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ACE2, a new regulator of the renin–angiotensin system

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Angiotensin-converting enzyme (ACE) is a zinc metalloproteinase and a key regulator of the renin–angiotensin system (RAS). ACE2 is a newly described enzyme identified in rodents and humans with a more restricted distribution than ACE, and is found mainly in heart and kidney. ACE2 cleaves a single residue from angiotensin I (Ang I) to generate Ang 1–9, and degrades Ang II, the main effector of the RAS, to the vasodilator Ang 1–7. The importance of ACE2 in normal physiology and pathophysiological states is largely unknown. ACE2 might act in a counter-regulatory manner to ACE, modulating the balance between vasoconstrictors and vasodilators within the heart and kidney, and playing a significant role in regulating cardiovascular and renal function.

Angiotensin-converting enzyme (ACE), a dipeptidyl carboxypeptidase, is a key enzyme in the renin–angiotensin system (RAS); it converts the inactive decapeptide, angiotensin I (Ang I; or Ang 1–10), to the active octapeptide and potent vasoconstrictor Ang II (or Ang 1–8) (Figure 1), and inactivates the vasodilator bradykinin [1]. Ang II is thought to be responsible for most of the physiological and pathophysiological effects of the RAS, and inhibitors of ACE that reduce the formation of Ang II have been highly successful in the management of hypertension, are standard therapy following myocardial infarction to delay the development of heart failure, and reduce the rate of progression of renal disease [2,3].

Recently, however, the classical view of the RAS has been challenged by the discovery of the enzyme ACE2 [4,5], in addition to the increasing awareness that many angiotensin peptides other than Ang II have biological activity and physiological importance [6]. The reported vasodilatory actions of Ang 1–7 [6], along with the potential involvement of ACE2 in both Ang II degradation and Ang 1–7 production, add another level of complexity to the RAS [7,8].

It is predicted that cardiovascular disease will be the leading cause of death by 2020, and although current approaches to block the RAS have been of major benefit in this area, it is now clear that other pathways and enzymes within the RAS can modulate its main effector, Ang II. We must question our understanding of the 'classical' RAS, and work towards unravelling the complexity of the 'new' RAS (Figure 1). This should result in alternative strategies for the treatment of cardiovascular disease in the future.

ACE2 structure and function

ACE2 is the first known human homologue of ACE, and was cloned from a human heart failure cDNA library [4] and a human lymphoma cDNA library [5]. Analysis of the genomic sequence of *ACE2* has revealed that the gene contains 18 exons and maps to chromosomal location Xp22 [5]. The full-length, human *ACE2* cDNA predicts a protein of 805 amino acids that has 42% homology with the N-terminal catalytic domain of ACE, and a hydrophobic region near the C-terminus, which probably serves as a membrane anchor. Like ACE, ACE2 is predicted to have the topology of a type 1 membrane protein, with the catalytic domain on the extracellular surface.

Unlike somatic ACE, ACE2 has only one active enzymatic site and functions as a carboxypeptidase rather

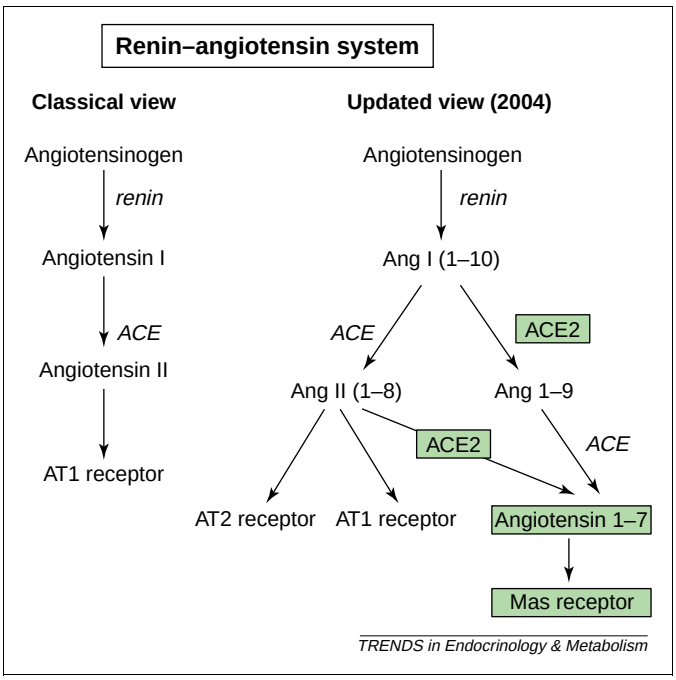

Figure 1. The renin–angiotensin system (RAS) pathway. Schematic diagram of the classical RAS (left), showing the main pathway for angiotensin II (Ang II) generation from Ang I via angiotensin-converting enzyme (ACE). In the classical view, Ang II mediates all known effects via the AT1 receptor. On the right is the updated view of the RAS, showing the role of ACE2 in degrading Ang I to Ang 1–9, and Ang II to the vasodilator Ang 1–7. In this version of the RAS, Ang II also mediates effects via the G-protein-coupled AT2 receptor, whereas Ang 1–7 acts through the Mas receptor.

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than a dipeptidyl carboxypeptidase. Thus, ACE2 removes a single C-terminal Leu residue from Ang I to generate Ang 1–9, a peptide with no known function. Although ACE2 was described originally for its ability to generate Ang 1–9 from Ang I [4], it also degrades Ang II to the biologically active peptide, Ang 1–7 [9] (Figure 1). Indeed, *in vitro* studies indicate that the catalytic efficiency of ACE2 for Ang II is 400-fold greater than for Ang I [9], indicating that the major role for ACE2 is the conversion of Ang II to Ang 1–7. The potential role of Ang 1–7 as a cardioprotective peptide with vasodilator, anti-growth and anti-proliferative actions has been recognized relatively recently [6,10]. Taken together, the data suggest that ACE2 might function to limit the vasoconstrictor action of Ang II through its inactivation, in addition to counteracting the actions of Ang II through the formation of the agonist, Ang 1–7. Recent studies [11] have identified the G-protein-coupled receptor encoded by the *MAS1* proto-oncogene, Mas, as the receptor for Ang 1–7 [11]. Genetic deletion of the Mas receptor abolished the binding of Ang 1–7 to whole mouse kidney, whereas the functional significance of Ang 1–7 and its receptor was demonstrated by studies showing that Mas-deficient mice could not respond with antidiuresis to Ang 1–7 after a water load, and that the aortas of Mas-deficient mice were unable to relax in response to Ang 1–7 [11].

In addition, an unexpected function of ACE2 has recently been identified and characterized; ACE2 is a functional receptor for coronaviruses, including the coronavirus that causes severe acute respiratory syndrome, and is involved in mediating virus entry and cell fusion [12,13]. Although not directly relevant to cardiovascular function, this would indicate that the RAS including ACE2 has multiple roles in physiology and various pathophysiological states [13].

ACE2 distribution

ACE2 has a much more restricted distribution compared with ACE, and in humans ACE2 transcripts have been identified in the heart, kidney and testis [4,5]. Like ACE, ACE2 is found in endothelial cells, and is present to a lesser degree in vascular smooth muscle cells. In the kidney, ACE2 is found in the proximal tubular epithelium. Recently, ACE2 has been identified in the gastrointestinal tract, brain and lung [14], suggesting a more ubiquitous distribution than initially thought.

ACE2 substrates

The precise physiological function of ACE2 is currently under intense investigation. There has been increasing interest in peptides that are either cleaved or generated by this enzyme. In addition to effects on the angiotensin peptides, ACE2 cleaves the C-terminal residue of the peptides des-Arg9-bradykinin, neurotensin 1–13 and kinetensin [4], and hydrolyses apelin-13 and dynorphin A 1–13 with as high a catalytic efficiency as Ang II [9]. ACE2 has no effect on bradykinin, in contrast to ACE, emphasizing the specificity of ACE2 [4,9]. Many of the *in vitro* substrates of ACE2 have actions that are relevant to cardiovascular regulation; apelin is a potent cardiac inotrope [15], dynorphin A is an endogenous opioid neuropeptide and des-Arg9-bradykinin binds to the bradykinin B1 receptor, which is activated by inflammation and tissue injury [16].

It is not yet known whether the *in vitro* substrates of ACE2 are also physiological *in vivo* substrates, and further studies are needed that address *in vivo* changes in the levels of putative substrates or products of ACE2 using *Ace2*-knockout mice [17], *Ace2* transgenic animals [18] and ACE2 agonists, in addition to potent, selective ACE2 inhibitors [19,20]. Interestingly, the *in vitro* enzymatic activity of ACE2 is unaffected by ACE inhibitors [4,5], but there are no data as to the effect of angiotensin receptor blockers on ACE2 activity. In the future, it will be of great interest to assess the *in vivo* effects of ACE2 inhibitors, particularly in light of the interesting findings with regard to cardiac function, which have been seen in both the *Ace2*-knockout and transgenic animals [17,18].

ACE2 and hypertension

It has been hypothesized that ACE2 might protect against increases in blood pressure and that ACE2 deficiency leads to hypertension. In several rat models of experimental hypertension, the gene for ACE2 maps to a defined quantitative trait locus on the X chromosome previously identified as a quantitative locus for blood pressure [17]. An association between ACE2 and blood pressure has also been reported; in the spontaneously hypertensive rat (SHR) and SHR stroke prone rats, renal ACE2 levels are reduced compared with normotensive Wistar-Kyoto rats (WKY) [17], and we have confirmed that renal *Ace2* mRNA is reduced in SHR compared with WKY [21]. It is not clear whether ACE2 deficiency plays a pathophysiological role to cause hypertension or is simply a consequence of increased blood pressure, and the precise role of ACE2 in hypertension requires clarification.

ACE2 and the heart

Evidence of the biological role of ACE2 in angiotensin peptide degradation comes from studies in *Ace2*-knockout mice, which lack ACE2 protein [17]. These mice develop abnormal heart function, with severely impaired cardiac contractility, and the decrease in function is both sex and time dependent, with more severe abnormalities in male than in female mice, and a more pronounced phenotype in older animals. The importance of ACE2 in cleaving and/or inactivating Ang II is indicated by the increase in plasma, cardiac and kidney Ang II levels in the *Ace2*-knockout animals, and confirmed by studies in which genetic ablation of *Ace*on an *Ace2* mutant background completely rescued the cardiac phenotype [17].

Hypoxia markers were also upregulated in the heart of the *Ace2*-knockout mice, suggesting that loss of ACE2 from the vascular endothelial cells resulted in coronary vessel constriction and reduced oxygen delivery to the myocytes [17]. Certainly, the localization of ACE2 in the rat and human heart to endothelial cells of intramyocardial blood vessels and smooth muscle cells supports a role for ACE2 in the control of local vasodilation. Although it was reported that ACE2 protein synthesis was similar in normal hearts and a failing heart from a single patient with idiopathic cardiomyopathy [4], in a study of 14

subjects with idiopathic cardiomyopathy, there was an increase in functional cardiac ACE2 activity assessed by the *ex vivo* formation of Ang 1–7 [22].

To date, there have been no published studies on cardiac ACE2 in the context of ischaemic heart disease. Given that marked stimulation of ACE and Ang II occurs after myocardial infarction [23], ACE2 probably increases after myocardial infarction; this increase would limit the adverse effects of raised cardiac Ang II by increasing levels of the vasodilator Ang 1–7. Support for this idea comes from studies in the rat myocardial infarction (MI) model, in which the development of heart failure was associated with increased Ang 1–7 immunoreactivity [24]. Furthermore, infusion of Ang 1–7 attenuated the development of heart failure after MI, confirming the functional significance of Ang 1–7 [25]. It is possible that the relative balance of vasoconstrictor and vasodilator angiotensin peptides is important in the modulation of both haemodynamic and trophic effects of these peptides in the context of ischaemic heart disease.

A word of caution is needed with regard to ACE2 and the heart. Although the data presented suggest that ACE2 and Ang 1–7 might have cardioprotective effects after myocardial injury, in transgenic mice with increased cardiac *Ace2* expression [18], there was a high incidence of sudden death, which correlated with transgene expression levels. Electrophysiology revealed severe, progressive conduction and rhythm disturbances, with sustained ventricular tachycardia, that progressed to fibrillation and death. Clearly, further studies are needed to determine the functional relevance of ACE2 in the heart.

ACE2 and the kidney

Immunohistochemical studies by our group [26] and others [4] have shown that in the kidney, both ACE2 and ACE protein are localized predominantly to epithelial cells of the distal tubule. The RAS has been implicated in the pathogenesis of diabetic complications, in particular diabetic nephropathy [27], and ACE inhibition provides significant renoprotection. We have recently characterized ACE2 in the kidney of a rodent model of type 1 diabetes mellitus and compared and contrasted it with ACE [26]. Previous studies in the kidney using this model of diabetes demonstrated that ACE is downregulated in the renal tubules and upregulated in the glomerulus. In a recent study, we found that both *ACE* and *ACE2* mRNA levels were decreased in diabetic renal tubules by ~50%. We also found that ACE2 protein synthesis is reduced in the diabetic kidney, and this reduction is prevented by ACE inhibitor therapy, suggesting that ACE2 might have a renoprotective role in diabetes [28]. Although there have been no other studies on the expression of *ACE2* after renal tissue injury, the synthesis of collectrin, a protein with significant homology to ACE2 is upregulated in a model of progressive renal injury [29].

Box 1. ACE2: Outstanding questions

- Anatomical localization and distribution in pathophysiology
- *In vivo* substrates and products
- Role of ACE2 in angiotensin 1–7 metabolism
- Regulation of Mas receptor
- Effects of pharmacological blockade of ACE2
- Effects of functional agonism of ACE2
- Potential deleterious effects of ACE2 to cause vasodilation and cardiac arrhythmias
- Role of ACE2 as a receptor for coronaviruses such as that responsible for severe acute respiratory syndrome

Conclusion

The RAS has proved to be an important regulator of cardiovascular and renal structure and function, in addition to salt and water balance. Blockade of the RAS with ACE inhibitors or Ang II type 1 receptor antagonists has clearly established its key role in the pathophysiology of an increasing number of diseases, including hypertension, heart failure, ventricular remodelling, renoprotection and diabetic complications. These beneficial results are thought to result from blocking the vasoconstrictor, hypertrophic and proinflammatory actions of Ang II. The discovery of a new enzyme in the RAS pathway, ACE2, which produces the vasodilatory and antihypertrophic peptide Ang 1–7, gives rise to the hypothesis that ACE2 provides a counter-regulatory system to Ang II, which might also contribute to the beneficial effects of RAS blockade.

The identification of ACE2 in the heart and kidney, its modulation in heart failure and diabetes, and the recent description that this enzyme plays a biological role in the generation and degradation of angiotensin peptides provides a rationale for the further exploration of its role in pathophysiological states, including myocardial ischaemia, renal failure, atherosclerosis, and diabetic complications. Many questions remain to be answered about ACE2 (Box 1), in particular the impact of ACE2 on the generation of bioactive peptides, but clarification of the role of ACE2 in health and disease will be assisted by the development of agents that modulate ACE2 activity. The discovery of ACE2 has opened up an exciting new area of cardiovascular and renal physiology, in addition to providing the possibility of identifying novel therapeutic targets.

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