Do Studies With ACE N- and C-Domain–Selective Inhibitors Provide Evidence for a Non-ACE, Non-Chymase Angiotensin II–Forming Pathway?

Ahsan Husain, Ming Li, Robert M. Graham

Angiotensin I–converting enzyme (ACE, EC 3.4.15.1) is a zinc metalloprotease of the gluzincin family.1 Gluzincins characterized to date include thermolysin-like enzymes in Gram-positive and -negative bacteria (M4 family), mycolysin from Streptomyces (M5), botulinum neurotoxin (M27), a family of aminopeptidases (M1), neprilysin-like enzymes (M13), and ACE (M2). Of these, some of the neprilysin-like enzymes, eg, neutral endopeptidase (NEP or neprilysin) and ACE possess peptidyl-dipeptidase character. Furthermore, sequence similarity between neprilysin-like enzymes and ACE suggests that the M13 family is the closest gluzincin relatives of ACE and that these enzymes are the more recently evolved of the gluzincin group.2 The Caenorhabditis elegans genome contains an ACE-like and several neprilysin-like sequences, but similar sequences have not been detected in unicellular eukaryotes, suggesting that an ACE/neprilysin ancestor arose during the Cambrian radiation about 530 million years ago. After divergence from neprilysin, ACE acquired a unique chloride activation mechanism that has been identified in invertebrates through to mammals.3 Some time before the divergence of bird and mammalian ancestors 310 million years ago, an internal duplication produced the vertebrate gene with two catalytic domains, N and C.4 Dual- and single-module ACEs acquired a C-terminal membrane anchor as retained in the ACE-like enzyme ACEH,5 although the acquisition dates are uncertain. Tick and mammalian sequences have hydrophobic—potentially membrane spanning—sequences, but C elegans and diterans sequences do not.

Most mammalian tissues contain ACE with two catalytic domains; however, the use of an internal promoter has additionally led to the expression of an ACE with a single catalytic domain (C-domain), in the testis.6 Vertebrate ACE is a type I membrane protein; its catalytic domains are extracellular. Proteolytic cleavage at a site near its extracellular transmembrane anchor domain causes the release of the two joined domains1 (Figure). This cleavage, which may be limited by the concentration of the membrane-bound ACE and by the membrane-associated or -bound secretase, is responsible for its release into the circulation. Proteolytic processing can, in some cases, lead to the release of the ACE N-domain.7 Membrane-bound ACE is the predominant form of ACE. Thus, gene duplication, multiple promoters, and posttranslational processing have diversified a single invertebrate ACE into a more complex vertebrate protein family. Evolutionary conservation of the ACE N- and C-domains suggests important distinct functions of these domains.

ACE inhibitors are widely used in the treatment of hypertension and congestive heart failure. It is thought that suppression of angiotensin II formation and bradykinin degradation is mechanistically important in the treatment of these diseases by ACE inhibitors. However, the possibility that other known or as yet unidentified substrates that are processed by ACE could contribute to the therapeutic benefits of ACE inhibition cannot be excluded.

At the heart of ACE function is the substrate specificity of its two catalytic domains. Although originally it was thought that ACE, a dipeptidyl peptidase, is a broad-specificity enzyme,8 studies over the last decade have demonstrated a notable degree of substrate discrimination between the ACE N- and C-domains.9,10 However, the physiological or pathological roles of these individual catalytic domains have remained obscure because clinically used ACE inhibitors, which were developed before it was realized that ACE has two catalytic domains, target both these domains with similar affinities.

A major advance in understanding the role of the two ACE catalytic domains has come from the development of the next generation of ACE inhibitors that selectively target one of these catalytic domains. In 1999, Dive and colleagues11 described an ACE N-domain–selective inhibitor and defined the important role of the ACE N-domain in processing AcSDKP (acetyl-Ser-Asp-Lys-Pro-COOH). AcSDKP is a negative regulator of hematopoietic stem cell proliferation.

In this issue of Circulation Research, Dive and colleagues12 describe an ACE C-domain selective inhibitor—the counterpart of the ACE N-domain selective inhibitor. Both these inhibitors are phosphinic peptides, which mimic structure of metalloprotease substrates in the transition state, and have been used previously to target other enzymes of this superfamily.13 Dive and colleagues12 use these inhibitors to address a fundamental physiological question that has interested many of us who study the renin-angiotensin and kinin systems (ACE plays a central role in both these hormone-processing enzymatic pathways). Which ACE catalytic do-

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main is important in vivo for converting angiotensin I to angiotensin II or for inactivating bradykinin?

The key findings are presented in the Table. For bradykinin and AcSDKP inactivation, there was a high concordance between the in vivo data with synthetic natural substrates of ACE given intravenously and the in vitro studies using pure ACE C- and N-domains. With the in vitro conversion of angiotensin I to angiotensin II, as expected, Dive and colleagues12 found that both inhibitors were simultaneously required to prevent angiotensin II formation. That is, in vitro, inhibition of angiotensin II formation by these N- or C-domain–selective inhibitors was partial, indicating a role for both domains in angiotensin II formation. However, unexpectedly, in the in vivo setting, either the N- or the C-domain inhibitor on its own could fully inhibit this conversion. Potential interpretations that could explain the seeming paradox between the in vitro and in vivo data for angiotensin I as follows.

First, the membrane-bound, or attached, ACE shows some sort of interaction between domains that is not seen in the soluble, unattached, enzymes used in the in vitro studies. However, if this were the case, the AcSDKP and bradykinin data in vitro would not parallel the AcSDKP and bradykinin data in vivo. Therefore, this explanation provided by Dive and colleagues12 does not appear to be operative. In fact, if this explanation of the data were to be correct, the entire endeavor would have been in vain, since these inhibitors would then be unable to provide physiological insights into the in vivo roles of the ACE N- and C-domains.

Second, the concentrations of the substrates used for the in vivo compared with the in vitro studies were not the same and, thus, that the different inhibition results are concentration-related. However, this explanation is also unlikely. Thus, if this difference creates an anomaly between the in vivo and in vitro data, the anomaly should exist for all three substrates, since in all cases, their in vitro concentrations were higher than those used for the in vivo experiments.

Third, metabolism of the inhibitors in vivo makes them nonspecific. Again, this is not a satisfactory explanation because the AcSDKP data in vitro parallels the AcSDKP data in vivo.

Fourth, the major enzyme responsible for converting circulating angiotensin I to angiotensin II in vivo is not ACE and it is not chymase. It is not ACE because of the lack of concordance between the in vivo and in vitro data. Chymase has angiotensin II–forming enzyme activity.14 However, it is not chymase because phosphinic peptide inhibitors mimic a structure that is unlikely to be complementary to the transition state of the serine protease chymase. Rather, it is tempting to speculate that it is an uncharacterized metalloproteidase that can be fully inhibited by either of these domain-selective ACE inhibitors as well as by nonglycosylated clinically used ACE inhibitors. In support of this notion that angiotensin II can be formed by enzymatic mechanisms other than ACE are the recent findings of Dell’Italia’s group.15 Using wild-type mice and mice with homozygous disruption of the ACE gene (−/−), these investigators show that angiotensin II concentration levels and angiotensin II:angiotensin I ratios in the kidney, heart, and lung were not different between these two genotypes. In ACE-null (−/−) mice, an increase in tissue chymase activity relative to wild-type was used to explain why angiotensin II generation was unabated despite an absence of the ACE gene.15 Although gene knockout studies with ACE have shown that this metalloprotease is needed to maintain a normal level of blood pressure,16 these earlier studies had not examined the influence of ACE gene deletion on angiotensin II levels. Thus, while the development of a new generation of ACE inhibitors by Dive and colleagues12 provides insights into the preservation of the two discrete ACE catalytic domains, it may also have serendipitously revealed the existence of an unexpected angiotensin II–generating enzyme system alternate to both ACE and chymase.

Effect of Selective ACE N- and C-Domain Inhibition on the In Vitro and In Vivo Hydrolysis of Angiotensin I, Bradykinin, and AcSDKP

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<th>ACE Inhibitor</th>
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References


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