2* gene maps to the human X chromosome and encodes an 805-amino-acid, type I membrane-bound glycoprotein with an apparent molecular weight of approximately 120 kDa. The amino-terminal domain of ACE2 shares around 40% sequence identity with the corresponding domain of somatic ACE and contains a single HEXXH zinc-binding motif [5]. Intriguingly, the carboxy-terminal domain displays 48% sequence homology with collectrin, an enzymically inactive glycoprotein of unknown function expressed exclusively in the kidney [7]. ACE2 initially appeared to be more limited in its tissue distribution than ACE, with significant levels only detected in the heart, kidneys, testes and gastrointestinal tract [5,8]. A recent study, however, detected significant levels of ACE2 expression in a wide variety of tissues [9].

4. Comparing the specificities of ACE and ACE2

On first inspection, owing to the high degree of sequence similarity that exists between ACE2 and ACE, it could be assumed that ACE2 might function, like ACE, as a dipeptidyl carboxypeptidase. Indeed, the only previously characterised ACE-like proteins, the *Drosophila* ACE orthologues AnCE and Acer, do display such an activity [10]. Surprisingly, however, ACE2 functions as a carboxypeptidase, cleaving only a single C-terminal residue from its peptide substrate (Table 1). Indeed, ACE2 is unable to cleave the archetypal ACE substrates, bradykinin and Hip-His-Leu, and is insensitive to the potent ACE inhibitors lisinopril and captopril [5]. Further characterisation using a panel of biologically active peptides has provided a consensus sequence of Pro-X-Pro-hydrophobic/basic for the protease specificity of ACE2 [11]. This consensus sequence is supported by the ACE2 active site model proposed by Guy et al. [11]. It has also been ascertained that the chain length of hydrolysed peptides is not limiting although the cleavage of dipeptides is very slow. The catalytic activity of ACE2, like ACE, is pH- and anion-dependent [11–13].

5. Comparing the active sites of ACE and ACE2

The structures of tACE [14] and the *Drosophila* homologue AnCE [15] have recently been elucidated. The minimum glycosylation requirement for expression and processing of enzymically active tACE was investigated by progressive deglycosylation [16]. A truncated, soluble form of tACE, lacking the first 36 residues at the N-terminus, expressed in CHO cells in the presence of the glycosidase-I inhibitor (*N*-butyldeoxynojirimycin) retained the activity of the native enzyme and yielded crystals that diffracted to 2.0-Å resolution. Natesh et al. [14] described the structure of tACE alone and in complex with the inhibitor lisinopril and thereby provided a detailed picture of the active site. The tACE molecule has an overall ellipsoid shape with a central groove that extends approximately 30 Å into the protein dividing it into two subdomains with the active site located towards the bottom of this groove. Simultaneously, the crystal structure of *Drosophila* AnCE was determined [15]. It is not clear from the information presented if a minimally glycosylated form of AnCE was expressed and purified for the crystallisation studies. However, a previous report by a separate group has shown

Table 1
Hydrolysis of biologically active peptides by ACE2

	Peptide sequence
Apelin-13	QRPRLSHKGPM P ⇑ F
Angiotensin I	DRVYIHPFH ⇑ L
Angiotensin II	DRVYIHP ⇑ F
des-Arg^9-Bradykinin	RPPGFS ⇑ F
β-Casomorphin-(1–7)	YPFVEP ⇑ I
Dynorphin A-(1–13)	YGGFLRRIRPKL ⇑ K
Ghrelin (C terminus shown)	. . .ESKKPPAKLQP ⇑ R
Neurotensin-(1–8)	pELYENKP ⇑ R
Neurotensin-(1–11)	pELYENKPRRP ⇑ Y

The major peptides that are hydrolysed by ACE2 are shown with their amino acid sequence. An arrow indicates the site of cleavage. pE is pyroglutamyl.

that AnCE does not require glycosylation for secretion or activity [17]. Data from the AnCE crystals revealed that, like ACE, a large internal channel encompasses the entire protein molecule and the active site is located at the bottleneck connecting two chambers of unequal size within this cavity.

Most recently, the crystal structures of the extracellular domain, lacking the transmembrane and cytosolic regions, of native and inhibitor-bound ACE2 have been determined to 2.2- and 3.0-Å resolution, respectively [18]. These data confirm the integrity of the active site model of ACE2 proposed by Guy et al. [11]. This model, based on the crystal structure of tACE, indicated that the catalytic mechanism of ACE2 resembles that of ACE. The predicted structural differences between the active site of ACE (dipeptidyl carboxypeptidase) and ACE2 (carboxypeptidase), responsible for the differences in specificity, were also verified by the ACE2 structure. In essence, the main differences occur in the ligand-binding pockets, particularly at the S2′ subsite of ACE and in the binding of the peptide carboxy-terminus. The cavity in tACE is larger than that of ACE2, allowing an extra amino acid to bind in the specificity pocket. Most importantly, the R273 to Q substitution between ACE2 and ACE appears to be largely responsible for the elimination of the S2′ pocket in ACE2. These structural differences offer an explanation as to why the classical ACE inhibitor lisinopril is unable to bind to ACE2.

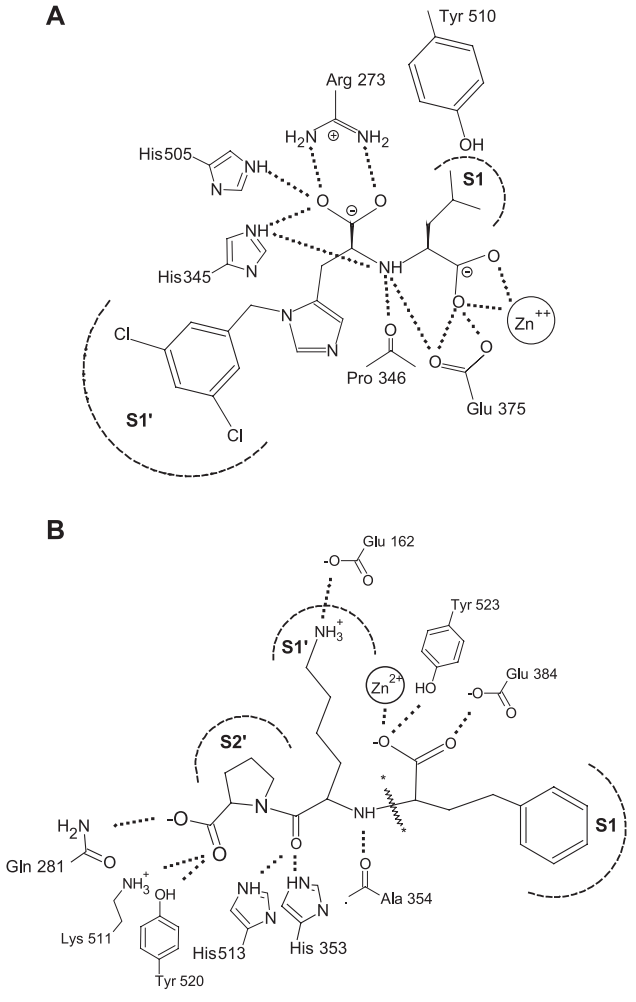

Fig. 1. Interaction of the catalytic sites of ACE2 and tACE with inhibitors. Schematic view of the binding interactions of (A) the inhibitor MLN-4760 at the active site of ACE2 (adapted from Ref. [18]) and (B) lisinopril at the active site of tACE (adapted from Ref. [11]). Hydrogen bonds to the ligand are shown (dotted lines). The different binding subsites are labelled.

The ACE2 crystals, grown in the presence of MLN-4760 [19], revealed an unanticipated inverse binding orientation of the inhibitor in the active site (Fig. 1). This can be rationalised by the Y510 to V substitution between ACE2 and ACE whereby the size of the S1 pocket, in ACE2, is considerably reduced and can no longer accommodate the bulky dichlorobenzyl group. Moreover, this explains why substrates like bradykinin, angiotensin-(1–9) and Leu-enkephalin are poor substrates for ACE2, but good substrates for ACE. Another surprising observation is the absence of a bound chloride in ACE2 in an identical position to the second binding site (CL2) of tACE [14], as an occupied CL2 site is largely responsible for the chloride activation of ACE [20]. ACE2 is clearly different from ACE and displays unique enzyme function despite the sequence similarity that exists between the two proteins and the common features of their activity profiles.

6. Developing inhibitors of ACE2

The first class of selective inhibitors for ACE2 includes a series of non-peptide compounds whose design was based upon the substrate specificity of ACE2 and the requirement of a centrally located carboxylate to coordinate the inhibitor [19]. The lead compound possesses sub-nanomolar affinity (IC_{50} 0.44 nM) for ACE2 and 220 000- and 22 000-fold less affinity for human tACE and bovine carboxypeptidase-A (CP-A), respectively. Using an alternative strategy, potent ACE2 peptide inhibitors have been identified through screening of constrained peptide libraries [13]. A stable peptide inhibitor of ACE2, DX600, displays a K_i of 2.8 nM with angiotensin I as substrate. DX600 is a 26-amino-acid N-terminal acetylated and C-terminal amidated peptide. In addition, the dipeptide Pro–Phe has been identified as an inhibitor of ACE2 activity [11]. This finding would allow for a peptide-based approach to inhibitor design by comparison of the inhibitory effect of Pro–Phe and Pro–Leu (not a substrate for ACE2 and a weaker inhibitor than Pro–Phe) with other dipeptides. These ACE2 inhibitor compounds are fundamental tools for exploring ACE2 biology and enzymology.

In comparison with ACE2, the way in which the very first inhibitors of ACE were discovered was quite different. Teprotide is a nonapeptide that owes its potency and

selectivity to natural selection, being a component of the venom of a Brazilian pit-viper. Captopril, on the other hand, owes its potency and selectivity to chemical design and this was guided not only by the hypothetical active site model but also by data on the substrate specificity of ACE and comparison with CP-A. For example, the study carried out by Cheung et al. [21] was a major contributing factor in the design of second-generation inhibitors of ACE. Here, the active site binding and other properties of substrates and competitive inhibitors of ACE were investigated. Using a series of simple tri- and dipeptides, it was determined that the binding of a substrate or inhibitor to ACE is wholly dependent upon the C-terminal residues and their subsite interactions. In this vein, the importance of an exhaustive study to determine structure–activity correlations for ACE2 is critical for the systematic development of inhibitor compounds. Although ACE inhibitors were designed in part on the basis of similarities between the enzymic properties of ACE and those of CP-A, no significant segmental homology was found with carboxypeptidases (M14 family) upon solution of the crystal structure of tACE [14].

7. The cell biology of ACE2

In contrast to the growing availability of data on the enzyme kinetics and possible physiological roles of ACE2, comparatively little is known about ACE2 at the cellular level. In keeping with predictions made from its peptide sequence and its interactions as a viral receptor, recombinant ACE2 expressed in CHO and HEK293 cells is localised predominantly in the plasma membrane. Analysis of media taken from cells expressing ACE2 has revealed the presence of a soluble form of ACE2, most likely a product of a proteolytic cleavage event. Indeed, proteolytic release of ACE2 has been observed from cardiomyocytes in which ACE2 is overexpressed [22], and a soluble form of ACE2 has been detected in urine (D.W. Lambert, G.I. Rice, N.M. Hooper and A.J. Turner, unpublished data). Preliminary evidence indicates that the secretase responsible for the shedding of ACE2 is distinct from that of ACE (D.W. Lambert, N.M. Hooper and A.J. Turner, unpublished data). In light of the ability of soluble ACE2 to prevent SARS CoV infection [23], further study of ACE2 secretion is of prime significance.

Analysis of the ACE2 sequence reveals the presence of seven potential *N*-glycosylation sequons [5]. The recently resolved crystal structure of ACE2 provided evidence that six of these sites (N53, N90, N103, N322, N432 and N546) are glycosylated; it is not clear from this data whether the most C-terminal site is also occupied [18]. This study reported that soluble ACE2 expressed in insect cells has a molecular weight of 90 kDa, compared to the predicted 83.5 kDa, a difference accounted for by the presence of the carbohydrate groups. This molecular weight of 90 kDa has also been reported for ACE2 in rat and mouse tissue [22,24]. It should be noted, however, that other studies have reported a molecular weight of 120–130 kDa in the same species [25,26]. Indeed, ACE2 overexpressed in mammalian cells has an apparent molecular weight of 120 kDa, which is reduced to approximately 85 kDa following enzymic deglycosylation [5]. Whilst the difference between the reported molecular weights of ACE2 in insect and mammalian cells can be assigned to species differences in posttranslational modification, the reasons for the remaining discrepancies are unclear. Disruption of normal glycosylation of ACE2 expressed in CHO cells, by treatment with the glycosidase inhibitor tunicamycin, prevented correct cellular sorting of full-length ACE2 and abolished secretion of a truncated mutant lacking the transmembrane and cytosolic domains (D.W. Lambert, K. Pogson and A.J. Turner, unpublished observations). The resulting unglycosylated protein was also catalytically inactive, indicating a requirement for glycosylation for membrane targeting and activity.

In vivo, ACE2 expression is confined to endothelial cells and renal tubular epithelium; it has also been identified in vascular smooth muscle cells and the Leydig and Sertoli cells of the testes [6, 25]. The very low expression level of ACE2 in a wide variety of cell lines (F.J. Warner, D.W. Lambert, N.M. Hooper and A.J. Turner, unpublished data) has impeded further studies of the intracellular processing of ACE2. Furthermore, identification of a suitable in vitro model is vital in order to gain insight into events regulating the expression of ACE2 and to elucidate further its role(s) in vivo.

8. The physiology and pathology of ACE2

The first direct evidence concerning the physiological role of ACE2 was provided by studies on transgenic ACE2 knockout mice (*ace2*$^{-/-}$) carried out by Crackower et al. [27]. These mice show no gross abnormalities, are fertile, and have normal blood pressure and renal function despite moderately elevated angiotensin II levels. Closer examination, however, revealed the mice suffered from severe cardiac dysfunction manifest in a profound decrease in cardiac contractility resulting from the thinning of the left ventricle wall and an increase in ventricle chamber dimensions. This disruption of heart function was associated with an up-regulation of genes induced by hypoxia and bore a remarkable resemblance to a condition resulting from cardiac surgery referred to as ‘cardiac stunning’. Significantly, ablation of the ACE gene against an ACE2 knockout background rescues the cardiac phenotype, suggesting that ACE2 serves as a regulator of the effects of ACE in the RAS. In a separate study, targeted disruption of the *ace2* gene resulted in viable, fertile progeny lacking gross anatomical/structural abnormalities [28] and displaying only moderately elevated systolic blood pressure. After chronic infusion of angiotensin II (at a level that does not induce

hypertension), increased blood pressure and decreased heart rate were evident in ACE2 null mice compared with wild-type littermates. Thus, it appears that in the absence of ACE2 the mice are more susceptible to angiotensin II-induced hypertension.

Transgenic mice in which ACE2 was overexpressed exclusively in cardiac myocytes exhibited complete atrio-ventricular block from 3 weeks of age [22]. The transgenic mice appeared healthy with a grossly normal heart structure and only slightly lowered blood pressure. However, the mice suffered from an increased susceptibility to sudden cardiac death, which directly correlated with the transgene dose. Detailed electrophysiological analysis revealed severe, progressive conduction and rhythm disturbances along with sustained ventricular tachycardia and terminal ventricular fibrillation. In light of the observation that the gap junction proteins connexin40 and connexin43 were down-regulated in the hearts of these transgenic mice, it was suggested that the electrical remodelling was a result of gap junction dysregulation. In surviving older mice the expression of human ACE2 in cardiac myocytes was progressively down-regulated (the down-regulation of proteins under the control of the heart-specific αMHC promoter is a phenomenon reported for other models). This coincided with the reciprocal up-regulation of connexin43 resulting in partial restoration of the cardiac phenotype. However, it is important to note that ACE2 is not normally expressed in cardiac myocytes but rather in cardiac endothelium.

The ability of ACE2 to cleave vasoactive peptides such as angiotensin II indicates a possible role in blood pressure regulation. This theory was given increased credence by the observation that the *ace2* gene maps to a candidate quantitative trait locus (QTL) in a salt-sensitive rat model of hypertension. Furthermore, these rats display reduced ACE2 mRNA and protein expression compared to healthy littermates [29]. Overlapping QTLs have also been identified in other rat models of hypertension. In addition, two single nucleotide polymorphisms (SNPs) were identified in the ACE2 locus, which have been associated with the risk of cardiovascular disease in a Caucasian population [30].

Chronic administration of omapatrilat, a dual inhibitor of the vasopeptidases ACE and neprilysin (NEP), in spontaneous hypertensive rats (SHRs) results in a significant reduction in blood pressure, marked diuresis and augmented renal excretion of the vasodilatory peptide angiotensin-(1–7) [31]. ACE2 is capable of producing angiotensin-(1–7) from angiotensin II in vitro and is abundantly expressed in the kidney [5]. Upon treatment of SHRs with omapatrilat, increased staining of both angiotensin-(1–7) and ACE2 is observed in the renal proximal tubules, with a simultaneous increase of ACE2 mRNA expression in the cortex. The authors suggest that omapatrilat exerts this effect by inducing ACE2 expression, resulting in a concomitant increase in angiotensin-(1–7) production from angiotensin II and an associated decrease in blood pressure, rather than via the inhibition of ACE and NEP, both of which are known to cleave angiotensin-(1–7). It has been demonstrated that the concentrations of angiotensin-(1–7) in kidney and urine are increased during normal pregnancy in rats [32]. In light of the evidence of renal ACE2 expression, Brosnihan et al. [33] investigated the possibility that changes in ACE2 expression may be associated with the previous finding that the levels of angiotensin-(1–7) increase during pregnancy. It was found that the renal distribution of angiotensin-(1–7) and ACE2 is similar and does not change during gestation; however, the intensity of staining for both was elevated (56% and 117%, respectively) in pregnant rats. These findings suggest that ACE2 is the primary candidate for the local production and increased angiotensin-(1–7) generation during pregnancy.

Further evidence of the role ACE2 may play in the RAS came from a study by Zisman et al. [34] in which they report up-regulation of ACE2 expression in failing human heart ventricles. In a parallel paper, the same group presented evidence of the involvement of a novel angiotensin-(1–7) activity in the intact failing heart [35]. The later study demonstrated that formation of angiotensin-(1–7) was correlated with the level of ACE2 expression and could be completely abolished by administration of an ACE2-specific inhibitor. These data indicated that in failing hearts the major route for the increased production of angiotensin-(1–7) is from angiotensin II via ACE2 rather than from angiotensin I via neprilysin, as had previously been suggested.

A separate study has implicated renal ACE2 in the pathogenesis of diabetic nephropathy [26]. In this study it was shown that ACE2 mRNA and protein expression levels were significantly reduced in the kidneys of 24-week-old streptozocin-induced diabetic rats. The reduction in ACE2 mRNA expression was not influenced by ACE inhibitor treatment. However, the decrease in ACE2 protein expression was prevented under the same conditions, thereby suggesting that ACE2 protein expression is associated with ACE activity. In contrast, renal ACE2 protein was found to be higher, with the absence of a concomitant increase in ACE2 mRNA levels, in young diabetic mice (db/db) before the onset of diabetic nephropathy [36]. The balance between ACE and ACE2 (low versus high protein levels, respectively) may be renoprotective in the early stages of diabetes with this balance being lost upon progression to renal damage. Hence, it is postulated that ACE2 is involved in the aetiology of diabetes via the RAS, a role for which has been previously characterised in this disease state (for a review, see Ref. [37]).

Collectively, these studies in vivo provide strong evidence of a role for ACE2 in the RAS through localised production of angiotensin-(1–7) from angiotensin II, perhaps serving as a negative regulator of the vasoconstrictive effects of ACE to maintain blood

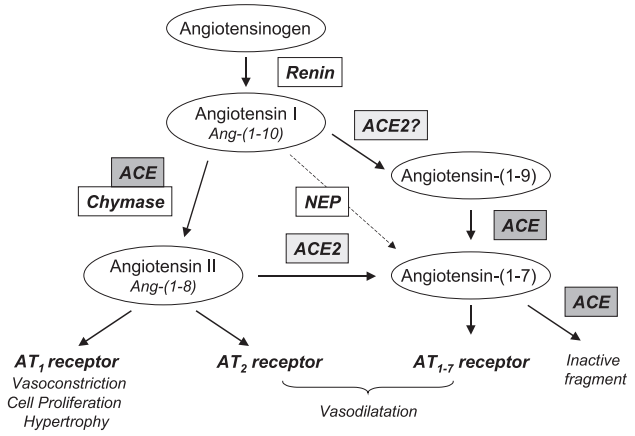

Fig. 2. Schematic representation of the renin–angiotensin system (RAS). ACE, angiotensin-converting enzyme; NEP, neprilysin; AT_1, angiotensin II type I receptor; and AT_2, angiotensin type II receptor. The conversion of angiotensin I to angiotensin-(1–9) by ACE2 is kinetically much less favourable than the conversion of angiotensin II to angiotensin-(1–7).

pressure homeostasis and maintain cardiac and renal function (Fig. 2).

9. A secret life

A surprising discovery is that ACE2 is a functional receptor for the SARS coronavirus, the causative agent of SARS. In a series of elegant experiments, Li et al. [23] co-immunoprecipitated the virus attachment glycoprotein (S1) with lysates from an African green monkey kidney cell line (Vero E6) and subjected the precipitated proteins to mass spectrometry. Among the sequences, a number were obtained corresponding to ACE2. Subsequently, it was demonstrated that recombinant soluble ACE2, but not ACE, was able to block the association between Vero E6 cells and the SARS CoV S protein. Expression of recombinant ACE2 and of S1 protein resulted in cell fusion and the formation of syncytia in 293T cells, a cell line normally resistant to SARS CoV infection. Furthermore, 293T cells expressing ACE2 permitted efficient replication of the coronavirus, with viral genome copies increased 100 000-fold over a 48-h period, compared to 10-fold in untransfected cells. An ACE2-specific antibody inhibited the cytopathicity of SARS-CoV in a dose-dependent manner.

The expression profiling of mRNA, performed by Harmer et al. [8], demonstrates the expression of low levels of ACE2 in the bronchus and lung parenchyma. This, taken together with evidence that the primary sites of murine ACE2 expression are in the lung and the kidney [38], led Li et al. [23] to suggest that the tissue distribution of ACE2 is consistent with the pathology of SARS-CoV. Furthermore, Hamming et al. [9] recently provided evidence that ACE2 is abundantly expressed in the epithelia of the lung and the small intestine, possible entry sites for the SARS virus.

Whilst research determining the receptor binding properties of the SARS CoV S protein has progressed at a rapid rate, little is known about the residues of ACE2 involved in binding. Preliminary experiments performed by Li et al. [23] indicate that disrupting the active site of ACE2 has no effect on S1 binding; further studies are required to understand the nature of the protein:protein interactions.

10. Future perspectives

Until recently, it was widely assumed that the intricacies of the RAS had largely been elucidated. Recent studies examining the properties of ACE2, however, have provided evidence that this crucial pathway may be significantly more complicated than previously thought. Whilst, as yet, the precise physiological role of ACE2 remains to be determined, it is becoming increasingly apparent that ACE2 may act as a counterbalance to the effects of ACE in the RAS. Given the significance of ACE inhibitors in the treatment of a number of pathophysiological disorders, the contribution of ACE2 to the regulation of the RAS cannot be ignored. Indeed, modulation of ACE2 activity may provide a significant new therapeutic opportunity in the treatment of cardiovascular disease, either in isolation or in conjunction with existing ACE inhibitors. This exciting possibility highlights the need for continuing efforts to elucidate the in vivo roles and biochemical properties of this emerging player in the RAS.

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References

[1] A.J. Turner, N.M. Hooper, The angiotensin-converting enzyme gene family: genomics and pharmacology, Trends Pharmacol. Sci. 23 (2002) 177.
[2] D.J. Campbell, Tissue renin–angiotensin system: sites of angiotensin formation, J. Cardiovasc. Pharmacol. 10 (1987) S1–S8.
[3] L.T. Skeggs, J.R. Kahn, N.P. Shumway, The preparation and function of the hypertensin-converting enzyme, J. Exp. Biol. 103 (1956) 295–299.
[4] H.Y.T. Yang, E.G. Erdos, Y. Levin, A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin, Biochim. Biophys. Acta 214 (1970) 374–376.
[5] S.R. Tipnis, N.M. Hooper, R. Hyde, E. Karran, G. Christie, A.J. Turner, A human homologue of angiotensin-converting enzyme, J. Biol. Chem. 275 (2000) 33238.
[6] M. Donoghue, F. Hsieh, E. Baronas, K. Godbout, M. Gosselin, N. Stagliano, M. Donovan, B. Woolf, K. Robison, R. Jeyaseelan, R.E. Breitbart, S. Acton, A novel angiotensin-converting enzyme-related

carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9, Circ. Res. 87 (2000) e1.
[7] H. Zhang, J. Wada, K. Hida, Y. Tsuchiyama, K. Hiragushi, K. Shikata, H. Wang, S. Lin, Y.S. Kanwar, H. Makino, Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homologue of ACE2 and is developmentally regulated in embryonic kidneys, J. Biol. Chem. 276 (2001) 17132.
[8] D. Harmer, M. Gilbert, R. Borman, K.L. Clark, Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme, FEBS Lett. 532 (2002) 107.
[9] I. Hamming, W. Timens, M.L.C. Bulthuis, A.T. Lely, G.J. Navis, H. van Goor, Tissue distribution of ACE2 protein, the functional receptor for SARS cornavirus. A first step in understanding SARS pathogenesis, J. Pathol. 203 (2004) 631.
[10] X. Houard, T.A. Williams, A. Michaud, P. Dani, R.E. Isaac, A.D. Shirras, D. Coates, P. Corvol, The Drosophila melanogaster-related angiotensin-I-converting enzymes Acer and Ance-distinct enzymic characteristics and alternative expression during pupal development 257 (1998) 599–606.
[11] J.L. Guy, R.M. Jackson, K.R. Acharya, E.D. Sturrock, N.M. Hooper, A.J. Turner, Angiotensin-converting enzyme-2 (ACE2): comparative modelling of the active site, substrate specificity and chloride sensitivity, Biochemistry 42 (2003) 13185.
[12] C. Vickers, P. Hales, V. Kaushik, L. Dick, J. Gavin, J. Tang, K. Godbout, T. Parsons, E. Baronas, F. Hsieh, S. Acton, M. Patane, A. Nichols, P. Tummino, Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase, J. Biol. Chem. 277 (2002) 14838.
[13] L. Huang, D.J. Sexton, K. Skogerson, M. Devlin, R. Smith, I. Sanyal, T. Parry, R. Kent, J. Enright, Q. Wu, G. Conley, D. DeOliveira, L. Morganelli, M. Ducar, C.R. Wescott, R.C. Ladner, Novel peptide inhibitors of angiotensin-converting enzyme 2, J. Biol. Chem. 278 (2003) 15532.
[14] R. Natesh, S.L.U. Schwager, E.D. Sturrock, K.R. Acharya, Crystal structure of the human angiotensin-converting enzyme–lisinopril complex, Nature 421 (2003) 551.
[15] H.M. Kim, D.R. Shin, O.J. Yoo, H. Lee, J.O. Lee, Crystal structure of *Drosophila* angiotensin I-converting enzyme bound to captopril and lisinopril, FEBS Lett. 538 (2003) 65.
[16] K. Gordon, P. Redelinghuys, S.L.U. Schwager, M.R.W. Ehlers, A.C. Papageorgiou, R. Natesh, K.R. Acharya, E.D. Sturrock, Deglycosylation, processing and crystallisation of human testis angiotensin-converting enzyme, Biochem. J. 371 (2003) 437.
[17] T.A. Williams, A. Michaud, X. Houard, M. Chauvet, F. Soubrier, *Drosophila melanogaster* angiotensin I-converting enzyme expressed in *Pichia pastoris* resembles the C domain of the mammalian homologue and does not require glycosylation for secretion and enzymic activity, Biochem. J. 318 (1996) 125.
[18] P. Towler, B. Staker, S.G. Prasad, S. Menon, J. Tang, T. Parsons, D. Ryan, M. Fisher, D. Williams, N.A Dales, M.A. Patane, M.W. Pantoliano, ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis, J. Biol. Chem. 279 (2004) 17996.
[19] N.A. Dales, A.E. Gould, J.A. Brown, E.F. Calderwood, B. Guan, C.A. Minor, J.M. Gavin, P. Hales, V.K. Kaushik, M. Stewart, P.J. Tummino, C.S. Vickers, T.D. Ocain, M.A. Patane, Substrate-based design of the first class of angiotensin-converting enzyme-related carboxypeptidase (ACE2) inhibitors, J. Am. Chem. Soc. 124 (2002) 11852.
[20] X. Liu, M. Fernandez, M.A. Wouters, S. Heyberger, A. Husain, Arg^{1098} is critical for the chloride dependence of human angiotensin I-converting enzyme C-domain catalytic activity, J. Biol. Chem. 36 (2001) 33518.
[21] H.S. Cheung, F.L. Wang, M.A. Ondetti, E.F. Sabo, D.W. Cushman, Binding of peptide substrates and inhibitors of angiotensin-converting enzyme, J. Biol. Chem. 255 (1980) 401.
[22] M. Donoghue, H. Wakimoto, C.T. Maguire, S. Acton, P. Hales, N. Stagliano, V. Fairchild-Huntress, J. Xu, J.N. Lorenz, V. Kadambi, C.I. Berul, R.E. Breitbart, Heart block, ventricular tachycardia, and sudden death in ACE2 transgenic mice with down-regulated connexins, J. Mol. Cell. Cardiol. 35 (2003) 1043.
[23] W. Li, M.J. Moore, N. Vasilieva, J. Sui, S.K. Wong, M.A. Berne, M. Somasundaran, J.L. Sullivan, K. Luzuriaga, T.C. Greenough, H. Choe, M. Farzan, Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus, Nature 426 (2003) 450.
[24] N. Li, J. Zimplemann, K. Cheng, J.A. Wilkins, K.D. Burns, The role of angiotensin converting enzyme 2 in the generation of angiotensin 1–7 by rat proximal tubules, Am. J. Physiol., Renal Physiol. (2004) (Online prepublication).
[25] G.C. Douglas, M.K. O'Brian, M.P. Hedger, D.K.L. Lee, M.A. Yarski, A.I. Smith, R.A. Lew, The novel angiotensin-converting enzyme (ACE) homologue, ACE2, is selectively expressed by adult leydig cells of the testis, Endocrinology 145 (2004) 4703–4711.
[26] C. Tikellis, C.I. Johnston, J.M. Forbes, W.C. Burns, L.M. Burrell, J. Risvanis, M.E. Cooper, Characterisation of renal angiotensin-converting enzyme 2 in diabetic nephropathy, Hypertension 41 (2003) 392.
[27] M.A. Crackower, R. Sarao, G.Y. Oudit, C. Yagil, I. Kozieradzki, S.E. Scanga, A.J. Oliveira-dos-Santos, J. da Costa, L. Zhang, Y. Pei, J. Scholey, C.M. Ferrario, A.S. Manoukian, M.C. Chappell, P.H. Backx, Y. Yagil, J.M. Penninger, Angiotensin-converting enzyme 2 is an essential regulator of heart function, Nature 417 (2002) 822.
[28] A.J. Allred, M. Donoghue, S. Acton, T.M. Coffman, Regulation of blood pressure by the angiotensin converting enzyme homologue ACE2, Am. J. Nephrol. 13 (2002) 52A.
[29] M.A. Crackower, R. Sarao, C. Yagil, Y. Yagil, J.M. Penninger, ACE2—A novel candidate gene for hypertension, Am. J. Hypertens. 14 (2001) A78.
[30] A.A. Chen, G. Barnes, A. Foti, E. Nolin, S. Lewitzsky, J. Metivier, J. Meyer, A. Parker, E. Topol, Two single nucleotide polymorphisms in the ACE2 locus are associated with cardiovascular disease, Genet. Epidemiol. 23 (2002) 272.
[31] M.C. Chappell, F. Jung, P.E. Gallagher, D.B. Averill, M.A. Crackower, J.M. Penninger, C.M. Ferrario, Omapatrilat treatment is associated with increased ACE2 and angiotensin (1–7) in spontaneously hypertensive rats, Hypertension 40 (2002) 409.
[32] L.A. Neves, A.F. Williams, D.B. Averill, C.M. Ferrario, M.P. Walkup, K.B. Brosnihan, Pregnancy enhances the angiotensin (Ang)-(1–7) vasodilator response in mesenteric arteries and increases the renal concentration and urinary excretion of Ang-(1–7), Endocrinology 144 (2003) 3338.
[33] K.B. Brosnihan, L.A.A. Neves, J. Joyner, D.B. Averill, M.C. Chappell, R. Sarao, J. Penninger, C.M. Ferrario, Enhanced renal immunocytochemical expression of Ang-(1–7) and ACE2 during pregnancy, Hypertension 42 (2003) 749.
[34] L.S. Zisman, R.S. Keller, B. Weaver, Q. Lin, R. Speth, M.R. Bristow, C.C. Canver, Increased angiotensin-(1–7)-forming activity in failing human heart ventricles. Evidence for up-regulation of the angiotensin-converting enzyme homologue ACE2, Circulation 108 (2003) 1707.
[35] L.S. Zisman, G.E. Meixell, M.R. Bristow, C.C. Canver, Angiotensin-(1–7) formation in the intact human heart. In vivo dependence on angiotensin II as substrate, Circulation 108 (2003) 1679.
[36] M. Ye, J. Wysocki, P. Naaz, M.R. Salabat, M.S. LaPointe, D. Batlle, Increased ACE 2 and decreased ACE protein in renal tubules from diabetic mice. A renoprotective combination? Hypertension 43 (2004) 1120.
[37] R.E. Gilbert, H. Krum, J. Wilkinson-Berka, D.J. Kelly, The renin–angiotensin system and the long-term complications of diabetes: pathophysiological and therapeutic considerations, Diabet. Med. 20 (2003) 607.
[38] T. Komatsu, Y. Suzuki, J. Imai, S. Sugano, M. Hida, A. Tanigami, S. Muroi, Y. Yamada, K. Hanaoka, Molecular cloning, mRNA expression and chromosomal localisation of mouse angiotensin-converting enzyme-related carboxypeptidase (mACE2), DNA Sequence 13 (2002) 217.