



Editorial

ACE and ACE2: a tale of two enzymes

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This editorial refers to 'Myocardial infarction increases ACE2 expression in rat and humans'[†] by Burrell *et al.*, on page 369

The cardiac renin–angiotensin–aldosterone system (RAAS) is an endocrine cascade, which results in the conversion of the inactive pro-hormone angiotensin I (Ang I) to the active peptide hormone Ang II, and may also function as an autocrine/paracrine system to modulate cardiac function and growth. Renin, the initial enzyme of this cascade, cleaves the amino terminus of the pre-pro-hormone angiotensinogen, thereby releasing the decapeptide pro-hormone Ang I. Angiotensin-converting enzyme (ACE) removes two additional amino acids to yield the active octapeptide hormone Ang II. Ang II, acting through the AT1 receptor, is a potent vasoconstrictor and stimulates cardiac growth. This cardiac growth may be related to myocyte hypertrophy and/or fibroblast proliferation with a concomitant alteration in the extracellular matrix. Ang II, by interaction with the neuroadrenergic and endothelin systems may cause an increase in local noradrenaline and endothelin levels, which in turn may have direct trophic and toxic effects on the cardiac myocyte. In addition, Ang II stimulates aldosterone secretion from the adrenal glands, which may further stimulate cardiac remodelling. The cellular events triggered by increased Ang II formation, while initially compensatory, may lead to eventual loss of cellular function, contractility, and viability. ACE-inhibitors have been shown to improve survival and cardiac function in patients with left heart failure.

In response to RAAS activation there is an increase, at the level of both gene and protein expression, of components which decrease the local concentration of Ang II and which generate bioactive compounds that counteract Ang II-mediated effects. ACE2, the recently described homologue of ACE, is a pivotal enzyme in this counter-regulatory response.^{1,2}

ACE2 contains a single HEXXH zinc-binding domain which is homologous to one of the active sites of ACE and has 40% overall identity to ACE. However, ACE2 is not inhibited by captopril or other 'classical' ACE-inhibitors. Recombinantly expressed ACE2 was first reported to hydrolyse the His–Leu bond in Ang I to release Ang-(1–9). Ang-(1–9) was a substrate for ACE, which hydrolysed it to Ang-(1–7). Of note, ACE2 did not hydrolyse Ang-(1–9) to form Ang II. Subsequent to these initial characterizations it was found that ACE2 had substrate preference for Ang II, which it hydrolysed to Ang-(1–7) at a high rate.³ Indeed, ACE2 purified from human heart hydrolysed Ang II, but not Ang I.⁴ Thus, ACE2 should function to decrease Ang II concentration where it is present and active. Northern blot analysis demonstrated that, in contrast to the near ubiquitous expression of somatic ACE, ACE2 gene expression was localized predominantly to the heart, kidney, and testes. Subsequent studies using quantitative PCR have shown that ACE2 gene expression also occurs in the gastrointestinal tract and, to a lesser extent, in other organs.⁵

ACE2 may be important as a counter-regulatory enzyme not only because it decreases local cardiac Ang II concentrations, but also because its product Ang-(1–7) mediates specific effects through its recently identified receptor, the mas oncogene product (MAS).⁶ Through this receptor, Ang-(1–7) may stimulate nitric oxide synthase (NOS) and counteract the effects of Ang II. The effects of Ang-(1–7) may also involve cross-talk with the angiotensin type 2 receptor (AT2R), and the bradykinin type two receptor (BK2R).

The importance of ACE2 in regulating cardiac function is highlighted by the phenotype of the ACE2 knockout mouse, which showed left ventricular dilation and impaired contractility.⁷ However, this cardiac dysfunction occurred in the absence of ventricular hypertrophy or the myosin heavy chain isoform switch typically found in other animal models of heart failure as well as in human heart failure. The phenotype was rescued by crossing with the ACE knockout mouse. This finding suggests that increased local cardiac Ang II was the

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cause of the cardiac abnormalities in the ACE2 knockout. However, it remains unclear why the model did not show any evidence for cardiac hypertrophy. One possible explanation is that ACE2 is critical in the development of the microvascular capillary bed of the developing heart. Indeed, hypoxia-induced genes were highly up-regulated in the hearts of ACE2 knockout mice.

Burrell *et al.*⁸ quantify ACE2 gene expression in the rat myocardial infarction model. The authors found a marked increase in ACE2 gene expression in the border/infarct zone as well as in viable myocardium in the post-myocardial infarction rat heart. However, ACE2 expression in viable myocardium increased later than in the border/infarct zone. These findings suggest that ACE2 gene expression can be activated by two distinct processes: (i) an acute inflammatory or wound-healing response focused at the border/infarct zone, and (ii) a relatively chronic response induced by such mechanisms as increased wall stress or other factors associated with remodelling. How such processes lead to an increase in ACE2 gene expression remains to be elucidated.

The initial description of ACE2 suggested that it was expressed solely in endothelial cells in the human heart.¹ However, in the setting of myocardial infarction, it now appears that ACE2 protein is found in multiple cell types including macrophages, smooth muscle cells, and cardiac myocytes in addition to endothelial cells.⁸ Furthermore, Burrell *et al.*⁸ identified ACE2 protein in cardiac myocytes from failing human hearts. However, it remains unclear whether ACE2 protein is actually produced by translation of mRNA in cardiac myocytes or is released from other cell types and diffuses to cardiac myocytes. Our laboratory has recently found that ACE2 interacts with integrin beta1D, a cardiac specific integrin isoform, a finding which raises this mechanism for localization as a distinct possibility.⁴ Techniques to sort out cell-type specific gene expression, such as *in situ* RT-PCR, or laser-capture micro-dissection, will be required to answer this question.

Although there are many peptidases which can hydrolyse Ang II to Ang-(1-7), ACE2 appears to be the major pathway for Ang-(1-7) formation from Ang II in the intact human heart.⁹ Furthermore, it has been shown that ACE2 gene expression and activity are markedly increased in failing human heart.^{10,11}

If ACE is the worst of enzymes, is ACE2 the best of enzymes? Cardiac specific over-expression of ACE2 resulted in abnormalities of connexin architecture and conduction.¹² Caution must be used in extrapolating the results of this over-expression model to the role of ACE2 in adult heart failure. Both the ACE2 transgenic over-expression model, and the ACE2 knockout model may have generated a cardiac phenotype by altering key steps in cardiac morphogenesis; effects that would not occur if ACE2 regulation was modified only after birth. ACE2 may have other important functions aside from its activity as an angiotensinase. A truncated form of ACE2, found in the kidney, did not have angiotensinase activity, but nevertheless was found to play a role in renal organogenesis.¹³ In addition, ACE2 has recently been shown to be a 'receptor' for the SARS virus (not a

good thing).¹⁴ It is possible that ACE2 may exert non-angiotensinase functions via binding to integrins. Further research will be required to understand the importance of this interaction.

Based on the initial characterization of ACE2, it was believed that inhibiting the enzyme could be a therapeutic strategy for cardiovascular disease. However, as our knowledge of this enzyme has grown, it appears that increasing its activity, and thereby the local formation of Ang-(1-7), may actually be beneficial under certain circumstances. Burrell *et al.*⁸ have made an important contribution to our understanding of ACE2 and its role in the counter-regulatory response to myocardial infarction. As these authors observe, the next key step is to develop strategies for modulating ACE2 expression and activity in adult animal models of heart failure.

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