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Editorials

See related article, Circ Res. 2000;87:e1–e9

A Pair of ACEs, for Openers?

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The renin-angiotensin system (RAS) has provided bountiful material for investigation by basic scientists and clinicians for more than 100 years. Renin, first identified in 1898 as a pressor substance present in kidney extracts,1 sits at the top of an enzymatic cascade that, in its simplest form, is now broadly known: renin cleaves the circulating precursor, angiotensinogen, to release the inactive angiotensin I, which is in turn cleaved by angiotensin-converting enzyme (ACE) to release angiotensin II, a very potent vasoconstrictor. This ability of ACE, initially purified from plasma, to mediate the cleavage of the decapeptide angiotensin I (Ang1-10) to the octapeptide angiotensin II (Ang1-8) was reported in 1956.2 Localization of the bulk of this conversion reaction to tissue ACE, particularly in the pulmonary vascular bed, was demonstrated in 1968.3

Despite the central position ACE has assumed as a target for antihypertensive therapies, the significance of angiotensin in blood pressure regulation was not fully appreciated until the identification of pharmacological inhibitors of ACE (for review, see Vane4). The first of these, a nonapeptide called teprotide,5 was derived from an extract of venom from the Brazilian viper Bothrops jararaca; once the link between angiotensin and hypertension was demonstrated through investigative6 and clinical7 administrations of teprotide, the utility of ACE inhibition was clear. Positing similarities of the active site of ACE to that of carboxypeptidase-A, investigators at the Squibb Institute for Medical Research designed, synthesized, and tested a series of about 60 compounds, an effort that led to the identification of captopril.8 Other orally available ACE inhibitors, including enalapril and lisinopril, soon followed. In addition to their effectiveness in treating hypertension, ACE inhibitors have been found in a series of large-scale clinical trials to lower the risk of death, myocardial infarction, stroke, coronary revascularization, heart failure, and complications due to diabetes (for review, see Francis9). In addition, there are now more than 10 different ACE inhibitors on the market, which together account for billions of dollars of prescription expense.10

In a recent issue of Circulation Research, Donoghue et al11 introduced a new player to this game with their report of the first known homologue of ACE, which they have named ACE2. The new gene was identified as a result of high-throughput sequencing of a human heart failure ventricle cDNA library. The predicted ACE and ACE2 protein sequences are 42% identical in their metalloprotease catalytic domains; the somatic isoform of ACE has two such domains, whereas ACE2, as in the case of the testicular isoform of ACE, has only one.

Was there reason from the vast amount of data collected on the RAS to suspect another ACE enzyme? Such suspicions may be aroused when inhibitors that work in vitro fail in vivo. In general, ACE inhibitors that work in vitro have been effective in vivo in cases in which the RAS is active; early work by Gavras et al7 demonstrated the lack of activity of ACE inhibitors in settings of sodium repletion and low renin activity, consistent with the working model of the RAS.7,12 Genetic studies may indicate redundancy when targeted deletion of a gene presumed to be critical fails to provide the expected (or any) change in phenotype, suggesting that a second gene is compensating for its absence. The results of ACE gene targeting in mice similarly do not provide strong support for (or against) the presence of a second ACE gene: homozygous null animals have low blood pressure, as might be expected, and heterozygotes have either slightly low13,14 or normal15 blood pressures. So, such analyses, even in retrospect, do not point to a physiological function that might be readily attributed to ACE2.

Indeed, the biochemical activities of ACE and ACE2 appear not to be redundant. The conversion of Ang1-10 to active Ang1-8 is mediated by a dipeptidase activity of ACE. Despite the structural similarities of the two enzymes, the newly described enzyme, ACE2, cleaves only a single residue from the carboxyl terminal of Ang1-10, resulting in the production of Ang1-9, and thus does not appear to function as a true converting enzyme. ACE2 also does not cleave bradykinin but removes the carboxy-terminal residue from other vasoactive peptides such as des-Arg bradykinin, neurotensin, and kinetensin. Ang1-9 may in turn be metabolized to Ang1-7, Ang1-5, and Ang1-4 but is not readily converted to Ang1-8.11 Of these products, Ang1-7 may have physiological effects that oppose Ang1-8,16 but the activities of the others are not well understood at present. Although there has been no shortage of other peptidases to explain the presence of these metabolites,16 the identification of ACE2 certainly provides further incentive to investigate their significance.

One potential scenario in which these metabolites, along with ACE2, may play a role is in the local RAS in the heart (for review, see Danser et al17). The substrate for the ACEs, Ang1-10, is present in the coronary circulation.18 ACE activity is present in cardiac homogenates19,20 and is limited to coronary vascular endothelial cells and the endocardium.21 ACE2 is also reported to be present in coronary vascular
endothelium. If ACE2 in fact acts on Ang1-10 to generate Ang1-9 and Ang1-7, this could represent a local mechanism for limiting the production or opposing the vasoconstrictive and hypertrophic effects of Ang1-8. In the absence of ACE inhibition, however, cardiac metabolism of Ang1-10 and Ang1-8 tracks together and is not separated by varying sodium loading conditions, at least in the angiographically normal hearts of individuals referred to cardiac catheterization for evaluation of atypical chest pain. There is evidence that alternative metabolites of Ang1-10, such as Ang1-9, do accumulate in the setting of ACE inhibition; although Ang1-10 can be converted to Ang1-7 by other endopeptidases, these alternative metabolites might also result from the activity of ACE2.

Which gene, ACE or ACE2, came first? Similar to the testicular isoform of ACE (t-ACE), ACE2 has a single catalytic domain, whereas somatic ACE (s-ACE) has two such domains. The two catalytic domains of s-ACE are 68% identical to one another, whereas ACE2 and t-ACE are 42% identical in this region. Although the phylogenetic analysis suggests that mammalian ACE and ACE2 are more closely related to one another than to Drosophila ACE, mammalian ACE and Drosophila ACE are both captopril-sensitive dipeptidases, whereas human ACE2 has monopeptidase activity. Indeed, inspection of the aligned sequences reveals regions of greater similarity between human t-ACE and Drosophila ACE than between human t-ACE and human ACE2. Between amino acids 300 and 564, the former pair shares 171 of 265 (65%) residues, whereas the latter pair shares 137 of 265 (52%). A BLAST alignment of the residues surrounding the putative active sites of human somatic ACE (amino acids 361 to 404) with other ACES shows at least 80% identity with other mammalian or chicken ACES and 73% with Drosophila ACE. The corresponding human ACE2 region is less similar, with only 60% sequence identity. These sequence differences likely underlie the differences in both enzymatic function and drug sensitivity of ACE2 and, of course, have great significance for the design of pharmacological inhibitors of ACE2. ACE, with its two catalytic domains, evolved by duplication of a single-domain ACE-like protein before the divergence of insect and mammalian lineages around 550 million years ago; comparisons of the sequences and peptidase activities suggest that the divergence of the primordial single-domain ACE2-like protein occurred even earlier. The functions of the single-domain molecules t-ACE and ACE2 may predate, in an evolutionary sense, the systemic or endocrine role of ACE, which is necessary to maintain hemodynamic performance in higher-vertebrate circulatory systems.

So, ACE2 has joined the game, and there is much to learn. As mentioned above, enzymes other than ACE2 can generate the same alternative metabolites of Ang1-10. Until ACE2 activity can be blocked with specificity, it will be difficult to prove its biochemical effect in vivo. Design of such inhibitors may be aided by structural analysis of the ACE2 active site, with particular attention to the residues that vary between ACE2 and the mammalian/insect ACE consensus. Cell types that may express ACE2 in vivo, such as endothelial, vascular smooth muscle, or renal epithelial cells, should be tested for ACE2 expression and activity in vitro, both in the presence and absence of ACE inhibition. Similarly, identification of these metabolites in the coronary circulation, particularly in the setting of ACE inhibition, would lend support to the case for ACE2 function. We would then need more information about the relative cellular distributions of ACE and ACE2, as well as comparisons of enzyme kinetics, to understand fully how ACE2 fits into the local cardiac RAS. Finally, the expression and activity of ACE2 in both acute and chronic tissue injury should be characterized.

Fortunately, the past century has seen, in addition to the elucidation of the RAS, the development of investigative tools that can be used to gather this information. We look forward to the identification of potential products of ACE2 activity in vivo, particularly in the setting of ACE inhibition, and to work that uses pharmacological inhibition of ACE2 and contemporary molecular genetic approaches to define the functional significance of ACE2 in normal physiology and pathophysiology. Putting the compelling pedigree of ACE2 aside, it will be the results of such investigations that provide a solid starting point for evaluating the potential of ACE2 as a therapeutic target.

References

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