Changes in our concepts of angiotensin I converting enzyme are reviewed briefly. The actions of this enzyme go beyond liberating angiotensin II from angiotensin I or inactivating bradykinin. Its very wide distribution in the body and its activity in vitro indicate involvement in the metabolism of other biologically active peptides. The recent molecular cloning of the human enzyme confirmed the existence of a hydrophobic C-terminal peptide that forms the short transmembrane domain of this plasma membrane-bound enzyme. The much longer external portion contains two homologous active site domains but probably only one functional active center. Finally, in spite of the great progress made in studying angiotensin converting enzyme, there are many challenging problems waiting to be solved.

**(Hypertension 1990;16:363-370)**

I have no doubt, that some diseases not yet understood may in time be transferred to the table of those known . . . If he enters with innocence that of the theory of medicine, it is scarcely possible he should come out untainted with error.

Thomas Jefferson, June 21, 1807

I was very much honored by the assignment to deliver the Lewis K. Dahl Memorial Lecture. The lecture is named after a scientist we all admired and all have, directly or indirectly, benefitted from the results of his pioneering work. Here, I briefly review an area of research in hypertension that developed in parallel to Dahl's basic work. My approach is, and has been, that of a biochemical pharmacologist whose job is made easier by the very successful clinical application of angiotensin I converting enzyme (ACE) inhibitors in hypertension or congestive heart failure.

My aim is not to survey the very extensive literature dealing with the use of ACE inhibitors but simply to show the changes in our concept of this enzyme and to point to some puzzling, still unsolved, issues.

Research on the basic properties of ACE goes back to the 1950s and 1960s. Skeggs et al.1,2 found a factor in horse plasma that converted the decapeptide hypertensin I to the octapeptide hypertensin II or, as the peptides were named later, activated angiotensin I to angiotensin II. They also noticed that their enzyme was inhibited by EDTA and activated by CT. Helmer3 also detected a factor in plasma that activated his angiotensin preparation.

Independent of these investigations, our laboratory studied the metabolism of bradykinin. We found first a "kininase," in human blood called carboxypeptidase N that inactivates bradykinin by releasing Arg9.4 Subsequently, another enzyme was discovered. This kininase splits the Pro7-Phe8 bond in bradykinin and releases the C-terminal Phe8-Arg9. The enzyme was concentrated from kidney microsomes,5 partially purified from human plasma,6 and called kininase II. Also in the 1960s, Vane7 and colleagues pointed out the importance of the pulmonary circulation in the metabolism of vasoactive substances, among them angiotensin I, bradykinin, and 5-hydroxytryptamine. They attributed both the activation of angiotensin I and the inactivation of bradykinin to the sequential degradation of the peptides by a carboxypeptidase N-type enzyme.8 Later, the two activities were assigned to two different enzymes.7 Application of inhibitors, which were first used to potentiate the actions of bradykinin on isolated tissues8 or in the circulation, was of help in studies on bradykinin and later on angiotensin I metabolism.
Werle and Grunz\textsuperscript{10} used cysteine in 1939 to potentiate the effect of bradykinin. We later\textsuperscript{11} used 10 compounds to potentiate the hypotensive effects of intravenously injected kinins in guinea pigs; eight of these compounds contained thiol groups. One of them was penicillamine, which is structurally related to captopril, as established much later. (With the present insight into the issues of injected kinins in guinea pigs; eight of these compounds contained thiol groups. One of them was penicillamine, which was also a substrate of ACE.\textsuperscript{14,15} Finally, the nonapeptide teprotide was sequenced and synthesized. It had the structurally important feature of two prolines at the C-terminus.\textsuperscript{14} The same C-terminal amino acids were also found in an inhibitor derived from Agkistrodon hahs blomhoffii venom by Kato and Suzuki.\textsuperscript{16,17} Proline then became a receptor. Nevertheless, various reports indicate that kinins contribute to the effect of ACE inhibitors. For example, in the experiments of Carbonell et al.\textsuperscript{28} the lowering of blood pressure by an ACE inhibitor in rats made hypertensive by renal ligation was attenuated by giving a synthetic bradykinin antagonist to block the effects of kinins (Figure 2). The beneficial effects of ACE inhibition on the reperfused rat heart is attributed to the effect of bradykinin on the myocardium.\textsuperscript{29}

The concept that the endothelium represents the only physiologically important location of ACE would be hard to reconcile with the fact that some epithelial cells contain a higher concentration of ACE than endothelial cells.\textsuperscript{30} ACE is purified from kidney,\textsuperscript{5,20} and human kidney homogenates have five to six times more ACE per weight than human lung, where it is mainly found in the vascular endothelium.\textsuperscript{30} Animal kidneys are also very rich in ACE, with the notable exception of the rat. In the kidney, ACE is also localized, in addition to the vascular endothelium, on
the surface of the brush border in the proximal tubules.\textsuperscript{31-34} Table 1 lists several tissues and biological fluids that contain active nonendothelial ACE.

ACE is membrane bound in most organs, but soluble ACE has been found in body fluids such as lymph, plasma, amniotic fluid, cerebrospinal fluid, seminal plasma, and in homogenates of prostate and epididymides.\textsuperscript{31,35} In addition to the kidney, various other microvillar structures, for example in the intestine, placenta, or choroid plexus, contain ACE.\textsuperscript{30,31,36-40} The pars recta of the proximal tubule is very rich in ACE where the microvilli of the brush border contain most of the ACE.\textsuperscript{34} Possibly the highest concentration of ACE in any tissue was found in the choroid plexus.\textsuperscript{39,40} (This must be a very fascinating finding, as it has been repeated and reported as startling news practically every year since its first description.)

The protein portions of the endothelial, epithelial, and neuroepithelial ACE appear to be the same. The immunological identity of ACE coming from endothelial, epithelial, or neuroepithelial cells was shown with polyclonal antibodies.\textsuperscript{30} Differences were found, however, in the carbohydrate moieties. For example, the sialic acid content of the human lung enzyme is much higher than that of the renal enzyme. This could be explained by the fact that the lung enzyme, when released into the circulation, is protected by the sialic acid residues against uptake by lectins in the liver, whereas the renal enzyme is excreted into the urine. ACE is a transmembrane peptidase with a short hydrophobic amino acid sequence near the C-terminus that anchors it in the bilayer of plasma membrane.\textsuperscript{30,31,36-38}

The function of ACE on the microvillar structures is less self-evident than in the vascular endothelium where it is in immediate contact with peptides released into the circulation. ACE on the proximal tubular brush border could have several functions and may be more involved in bradykinin than in angiotensin metabolism at this location. Such an inactivation of kinins, which enter the nephron after glomerular filtration, could make it possible for kal-likrein, localized and released distally,\textsuperscript{41} to participate in renal autoregulation. The intrarenal kinins liberated by kal-likrein can affect water and sodium absorption and prostaglandin synthesis. On the other hand, angiotensin II released from angiotensin I could participate in reabsorption at the level of the proximal tubules. For example, angiotensin II stimulates bicarbonate absorption by depressing intracellular cyclic adenosine monophosphate.\textsuperscript{42} Another function of ACE on brush border structures would be to participate in the reabsorption of amino acids. The same role has been suggested for ACE on the intestinal brush border.\textsuperscript{43} In the choroid plexus where ACE faces the cerebrospinal fluid and in the subfornical organ, ACE may facilitate the production of angiotensin II, which is a potent dipsogen. In addition the choroid plexus may be the source of ACE in cerebrospinal fluid.

The very wide distribution of ACE in the body also indicated, or it should have, that it cleaves not only angiotensin I or bradykinin but additional biologically active peptide substrates. The first one found was enkephalin. Of the biologically active substrates tested, [Met\textsuperscript{5}]enkephalin had the highest turnover number with human ACE (3,500 min\textsuperscript{-1}). As the $K_m$ is also high (1 mM), the specificity constant ($K_m/K_{cat}$) would seem to be unfavorable for metabolism in vivo.\textsuperscript{36} However, Benuck and Marks\textsuperscript{44} showed with rat brain ACE bound to an immunoaffinity support that the $K_m$ of [Met\textsuperscript{5}]enkephalin was 10-fold lower than with soluble purified human ACE. Other opioid peptides such as β-neoendorphin and dynorphins are also good substrates of ACE. In addition, rat brain ACE removed the C-terminal dipeptide of [Met\textsuperscript{5}]enkephalin-Arg\textsuperscript{6}-Gly\textsuperscript{7}-Leu\textsuperscript{8}.\textsuperscript{36}

The chemotactic peptide N-formyl-Met-Leu-Phe (F-Met-Leu-Phe) is another active peptide cleaved by peptidylglycine alpha-hydroxylating monooxygenase (PROHOMO). ACE cleaves it efficiently with a $K_m$ of 82 µM and $k_{cat}$ of 270 min\textsuperscript{-1}; because mono-

![Figure 2](https://example.com/f2.png)

**FIGURE 2.** Line graph showing effect of angiotensin converting enzyme inhibitor (CEI) (enalaprilat 60 µg/kg) on mean blood pressure of rats with severe hypertension infused with either kinin antagonist (K. Ant) (40 µg/kg/min) or vehicle (Veh) (0.9% NaCl). Values are mean±SEM. Reproduced with permission.

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**TABLE 1. Distribution of Angiotensin Converting Enzyme**

<table>
<thead>
<tr>
<th>Source of ACE</th>
<th>Membrane-bound</th>
<th>Soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epithelial cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microvilli; brush border of placenta, kidney, intestine, choroid plexus</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Neuroepithelial cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subfornical organ, pallidonigral dendrites, median eminence, etc.</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Male genital tract</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testes</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Prostate, epididymides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Seminal plasma</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><strong>Body fluids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood, urine, lung edema, amniotic fluid, CSF, lymph</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

ACE, angiotensin I converting enzyme; CSF, cerebrospinal fluid. Modified from Reference 31.
TABLE 2. Specificity of Hydrolysis of Active Peptides by Angiotensin Converting Enzyme

<table>
<thead>
<tr>
<th>Peptide structure</th>
<th>Cleavage product</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free C-term. end</td>
<td>C-term. dipeptide</td>
<td>Angiotensin I, kinins, enkephalins, neurotensin, FMet-Leu-Phe</td>
</tr>
<tr>
<td>Free C-term. end</td>
<td>C-term. tripeptide</td>
<td>des-Arg&lt;sup&gt;9&lt;/sup&gt;-bradykinin</td>
</tr>
<tr>
<td>(Ser/Ala-Pro-XXX)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protected C-term.</td>
<td>C-term. tripeptide</td>
<td>Substance P, LH-RH</td>
</tr>
<tr>
<td>end</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protected C-term.</td>
<td>C-term. dipeptide</td>
<td>Substance P</td>
</tr>
<tr>
<td>end</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protected N-term.</td>
<td>N-term. tripeptide</td>
<td>LH-RH</td>
</tr>
<tr>
<td>end</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Term., terminal; FMet-Leu-Phe, N-Formyl-Met-Leu-Phe; LH-RH, leutinizing hormone-releasing hormone. Modified from Reference 31.

cytes contain ACE, it may have a role in these cells of metabolizing chemotactic peptides. Endothelial ACE could also inactivate chemotactic peptides at sites of inflammation.

Because ACE had long been considered a dipeptidyl carboxypeptidase (or peptidyl dipeptidase) requiring substrates with a free C-terminus, it was at first difficult to accept reports stating that ACE inhibitors prevented the inactivation of substance P or that substance P inhibited the hydrolysis of other substrates by ACE. It was generally concluded that these effects were not due to hydrolysis of substance P by ACE because this peptide has a blocked C-terminal amino acid that presumably rendered it resistant to ACE. Nevertheless, the colocalization of ACE and substance P in the substantia nigra and globus pallidus was puzzling.

The possibility that the specificity of ACE was not restricted to its known action as a peptidyl dipeptidase was suggested by other experiments. For example, ACE cleaved a tripeptide from [des-Arg<sup>9</sup>] bradykinin (Table 2), and it hydrolyzed the penultimate peptide bond of substrates in which the last amino acid was replaced by nitrobenzylamine.

Homogeneous human ACE indeed does cleave substance P at two different sites: at Phe<sup>4</sup>-Gly<sup>5</sup> and at Gly<sup>9</sup>-Leu<sup>10</sup>, to release either the C-terminal tripeptide or dipeptide (Tables 2 and 3). However, the release of the tripeptide is clearly favored by a ratio of 4:1. After initial removal of the C-terminal tripeptide or dipeptide, ACE sequentially cleaves dipeptides from the remaining N-terminal fragment. When substance P with a C-terminal methyl ester ([Met<sup>11</sup>-OCH<sub>3</sub>]) was used as substrate, the major product of the initial cleavage was again the C-terminal tripeptide; however, the free acid of substance P was hydrolyzed about seven times faster (at 0.1 mM concentration) than substance P and here only the C-terminal dipeptide was released.

Because the decapptide luteinizing hormone-releasing hormone (LH-RH) also has a blocked C-terminal amino acid and a blocked N-terminus and is found in tissues known to contain ACE, it was tested as a substrate for human ACE. ACE quite unexpectedly cleaved LH-RH in vitro at both the N- and C-terminal ends to release several peptides. A major product was the N-terminal tripeptide pGlu<sup>1</sup>-His<sup>2</sup>-Trp<sup>3</sup>; another was LH-RH<sup>4-10</sup> heptapeptide, indicating that the Trp<sup>3</sup>-Ser<sup>4</sup> bond is cleaved to release the N-terminal tripeptide (Table 3). ACE also released the C-terminal tripeptide Arg<sup>8</sup>-Pro<sup>9</sup>-
**FIGURE 3.** Schematic representation of the human testicular and endothelial angiotensin converting enzyme (ACE). Middle: Diagram showing the cysteine positions, the potential asparagine-linked glycosylation sites, and the positions of the putative residues of the active site of the two enzymes (HEMGH). Beyond the point of divergence, the testicular enzyme is figured on the upper line and the endothelial enzyme on the lower line. Top and bottom: Hydropathy plots of the predicted testicular (top) and endothelial (bottom) amino acid sequences. Negative values indicate increasing hydrophobicity. Amino acid numberings are presented above and under the hydropathy plots. Reproduced with permission. 10

Gly\(^{10}\)-NH\(_2\) (Table 3). NaCl affected this hydrolysis: in 500 mM NaCl, 86% of the hydrolysis of LH-RH was due to the release of the N-terminal tripeptide, whereas in 10 mM NaCl, cleavage at the C- and N-termini occurred about equally. 36 Recent studies have shown the same cleavage pattern of LH-RH by ACE purified from rat brain. 46 These in vitro experiments raised the question of whether ACE contributes to the inactivation of some peptides mentioned above, after either local or systemic administration to laboratory animals. There are reports that indicate that ACE participates in the metabolism of peptides other than angiotensin I and bradykinin in vivo. For example, ACE inhibitors partially blocked the hydrolysis of substance P and neurotensin in the stomach. 47 Administration of captopril inhibited the metabolism of enkephalin heptapeptide after intracerebroventricular injection. 48 ACE inhibitors also reduced the peripheral degradation of enkephalins and potentiated the analgesic effects of enkephalin-Arg\(^6\)-Phe\(^7\) in the mouse. 36 In the cerebrospinal fluid, ACE is the major enzyme that inactivates enkephalins. 35 In the guinea pig plasma, captopril considerably enhanced the level of injected substance P. ACE inhibitors also potentiated the constrictor effects of substance P in the guinea pig lung. 49

**FIGURE 4.** Homology among amino acid sequences (one-letter notation) of human angiotensin converting enzyme (hACE), thermolysin from Bacillus thermoproteolyticus (THERM), rat or rabbit neutral endopeptidase (rNEP), and human skin fibroblast collagenase (hCOLL). First and second lines refer to first and second domains of ACE. Numbers refer to last amino acid position in each protein segment. Identical residues are boxed; conservative amino acid changes are indicated by broken lines. Gaps are indicated by dashes. Reproduced with permission. 30

The molecular cloning and sequencing of the complementary DNA for human 50 and mouse 51 ACE certainly changed some concepts and provided answers to a number of outstanding questions. It revealed the surprising fact that ACE has two homologous domains, each containing a putative zinc binding site and active center. Presumably, only one active site is functional, as shown by inhibitor binding studies 52 and by the fact that each ACE molecule contains one zinc atom. 50 The synthesis of ACE is directed by a single gene in the body, including that of the testicular ACE. 53, 54 This form of the enzyme is
shorter than the endothelial ACE (732 versus 1,306 residues). The N-terminal 67 amino acids of the human testicular enzyme are not found in the endothelial enzyme; however, the last 665 residues are identical with the C-terminal portion of ACE cloned from an endothelial cell library. Because the testicular enzyme lacks the N-terminal active site domain but is enzymatically as active as the endothelial or epithelial ACE, the C-terminal domain likely contains the functional active site in both forms. Besides the human enzyme, this also applies to the rabbit testicular ACE.54 ACE has active site residues common to other zinc-containing metalloenzymes, including the conserved residues (His, Glu, His) that bind the zinc cofactor (Figure 3).

Interestingly, although ACE was initially considered to be a dipeptidyl carboxypeptidase, its primary sequence has no homology with carboxypeptidase A.50 The synthesis of its first orally active inhibitor, captopril, was based on the presumed structural similarities in the active centers of carboxypeptidase A and ACE.55 Although the hypothesis was erroneous (the two enzymes neither cleave substrates the same way nor are they inhibited by the same specific inhibitor), it obviously did not keep captopril from becoming a very effective inhibitor. On the other hand, alignment with the sequence of other zinc peptidases and proteases revealed short segmental identities in regions comprising residues involved in catalytic activity56 (Figure 4). This may partially explain why ACE can also act as an endopeptidase on N- and C-terminally protected peptides.

Unresolved Questions

This brief, and certainly not comprehensive, review was written to reflect how our concept of an enzyme may change through the years as we learn more about it. ACE has been purified and cloned, the cleavage of many of its substrates characterized, and specific potent inhibitors that are effective in animal experiments and are very useful therapeutic agents have been developed. The obvious question at this point is, are more experiments really needed? Researchers usually prefer to work on unsolved problems. Obviously, a success story such as the development of ACE inhibitors is a hard act to follow. Although teaching medical students taught me not to end a lecture with unanswered questions or unsolved problems, I will deviate from this rule to show that we are not at the end of history. As an epilogue, I would like to point out issues, as I see them, that still beg for a final solution.

For example, ACE activity was reported to be enhanced after prolonged treatment with an inhibitor.56 Is that enhancement always due to a true induction of enzyme synthesis, or can the second domain of ACE somehow be activated? In that case, ACE may have two active centers, similar to other enzymes such as the complete tetrameric carboxypeptidase N.57 How much does the potentiation of the effects of peptides (e.g., enkephalin, substance P, and bradykinin) contribute to the therapeutic actions of ACE inhibitors14,17,26,28? This is more likely to happen locally than in the systemic circulation.

Do enzymes other than ACE release angiotensin II from angiotensin I in vivo after the administration of an ACE inhibitor? This could be done by the sequential cleavage of Leu10 and His9 from angiotensin I by a carboxypeptidase-type action59,60 or through the release of the His-Leu dipeptide by an enzyme that acts similarly to chymotrypsin.61,62

Why is there such a big interspecies variation in the plasma level of ACE (e.g., guinea pig plasma versus dog plasma) or in its concentration in tissues (e.g., human kidney versus rat kidney)?

Finally, ACE was initially considered to be a dipeptidyl carboxypeptidase, or more correctly a peptidyl dipeptidase; nevertheless it also cleaves blocked N- and C-terminal tripeptides. Thus, how do you name an enzyme that converts, as its name indicates but is also a kininase and an enkephalinase and has all the above mentioned actions as well?

Acknowledgment

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**KEY WORDS** • angiotensin • bradykinin • enkephalins • substance P • arginine carboxypeptidase • cloning, molecular
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E G Erdös

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