Effects of the N-Terminal Sequence of ACE on the Properties of Its C-Domain

Branislav Marcic, Peter A. Deddish, Herbert L. Jackman, Ervin G. Erdős, Fulong Tan

Abstract—Angiotensin I–converting enzyme (ACE, kininase II) has 2 active domains (N and C) in a single peptide chain. Because we found its N-domain more stable than its C-domain, we investigated the effect of the amino-terminus of human ACE on the C-domain with a molecular construct expressed in Chinese hamster ovary cells (CHO) cells and transiently in HEK293 cells. This active N-deleted ACE contained only the first 141 amino acids of the human N-domain but not its active center and was linked to the active C-domain containing the transmembrane and cytosolic portions of ACE. The CHO cells were also transfected with human B2 bradykinin receptor. ACE inhibitors (5 nmol/L or 1 µmol/L) augmented bradykinin (100 nmol/L) effects, elevated B2 receptor numbers, and resensitized the receptor desensitized by agonist as measured by arachidonic acid release or [Ca²⁺]i mobilization. Arachidonic acid release was mediated by pertussis toxin–sensitive Gαi and [Ca²⁺]i mobilization was mediated by pertussis-insensitive Gαq protein receptor complex. The properties of the construct were compared with wild-type ACE and separate N- and C-domains. The N-deleted ACE differed from wild-type in activation by Cl⁻ and [SO₄]²⁻ ions, hydrolysis ratios of substrates (both short synthetic and endogenous peptides) and heat stability. Thus, the N-terminal peptide of ACE affected the characteristics of the C-domain active center. ACE inhibitors acting on N-deleted ACE, which had only a single C-domain active center anchored to plasma membrane, induced cross-talk between the enzyme and the B2 receptor (eg, the inhibitors resensitized the receptor) independent of blocking bradykinin inactivation. (Hypertension. 2000;36:116-121.)

Key Words: angiotensin ■ bradykinin ■ angiotensin-converting enzyme inhibitors ■ receptors, bradykinin ■ arachidonic acid ■ calcium

Angiotensin I–converting enzyme (ACE, or kininase II) is a single-chain protein with 2 domains (N- and C-domain) named according to their proximity to the N- or C-terminal end.1,2 The 2-domain, widely distributed enzyme is called somatic ACE, in contrast to the testicular ACE (t-ACE), which represents the C-domain with an additional N-terminal sequence.3 This originates from an alternate initiation site in intron 12 of the ACE gene.4 The synthesis of both enzymes is directed, however, by a single gene. Although the homogeneity around the active centers is ≈89%,4 the 2 active sites cleave some substrates at different rates. In addition to active centers and substrate binding sites, the positions of all 7 cysteine residues in each domain are highly conserved (there are no cysteine residues in the other regions of the molecule), which indicates that both domains have some similarity in topological structure. The most important substrates, bradykinin (BK) and angiotensin (Ang) I, are hydrolyzed at both sites,2,5 but some of the others are cleaved preferentially by 1 of the sites, which is frequently on the N-domain. Of peptide hormones, for example, the luteinizing hormone-releasing hormone (LHRH) is inactivated mostly by the release of <Glu⁴-His⁵-Trp⁶,7 and the enkephalin precursor Met⁶-Enk⁸-Arg⁹-Phe⁷ is converted primarily to enkephalin by the N-domain site.8,9 Ang 1-710 and the tetrapeptide AcSer-Asp-Lys-Pro (AcSDKP)11,12 are also cleaved by the N-domain.

Although the role of individual amino acids in the active center has been well studied,4 the function of the N-terminal sequence in preserving the integrity of the N-domain of ACE is not known. We have been puzzled by our findings that although human intestinal brush border is quite rich in somatic ACE,13 ileal fluid contains the highly active intact N-domain of ACE only, but no C-domain is detected there.14 This separate N-domain has an estimated sequence from 1 to 443 amino acids. Thus, we questioned whether the N-terminal end preceding the active center of the N-domain would lend stability to ACE in the protease-rich milieu of the intestine. The integrity of the C-domain attached to plasma membrane can be protected by the N-domain on 1 side and by the transmembrane anchor portion of ACE on the other side.1,15 To learn more on the importance of structural elements in ACE, we constructed an ACE molecule that contained the first 141 amino acids of the N-domain without an active center (N-del ACE) and coupled it to the C-domain...
of human ACE, having the active center and the transmembrane and cytosolic portions of the wild-type ACE (WT-ACE). Because the 2 domains of ACE differ in substrate specificity and presumably in stability, we investigated how these properties are affected by the N-terminal peptide chain, ie, whether, in addition to the individual amino acids in and around the active center, other factors may influence ACE activity. We also wanted to establish whether ACE inhibitors can still potentiate BK effects on its receptor by acting on N-del ACE, having only the C-domain active site.

**Methods**

Materials BK, Ang, Ang 1-7, AcSDKP, hippuryl-His-Leu (Hip-His-Leu), tissue culture medium, buffers, reagents, and gel filtration columns were obtained from Sigma Chemical Co or from Pharmacia. Fetal bovine serum (FBS) was obtained from Atlanta Biologicals. Benzoyloxycarbonyl (Z)-Phe-His-Leu was obtained from Bachem. Enalaprilat and lisinopril were provided by the Merck, Sharp and Dohme Research Division (West Point, Pa). Keto-ACE (5 S,5-benzamido-4-oxo-6-phenylhexanoyl-L-proline) was a gift from Dr R.G. Ahlquist at the Stanford Research Institute (Palo Alto, Calif). The wild-type cDNA of ACE was kindly donated by Prof P. Corvol, College de France, Paris, France. Chinese hamster ovary (CHO) cells and human embryonic kidney (HEK293) cells were from the American Type Culture Collection. Transfection reagents were from Qiagen. The human active, separate N-domain and C-domain and full C-domain, including the transmembrane anchor and cytoplasmic C-terminus. The N-domain contributes 2 cysteine residues, and the C-domain supplies 5 cysteine residues (Figure 1).

**Transfection With Human B2 Receptor cDNA**

The selected clones were transfected with pcDNA3 vector containing human B2 BK receptor cDNA by use of the Superfect transfection method. The cells expressing both ACE and B2 receptors were marked CHO/AB.

**Radioligand Binding on Selected Clones**

To select the clone with the highest expression of B2 receptors, [3H]BK saturation binding was performed on whole CHO/AB cell monolayers. Clones with high expression of B2 receptors were used further.

**Effect of Pertussis Toxin**

CHO cells expressing WT-ACE and B2 receptor were incubated with 500 ng/mL pertussis toxin in Ham’s F-12 medium at 37°C for 18 hours. Afterwards, either 3H arachidonic acid (AA) release induced by 100 mM BK or 100 mM BK +1 μM ramiprilat or the effect of BK and ramiprilat on [Ca2+]i was measured.

**Enzyme Solubilization and Assay**

Transfected HEK293 or CHO/AB cell monolayers expressing ACE were washed twice with PBS and were then suspended by scraping into 50 mM Tris maleate buffer (pH 7.4) plus 0.5% CHAPS detergent. The cell suspension was incubated at 4°C for 18 hours and then centrifuged at 100 000g for 1 hour. The supernatant, which contained the detergent solubilized enzyme, was then assayed.

In general, enzymatic activity of ACE was assayed with Hip-His-Leu or Z-Phe-His-Leu. The released His-Leu was measured after adding 100 μL of 20 mg/mL o-phthaldialdehyde.

**Hydrolysis of Peptides**

The rates of hydrolysis of BK and Ang were generally determined at 37°C in 50 mM Tris maleate buffer (pH 7.4) containing 150 mM NaCl, 100 μM substrate, and 10 μL of solubilized enzyme from HEK293 cells in the absence or presence of 1.0 μM enalaprilat.

The hydrolysis of the various peptides by the ACE enzymes was assayed by high-pressure liquid chromatography. It was established that cleavage of the substrates followed zero-order kinetics to the time point of the assay.

**Effect of Cl− on Activity**

The hydrolysis of Z-Phe-His-Leu by solubilized enzymes was measured in NaCl added to assay buffer in concentrations ranging from 0 to 800 mM/L.

**Effect of [SO42−] on Activity**

The hydrolysis of Hip-His-Leu was measured in assay buffer containing 150 mM/L NaCl and either 0 or 900 mM/L Na2SO4.

**Keto ACE Inhibition**

The inhibition of the enzymatic hydrolysis of 1 mM/L Hip-His-Leu by keto-ACE was determined by preincubating the enzymes for 30
minutes at 4°C with $10^{-5}$ to $10^{-4}$ mol/L keta-ACE before the addition of substrate.10

Measurement of [Ca$^{2+}$] and $[^3$H] AA
Free cytosolic calcium [Ca$^{2+}$], was measured by means of a microspectrofluorometer (PTI Deltascan or Attofluor Ratiovision) with fura-2/AM reagent.17 In some experiments, [Ca$^{2+}$] was recorded in a single cell or in 100 cells simultaneously by means of an Attofluor Ratiovision microspectrofluorometer.5 $[^3$H] AA release was determined as described.3,17

Desensitization and Resensitization of the B$_2$ Receptor
After desensitization of the receptor by initial exposure of cells to BK (0.1 to 1.0 μmol/L), the restoration of sensitivity to the agonist (resensitization) was established by measuring either $[^3$H] AA release or mobilization of [Ca$^{2+}$]. 15 For example, monolayers of transfected CHO cells loaded with $[^3$H] AA were stimulated with BK for 30 minutes. Then, without removal of BK from medium or the addition of more BK, cells were exposed to an ACE inhibitor (5 μmol/L to 1 μmol/L) or, as control, to BK for an additional 5 minutes. AA release by or [Ca$^{2+}$], mobilization in the cells was registered.

ACE-Derived Enzymes
To obtain separate active N- and C-domains, purified human kidney ACE20 was digested with endoproteinase AspN (Sigma) for 18 hours at 37°C.21 The fragments were separated and purified by differential elution from a lissopril-Sepharose column16 and by gel filtration on a Superdex 200 FPLC column.

Heat Stability
To compare the heat stability, separate N-domain ACE, C-domain ACE, N-del ACE, and WT-ACE were used. Enzyme samples were diluted 1:10 into 50 mmol/L Tris-maleate (pH 7.4) containing 150 mmol/L NaCl and were incubated for 25 minutes at temperatures ranging from 25°C to 60°C. The enzymatic activity remaining in the aliquots was then measured with Z-Phe-His-Leu.

Protein Assays
Protein concentrations were assayed as before.16

Statistics
Probability values were calculated by the unpaired t test by means of SlideWrite software (Advanced Graphics Software).

Results
Hydrolysis of Hip-His-Leu and Z-Phe-His-Leu
The ratio of rates of hydrolysis of Z-Phe-His-Leu to that of Hip-His-Leu22 was determined for the N-del ACE from HEK293 cells, WT-ACE, N-domain ACE, and t-ACE (Table 1). The N-del ACE hydrolyzed Z-Phe-His-Leu $\approx$11 times faster than it did Hip-His-Leu. This ratio was $\approx$3 times larger than with N-domain ACE and 12 times larger than with t-ACE. Clearly, inserting the C-domain into the site of the active portion of the deleted N-domain created an enzyme that hydrolyzes substrates at a different ratio than does WT-ACE, N-ACE, or t-ACE.

Activation of B$_2$ Receptor
The recombinant ACE with partial N-domain deletion (N-del ACE) has the first 141 amino acids of the N-terminal end of ACE but is missing most of the N-domain sequence (residues 142 to 739). The missing part includes the active center and the so-called “bridge section”14 connecting both domains. The CHO clone used in the experiments expressed 60 000 B$_2$ receptors and 10 000 ACE molecules per cell.

The cells expressing both N-del ACE and B$_2$ receptor were then tested with enalaprilat to potentiate the BK-induced $[^3$H] AA release. Enalaprilat (5 mmol/L) potentiated BK-induced $[^3$H]AA release 1.7 $\pm$0.4-fold (n=3, P<0.05), whereas 1 μmol/L enalaprilat enhanced it 2.8 $\pm$0.6-fold (n=3, P<0.05). Enalaprilat also resensitized the receptor when added to the cells desensitized by the agonist; it reactivated the receptor to the desensitizing dose of BK still present in the medium. Enalaprilat (5 mmol/L) increased the release of AA 3.4$\pm$0.9-fold (P<0.05), and 1 μmol/L enalaprilat increased it (7.4$\pm$1-fold) (n=3, P<0.005) compared with the effects of control buffer or a second inactive dose of BK taken as 1 (n=3; P<0.005).

The effect of enalaprilat on the B$_2$ receptor number in CHO/AB cells was also tested. Pretreatment with 5 mmol/L enalaprilat increased the specific $[^3$H]BK binding insignificantly, only an average of 1.2-fold (range 1.18 to 1.24), and 1 μmol/L enalaprilat elevated the $[^3$H]BK binding sites 2.5-fold (range 2.34 to 2.68) (n=2). Because these results with CHO/AB cells were in agreement with those obtained with WT-ACE and B$_2$, they were not pursued further.16

Another interaction between N-del-ACE and B$_2$ receptors was also investigated by using ramiprilat to resensitize receptor to BK and measuring [Ca$^{2+}$], mobilization. Ramiprilat (1 μmol/L), the active form of another ACE inhibitor, resensitized the receptor that was desensitized to BK 1.37$\pm$0.21-fold (n=5) compared with the first response to BK. When instead of the ACE inhibitor a second dose of BK was given, it was inactive, as noted before.16,17 Obviously, this resensitization of the receptor to BK by ACE inhibitor cannot be explained by the inhibition of its enzymatic breakdown.

Consequently, the single active C-domain of N-del ACE anchored to plasma membrane was sufficient to mediate an interaction of ACE with B$_2$ receptors induced by ACE inhibitor.

Effect of Pertussis Toxin
Treatment of CHO cells expressing both WT-ACE and B$_2$ with pertussis toxin to block protein abolishes the release of AA. Neither 100 mmol/L BK alone (1.05$\pm$0.22-fold over basal rate) nor in combination with ramiprilat (1 μmol/L) added to treated CHO cells expressing WT-ACE and B$_2$ receptor (Figure 2A) released any significant amount of AA over baseline (n=3). Pertussis toxin, however, did not affect the elevation of [Ca$^{2+}$] by BK, which was stimulated 2.47$\pm$0.6-fold over basal level (n=3, P<0.05). Furthermore, after the initial B$_2$ receptor response, the receptor desensitized

### TABLE 1. Ratio of Z-Phe-His-Leu to Hip-His-Leu Hydrolysis by ACE Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-del ACE</td>
<td>10.6±2.7</td>
</tr>
<tr>
<td>WT-ACE</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>N-ACE</td>
<td>4.2±1.1</td>
</tr>
<tr>
<td>t-ACE</td>
<td>0.9±0.2</td>
</tr>
</tbody>
</table>

Substrate was 1.0 mmol/L, and Cl$^-$. 150 mmol.

*Ratio=Mean±SEM (n=3).
by BK was resensitized to the BK in the medium by 1 \text{ mmol/L} ramiprilat and increased [Ca\textsuperscript{2+}] \textsubscript{i} 3.4 \pm 0.4-fold over basal level (n = 3, P < 0.05) (Figure 2B).

Thus the BK-induced AA release and potentiation of this response by ramiprilat are mediated by protein inactivated by pertussis toxin. On the other hand, the BK-induced increase in [Ca\textsuperscript{2+}] \textsubscript{i} as well as the resensitization of this response by ramiprilat are mediated by the toxin-insensitive G\textsubscript{a}\textsubscript{q}.

Inhibition by Keto-ACE

Keto-ACE is a relatively specific inhibitor of the C-domain active site of ACE.\textsuperscript{10} Therefore, the effectiveness of this inhibitor was determined for the N-del ACE as compared with N-domain and t-ACE. Keto-ACE inhibited the N-del ACE similarly to t-ACE. The IC\textsubscript{50} of keto-ACE for N-del ACE (0.09 \pm 0.002 \text{ mmol/L}) or t-ACE (0.12 \pm 0.03 \text{ mmol/L}) was much lower than the IC\textsubscript{50} for N-domain ACE (15 \pm 7.0 \text{ mmol/L}; P < 0.005); thus, the inhibition of N-del ACE active center did not differ from that in C-domain.

Activation of ACE Enzymes by Chloride (Cl–)

Chloride ions activate ACE\textsuperscript{24,25} but affect the 2 active sites of ACE unequally.\textsuperscript{4,8} We tested the effect of Cl– on N-del ACE activity between 0 and 800 mmol/L NaCl concentrations (Figure 3). Cl– activates N-del ACE differently from either the isolated N– or C-domain or WT-ACE. The N-del ACE has an optimum Cl– of 200 mmol/L, and higher Cl – concentrations inhibit hydrolytic activity. WT-ACE reached a Cl– optimum at 800 mmol/L (Figure 3).

Activation by Sulfate [SO\textsubscript{4}\textsuperscript{2–}]

Na\textsubscript{2}SO\textsubscript{4} accelerates the hydrolysis of Hip-Gly-Gly while inhibiting the cleavage of other substrates by ACE.\textsuperscript{2} The activity of N-del ACE, t-ACE, and N-domain ACE was measured with and without added 900 mmol/L Na\textsubscript{2}SO\textsubscript{4} with 1.0 mmol/L Z-Phe-His-Leu (Table 2). Na\textsubscript{2}SO\textsubscript{4} enhanced the hydrolysis of Hip-His-Leu by the N-del ACE 3.3-fold and strongly inhibited (\textgtrless 80% or more) the cleavage by the other ACE congeners tested, including WT-ACE, N-domain of ACE, and t-ACE.

Hydrolysis of Natural Substrates

We compared the rates of hydrolysis of 4 naturally occurring peptide substrates of ACE (Ang I, Ang1-7, BK, and Ac-SDKP) by both N-del ACE (HEK293 cells) and WT-ACE (Table 3). N-del ACE cleaved AcSDKP poorly (0.7 \pm 0.5 mmol/min per mg protein) relative to the other peptides (30.8 \pm 4.7 mmol/min per mg for BK and 19.2 \pm 2.3 mmol/min

<table>
<thead>
<tr>
<th>TABLE 2. Effect of [SO\textsubscript{4}\textsuperscript{2–}] on the Activity of ACE Enzymes</th>
<th>Ratio of Hydrolysis Rates\textsuperscript{*}</th>
<th>Substrate=1.0 mmol/L Hip-His-Leu.</th>
<th>*Ratio=Mean±SEM (n=3).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>+Na\textsubscript{2}SO\textsubscript{4}/−Na\textsubscript{2}SO\textsubscript{4}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-del ACE</td>
<td>3.3±0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT-ACE</td>
<td>0.09±0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-ACE</td>
<td>0.21±0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-ACE</td>
<td>0.02±0.01</td>
<td></td>
<td></td>
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</tbody>
</table>

| TABLE 3. Hydrolysis of Peptides |
|---|---|---|---|---|
| Enzyme | Rate of Hydrolysis, mmol \cdot min\textsuperscript{-1} \cdot mg\textsuperscript{-1} protein | |
| --- | --- | --- | --- |
| N-del ACE | 4.1±0.7 | 19.2±2.3 | 30.8±4.7 | 0.7±0.5 |
| WT-ACE | 2.5±0.06 | 38.1±7.0 | 21.0±4.9 | 11.8±0.2 |

Substrate was 100 \text{ mmol/L} and Cl–, 150 mmol/L. Values are mean±SEM (n=3).
per mg for Ang I). However, N-del ACE cleaved BK faster than Ang I, and WT-ACE acted in the opposite way (Ang I 38.1±7.0 nmol/min per mg and BK 21.0±4.9 nmol/min per mg).

Ang 1-7 is cleaved by the N-domain of ACE to Ang 1-5 and a dipeptide,10 but it inhibits the C-domain of ACE. In this study, the C-domain in N-del ACE was active and cleaved Ang 1-7 (4.1±0.7 nmol·min⁻¹·mg⁻¹ protein) 8 times slower than BK (Table 3). Therefore, N-del ACE, in contrast to the results obtained with C-domain ACE,10 acted similarly to N-domain ACE and hydrolyzed Ang 1-7 at a significant rate, but not AcSDKP, another substrate of the N-domain.

Heat Stability

The activity of the N- and C-domain of ACE and that of somatic WT-ACE and N-del ACE was measured after they had been incubated 15 minutes at temperatures ranging from 25°C to 60°C (Figure 4). The stability of the various ACE molecules differed considerably. C-domain ACE was the least stable; it retained 23.3±2% activity after 15 minutes at 47°C. The N-domain ACE was most stable (55±6% at 47°C), and WT-ACE, obviously stabilized by N-domain, was similar to N-domain ACE (54±6% at 47°C). The N-del ACE was more sensitive to denaturation by heat than either the N-domain or WT-ACE (43±4%) but was less sensitive than C-domain alone (P>0.005).

Discussion

We investigated how the N-terminal sequence of ACE, normally distal to cell membrane and facing body conduits, can change some properties of ACE and thus how structural factors can alter the functions of this single-chain, 2-domain enzyme. We constructed an ACE molecule to incorporate the first 141 amino acids of ACE, the mutant (N-del ACE) linked to the results obtained with C-domain ACE,10 acted similarly slower than BK (Table 3). Therefore, N-del ACE, in contrast to the N-domain of WT-ACE,10 was cleaved at an appreciable rate by this modified C-domain.

Z-Phe-His-Leu and Hip-His-Leu are hydrolyzed by both sites of ACE but at different ratios of rates: 1.3 for the WT-ACE and 0.9 for t-ACE.22 However, the ratio for N-del ACE was entirely different; it cleaved Z-Phe-His-Leu ≈11 times faster than the t-ACE representing the unmodified C-domain (Table 1).

ACE is a chloride-activated enzyme, but activation depends on the structure of the substrate.7,8 The hydrolysis of BK is enhanced less by Cl⁻ than that of Ang I,24,25 although BK is cleaved more by the C-domain than by the N-domain. In somatic ACE, the N-domain active center is activated at much lower Cl⁻ concentration (10 mmol/L) than that of the C-domain (up to 800 mmol/L).26 N-del ACE differed, because it was most active in 200 mmol/L NaCl, and higher concentrations of NaCl inhibited N-del ACE. In contrast, the optimal Cl⁻ concentration for WT-ACE was 800 mmol/L.26

Added Na₂SO₄ also distinguished N-del-ACE. Sulfate ions enhance the cleavage only of substrates containing C-terminal Gly-Gly27,28; others are inhibited by this anion. N-del-ACE, however, cleaved Hip-His-Leu 3 times faster in the presence of [SO₄²⁻], and other ACE congeners were inhibited by ≈80% by Na₂SO₄ (Table 3).

When the inactivation of ACE by heating was determined, the isolated N-domain ACE was most stable, and the isolated C-domain was the most labile, N-del ACE was considerably more stable at 47°C than was the isolated C-domain.

The carbohydrate content of N-del ACE would also contribute some of the observed differences because the 1-141 sequence of N-del ACE contains 6 putative sites for N-linked carbohydrate in addition to the sites on the C-domain of ACE.4

ACE inhibitors (eg, ramiprilat, enalaprilat) potentiate the effects of BK and even of its ACE-resistant analogues on the B₂ receptor indirectly.6,16,17,29 They are inactive on cells that have B₁ receptor but lack ACE. This cross talk between the enzyme and the receptor is induced also by agents that react only with the N-domain17 active center. As shown, ACE inhibitors potentiated BK effects on B₂ receptor in the cells that expressed N-del ACE. Consequently, inhibitors that react with either active center of ACE can augment the action of BK and its ACE-resistant analogues on the B₂ receptor.16,17 This indirect activation of the B₂ receptor by ACE inhibitors depends on the steric relationship of the enzyme to the receptor.29

The release of prostaglandins and nitric oxide are among the most important indirect effects of BK30,31; they are initiated by the complexing of the B₂ receptor with the Gα or Gαₚ protein.31,32 ACE inhibitors augmented AA release at a lower concentration16,17 than enhanced [Ca²⁺], elevation by
BK. The involvement of the 2 different G proteins in the activities of B2 receptors was tested with pertussis toxin\textsuperscript{19,23} in CHO WT-ACEB cells. The toxin abolished the release of AA by the cells, by BK or by BK, and by ACE inhibitor; thus, the activation of phospholipase A\textsubscript{2} was blocked. On the other hand, [Ca\textsuperscript{2+}], mobilization\textsuperscript{17,23} and its enhancement by ACE inhibitor were not affected (Figure 2). The release of [Ca\textsuperscript{2+}], a consequence of phospholipase C activation, depends on the complexion of the receptor with G\textsubscript{a} protein.\textsuperscript{17,19}

Our findings are consistent with the observations of Williams et al.,\textsuperscript{22} who created chimeric N- and C-ACE domains in which 13 amino acids within the 60 central amino acid sequence that differ between the domains were exchanged for a corresponding sequence of the other domain. The hydrolysis rates of 2 substrates by the 2 domains indicated that this depended on the sequence surrounding the central regions in each domain rather than on the central regions themselves.

In conclusion, the properties of the C-domain active center were significantly altered by the added N-terminal peptide chain in N-del ACE. Thus, in addition to the amino acids in and around the active center, the structural elements of the N-domain can effectively influence activity at the C-domain.

Acknowledgments

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Marcic et al. N-Terminus of ACE Affects C-Domain 121

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