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Physiological roles of angiotensin-converting enzyme 2

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Abstract. Angiotensin-converting enzyme 2 (ACE2) is a recently discovered homologue of the key enzyme of the renin-angiotensin system, the angiotensin-converting enzyme. The ACE2 enzyme is mainly expressed in cardiac blood vessels and tubular epithelia of the kidneys. Together with ACE2's unique metallocarboxypeptidase activity, the restricted tissue distribution suggests a distinctive physiological function in blood pressure, blood flow and fluid regulation. The *ace2* gene was mapped to quantitative trait loci affecting susceptibility to hypertension in rats. Furthermore, ACE2 appears to be a negative regulator of ACE in the heart. ACE2 messenger RNA and protein levels are substantially regulated in the kidney of diabetic and pregnant rats. The mechanism of ACE2 function and its physiologic significance are not yet fully understood; however, as ACE2 differs in its specificity and physiological role from ACE, this opens a new potential venue for drug discovery aimed at cardiovascular disease, hypertension and diabetic complications.

Key words. Angiotensin-converting enzyme 2; knockout mice; renin-angiotensin system.

Introduction

For the last 50 years, angiotensin-converting enzyme (ACE) has assumed a central position in the renin-angiotensin system (RAS). The RAS is a major regulatory network that maintains blood pressure, fluid and electrolyte balance and electrolyte homeostasis. ACE functions primarily as a 'peptidyl dipeptidase', removing dipeptides from the C-terminus of peptide substrates [1]. Its primary substrate was identified as angiotensin I. ACE processes the decapeptide angiotensin I to the eight-amino-acid peptide angiotensin II, which functions as a strong vasoconstrictor. In parallel, ACE also inactivates the vasodilator peptides bradykinin and kallidin, and thus potentiates the vasopressor response mediated by angiotensin II [1]. Inhibition of ACE's enzymatic activity has a powerful effect in reduction of blood pressure; thus small molecule inhibitors of human endothelial ACE are used for antihypertensive therapies [2]. In addition to their effectiveness in treating hypertension, ACE inhibitors have been found to lower the risk of coronary heart disease and stroke. Furthermore, they improve the prognosis of patients with cardiac failure and diabetic nephropathy (for review, see [3]).

With the discovery of ACE 2/ACEH by Donoghue [4] and Tipins [5], a new level of complexity was added to the RAS. The ACE2 gene is located in the region of the X chromosome (Xp22), which maps to quantitative trait loci (QTL) in hypertensive rats [6–8]. Consistent with a possible role in cardiorenal function, ACE2 was found to be predominantly expressed in endothelia of the heart and in tubular epithelia of the kidney [4, 5]. In humans, ACE2 was also found in the gastrointestinal tract [9]. Additionally, in mouse, ACE2 has been detected in lungs [10]. While ACE and ACE2 protein are similar in their metalloprotease catalytic domains, they differ in their substrate specificity [11]. Analysis of ACE2 expression and the

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physiological role of its substrates suggest that ACE2 may act as a tissue-specific negative feedback regulator of the RAS [3]. Furthermore, the differences observed in phenotype between the genetically engineered *ace* and *ace2* mice [12] all suggest a role for ACE2 in heart pathophysiology. Moreover, since it has been shown that ACE2 acts not only on the angiotensin I and angiotensin II peptides, but also efficiently cleaves the C-terminal residues from several other peptides such as apelin-13 and dynorphin A 1–13, unrelated to angiotensin I [4], ACE2 function may not be limited only to RAS.

Substrate specificity of ACE2

In the classic pathway of RAS, angiotensin I is generated from the circulating precursor angiotensinogen by the action of renin, an enzyme secreted from juxtaglomerular cells at the renal afferent arterioles [13]. Angiotensin I has little effect on blood pressure and is converted by ACE to angiotensin II. Angiotensin II, a potent vasopressor, acts on the blood vessels and the kidneys by binding to the G-protein-coupled receptors AT_1 and AT_2. In contrast, ACE2 cleaves the C-terminal amino acid of angiotensin I to a nonapeptide angiotensin 1–9 [4]. In rat and human plasma angiotensin 1–9 levels are twice those of angiotensin II [14, 15], and angiotensin 1–9 accumulates in animals treated with ACE inhibitors [16]**.** The biological function of angiotensin 1–9 is still not well defined. However, angiotensin 1–9 is thought to potentiate angiotensin II-mediated vasoconstriction in isolated rat aortic rings and to have pressor effects in awake rats [17]. Angiotensin 1–9 was also shown to have weak pressor effects in anesthetized rats and dogs, and vasoconstricting activity in isolated rat aorta [17].

ACE2 directly converts angiotensin II to angiotensin 1–7 [4, 18]**.** In animals, angiotensin 1–7 has been proposed to be an important regulator of cardiovascular function, promoting vasodilatation, apoptosis and growth arrest [19, 20]. However, its functional significance in humans is still controversial. Aside from the degradation of the vasoconstrictor angiotensin II, the formation of the vasodilatatory angiotensin 1–7 might reflect the negative regulatory function of ACE2 in the presence of an activated RAS.

In addition to its activity as angiotensin-converting enzyme, ACE2 can remove in in vitro assays the C-terminal residue from other vasoactive peptides, including neurotensin, kinetensin (a neurotensin-related peptide) and des-Arg bradykinin (fig. 1). The kinin metabolites, des-Arg^{10}-kallidin (des-Arg^{10}-Lys^{1}-bradykinin) and des-Arg^{9}-bradykinin activate the G-protein-coupled B_1 receptor [21], which is upregulated in response to tissue injury and may be important in mediating inflammatory responses. Furthermore, ACE2 also acts on apelin-13 and apelin-36 peptides with high catalytic efficiency [18]. These two forms of apelin were recently identified as endogenous ligands for the human APJ receptor, which is a homolog of the angiotensin receptor AT1 [22]. The role of the apelins is not fully understood. Whereas systemic administration of apelin-13 promotes hypotension in rats [23], it has been shown that apelin-13 promotes vasoconstriction in endothelium-denuded coronary arteries [23]. Intraperitoneal injection of apelin-13 in rats increases water intake [23]. Two opioid peptides, dynorphin A 1–13 and

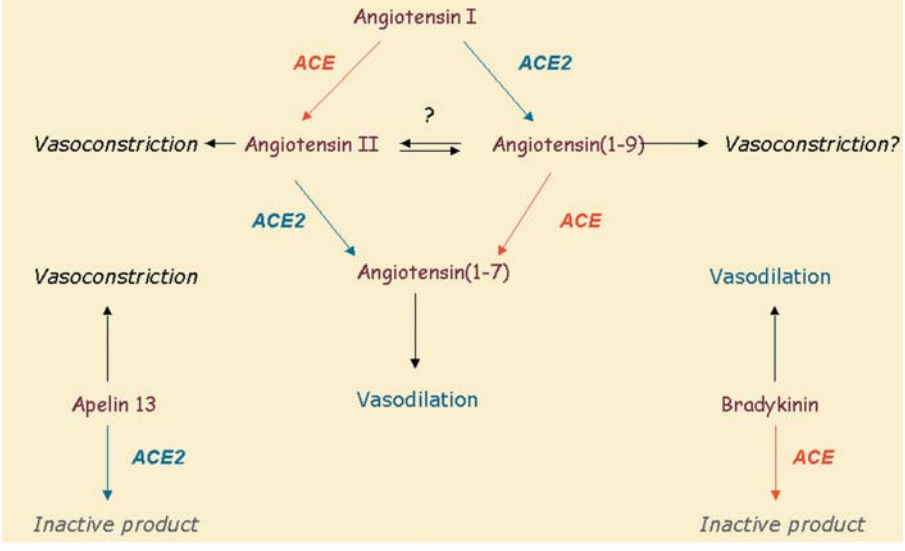

Figure 1. Hypothetical model of ACE and ACE2 functions. Angiotensin I serves as a substrate for both ACE and ACE2. Angiotensin II is known to act as vasoconstrictor in vivo. The function of Angiotensin (1–9) is still not well understood. Both ACE and ACE2 are involved in the production of the vasodilator peptide angiotensin (1–7). From genetic experiments it appears that ACE and ACE2 have complementary functions by negatively regulating each other in the RAS.

Table 1. ACE2 functions as a carboxymonopeptidase with a preference for C-terminal hydrophobic or basic residues. The ACE2 substrates, the amino acids cleaved by ACE2 (underline) and the receptors of some of the ACE2 substrates are indicated. The physiological functions are not always well defined.

ACE2 substrates/products	Receptor	Physiological functions of ACE2 substrate/product
Angiotensin I *Asp Arg Val Thr Ile His Pro Phe His Leu*		unknown/vasoconstrictor?
Angiotensin II *Asp Arg Val Tyr Ile His Pro Phe*	G-protein-coupled receptors AT_1 and AT_2	vasoconstrictor/vasodilator
Apelin-36 *c-term-Gin Arg Pro Arg Leu Ser His Lys Gly Pro Met Pro Phe* Apelin-13 *Gin Arg Pro Arg Leu Ser His Lys Gly Pro Met Pro Phe*	APJ receptor (homolog of AT_1)	vasoconstriction, vasodilation, water intake/inactive product
[Des-Arg]^{9}Bradykinin *Arg Pro Pro Gly Phe Ser Pro Phe* Lys [Des-Arg]^{9}Bradykinin *Lys Arg Pro Pro Gly Phe Ser Pro Phe*	G-protein-coupled receptors B_1	tissue injury/inflammatory responses/inactive product
Neurotensin *pGlu-Leu Tyr Glu Asn Lys Pro Arg* Dynorphin A *Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys* B casamophin *Tyr Pro Phe Val Glu Pro Ile*	kapa and delta G-protein-coupled opioid receptor	pain perception

β-casamorphin are also substrates of ACE2 [18] (table 1). These peptides activate kappa and delta G-protein opioid receptors that regulate pain perception and among other functions may have negative effects on cardiomyocyte contractility [24]. ACE2, however, failed to cleave bradykinin and 15 other unrelated vasoactive and hormonal peptides [4]. Although the biological peptides angiotensin II, apelin-13, dynorphin A 1–13 and des-Arg^9-bradykinin are good ACE2 substrates in vitro, their role as physiological substrates of ACE2 is still unclear.

ACE2 and blood pressure

Based on the potential in vivo functions of angiotensin 1–9, angiotensin 1–7 and des-Arg bradykinin, it is tempting to speculate that ACE2 plays a role in the regulation of blood pressure homeostasis (fig. 1). Indeed, the *ace2* gene is located in the region of the X chromosome (Xp22), which maps to a QTL in the Sabra, SHR and SHRSP rat models of hypertension [6–8, 25]. These QTLs carry a significant logarithm-of-the-odds score that suggests the presence of a hypertension-related gene within the chromosomal span demarcated by the QTLs.

In the Sabra rat model of salt-sensitive hypertension, ACE2 messenger RNA (mRNA) and protein levels are diminished in the hypertension-prone SBH/y strain when compared with the hypertension-resistant SBN/y strain [6]. Baseline systolic blood pressure is 10–20 mm Hg higher in the Sabra hypertension-prone SBH/y strain than in hypertension-resistant SBN/y rats. [7] Also, during salt loading, ACE2 levels are diminished even further in SBH/y animals, which become overtly hypertensive, whereas the levels remain unchanged in SBN/y rats, which remain normotensive [6, 7]. Spontaneously hypertensive rats (SHR) and spontaneously hypertensive stroke-prone rats (SHRSP) develop hypertension without an apparent external hypertensive stimulus. ACE2 expression is consistently lower in both hypertensive SHR and SHRSP strains compared to the normotensive Wistar Kyoto (WKY) control rats [26]. In all of these rat models of hypertension, ACE2 mRNA and protein levels were greatly reduced in the kidneys in association with increased blood pressure [6].

These genetic data together with the in vitro biochemical data imply a pathophysiologic role of ACE2 in essential hypertension. It is thought that a reduction in ACE2 levels results in impaired degradation of angiotensin II, and reduced formation of vasodilator by-products on the level of the kidney endothelium, thus promoting a blood pressure increase [26]. However, the in vivo studies of two recent knockout mice report conflicting results. Crackower et al. report that *ace2* null mice do not show increased blood pressure compared with control littermates, despite increased angiotensin II plasma and tissue levels [6]. In fact, at 6 months of age, male *ace2* knockout mice had reduced blood pressure as measured using the tail-cuff technique in conscious restrained mice [6]. These results were confirmed using invasive hemodynamic measurement in anesthetized *ace2* null mice that showed reduced systolic blood pressure and mean blood pressure as compared with littermate controls [27]. However, since this

reduction in blood pressure coincided with impaired cardiac function, it is difficult to separate these independent effects on systemic vascular response in *ace2* null mice. In contrast, Allred et al. [28] reported slightly elevated baseline blood pressure levels in a second knockout mouse line lacking the *ace2* gene. These *ace2* null mice also showed a significantly enhanced vasopressor response upon angiotensin II infusion compared to wild-type controls. The higher baseline blood pressure in *ace2*-deficient mice is consistent with the findings in the Sabra model, in which SBH/y with the lower ACE2 expression display higher baseline blood pressure [26].

However, blocking ACE2 by the strong peptide inhibitor DX512 in spontaneously hypertensive rats results in a dose-dependent blood pressure decrease and reflex tachycardia with the maximal average depressor response at 70.5 ± 4.6 mm Hg from an average mean arterial pressure of 155 ± 10 mm Hg at baseline [17]. This in vivo demonstration of the antihypertensive effect of an ACE2 inhibitor contradicts Allred's observation of increased blood pressure in *ace2* null mice. However, since essential hypertension depends on the concerted contribution of multiple genetic and environmental factors, the conflicting data from in vivo studies with ACE2 antagonists and *ace2* null mice might reflect effects of genetic background, age, gender and experimental setup [27].

Taken together, it still remains unclear what the net effect is of the interplay between angiotensin II and the ACE2-mediated peptides angiotensin 1–7 and angiotensin 1–9. It has to be clarified whether, in the relative absence of ACE2, an angiotensin II effect predominates, leading to vasoconstriction and hypertension, or whether compensating mechanisms maintain normal or lower blood pressure dependent on defined genetic backgrounds. The mechanism that regulates blood pressure through the production of angiotensin II was thought to be well understood, but given the complexity of the systems involved, additional studies on mutant mice and specific blocking agents are needed to further our understanding of the physiologic role of ACE2 in blood pressure regulation.

Loss of ACE2 impairs heart function

Experiments with inhibitors of ACE and angiotensin II receptors suggest the involvement of the RAS in the regulation of heart function and cardiac hypertrophy. However, neither *ace* [29,30] nor *angiotensinogen* [31] deficient mice show defects in heart development or are prone to heart disease. In contrast, *ace2* deficient mice exhibit a reduction in cardiac contractility and a significant decrease in aortic and ventricular pressure [6]. Therefore, ACE2 appears to be an important regulator of heart function in vivo. The observed phenotype closely resembles cardiac stunning/hibernation in human and animal models [32]. Cardiac stunning and hibernation reflect adaptive responses to prolonged tissue hypoxia that occurs in coronary artery disease or following bypass surgery [33]. Accordingly, the hearts of *ace2* null mice show upregulation of mRNA expression of hypoxia-inducible genes such as *BNIP3* [34] and *PAI-1* [35]. The magnitude of increased expression of these hypoxia-inducible genes resembles previously observed levels in other hypoxic models, such as myocyte-specific vascular endothelial growth factor mutant mice [36].

Interestingly, ablation of ACE expression on an *ace2* mutant background completely abolished the cardiac dysfunction phenotype of *ace2* single knockout mice [6]. In fact, the heart function of *ace/ace2* double mutant mice was similar to *ace* single mutant and wild-type littermates. The normal cardiac functions of *ace/ace2* double mutant mice suggest that an ACE product, most likely angiotensin II, accounts for the observed cardiac dysfunction of *ace2* single mutant mice. In fact, cardiac myocytes express angiotensin II receptors and undergo hypertrophy in response to angiotensin II. Taken together, it is intriguing to speculate that an excess in the vascular tone of *ace2* null hearts due to unopposed angiotensin II-mediated effects is responsible for the observed heart phenotype.

Renal function of ACE2

In the kidneys the local RAS plays a significant role in the control of organ function and blood pressure regulation. Reduction in systemic blood pressure, decrease in extracellular volume and pathophysiologic conditions affecting the renal arteries all result in reduced glomerular filtration and decreased amounts of sodium entering the proximal tubuli. As a result, renin secretion is stimulated in the kidneys. This mechanism, termed tubuloglomerular feedback (see [37] for review) ultimately results in increased angiotensin II and aldosteron production, counterbalancing reduced blood pressure and/or a decreased extracellular volume.

Within the kidney, ACE2 has a distribution similar to ACE. ACE2 is present in distal tubules and to a much lesser extent in glomeruli, as assessed by both gene and protein expression [38]. ACE2 levels are reduced in experimental diabetic nephropathy [38]. In the context of essential hypertension, previous studies demonstrated that the ACE2 product angiotensin 1–7 counteracting the pressor, trophic and antinatriuretic actions of angiotensin II was elevated in untreated essential hypertensive subjects [39]. In pregnant rats angiotensin 1–7 levels are increased in association with increased ACE2 expression, suggesting that ACE2 may contribute to the local production and overexpression of angiotensin 1–7 in the kidney [40]. Taken together, these findings suggest that angiotensin 1–7 might be a critical link in mediating the negative regulatory feedback between ACE and ACE2. From a more general point of view, it is possible that the

relative balance of vasoconstrictor and vasodilatory angiotensin peptides modulates both hemodynamic and trophic effects within the kidney. Nevertheless, the physiological role of ACE2 remains to be determined. Of note, *ace2* deficient mice have normal renal medullar development and normal renal achitecture [6]. So far, it is not known whether *ace2* null mice exhibit functional alterations in terms of altered tubuloglomerular feedback mechanism, urine concentration or electrolyte balance.

Concluding remarks

Almost 50 years after the discovery of ACE, our understanding of the pathways contributing to the formation of biologically active forms of angiotensinogen peptides are challenged with the discovery of ACE2. It has become apparent that additional intermediates are involved in a feedback regulation of the RAS. Furthermore, ACE2 was shown to function not only in the metabolism of angiotensin I but also in the catalysis of opioid peptides, apelin, neurotensin and kinetensin. In addition, ACE2 has gained recognition as an important regulatory enzyme in blood vessels supplying the heart and in the arterioles and tubules of the kidneys. High blood pressure is a major risk factor for myocardial infarction, cerebrovascular disease and stroke. The elucidation of the physiological role of ACE2 and the characterization of ACE2 substrates and its products may ultimately lead to the development of new therapeutics against hypertension and heart failure.

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