N-Domain–Specific Substrate and C-Domain Inhibitors of Angiotensin-Converting Enzyme

Angiotensin-(1–7) and Keto-ACE

Peter A. Deddish, Branislav Marcic, Herbert L. Jackman, Huan-Zhu Wang, Randal A. Skidgel, Ervin G. Erdös

Abstract—We used the isolated N- and C-domains of the angiotensin I–converting enzyme (N-ACE and C-ACE; ACE; kininase II) to investigate the hydrolysis of the active 1–7 derivative of angiotensin (Ang) II and inhibition by 5-S-5-benzamido-4-oxo-6-phenylhexanoyl-L-proline (keto-ACE). Ang-(1–7) is both a substrate and an inhibitor; it is cleaved by N-ACE at approximately one half the rate of bradykinin but negligibly by C-ACE. It inhibits C-ACE, however, at an order of magnitude lower concentration than N-ACE; the IC₅₀ of C-ACE with 100 µmol/L Ang I substrate was 1.2 µmol/L and the Kᵢ was 0.13. While searching for a specific inhibitor of a single active site of ACE, we found that keto-ACE inhibited bradykinin and Ang I hydrolysis by C-ACE in approximately a 38- to 47-times lower concentration than by N-ACE; IC₅₀ values with C-ACE were 0.5 and 0.04 µmol/L. Furthermore, we investigated how Ang-(1–7) acts via bradykinin and the involvement of its B₂ receptor. Ang-(1–7) was ineffective directly on the human bradykinin B₂ receptor transfected and expressed in Chinese hamster ovary cells. However, Ang-(1–7) potentiated arachidonic acid release by an ACE-resistant bradykinin analogue (1 µmol/L), acting on the B₂ receptor when the cells were cotransfected with cDNAs of both B₂ receptor and ACE and the proteins were expressed on the plasma membrane of Chinese hamster ovary cells. Thus like other ACE inhibitors, Ang-(1–7) can potentiate the actions of a ligand of the B₂ receptor indirectly by binding to the active site of ACE and independent of blocking ligand hydrolysis. This potentiation of kinins at the receptor level can explain some of the well-documented kininlike actions of Ang-(1–7).

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Key Words: bradykinin • receptors • signal transduction • arachidonic acid • enzymes

Ang-(1–7) is an active metabolite released by peptidases from Ang I or II.¹ Neutral endopeptidase 24.11 (neprilysin) cleaves the decapeptide Ang I to a tripeptide and to Ang-(1–7).² Prolylendopeptidase and metalloendopeptidase 24.15 catalyze the same reaction.³,⁴ Ang II is converted by prolylcarboxypeptidase to a heptapeptide at its Pro⁷-Phe⁸ bond.⁵ The resulting heptapeptide is biologically active, but its actions differ from those of the parent peptide. It is not dipsogenic and does not stimulate aldosterone release. Instead of being a vasoconstrictor as Ang II is, it is a vasodilator on coronary arteries. In addition, the heptapeptide is antiproliferative and releases neurotransmitters and vasopressin.⁶-⁹

Although the existence of a receptor for Ang-(1–7) was reported,¹⁰-¹² many of its effects involve BK, and they cannot be explained by the presence of a specific receptor for the heptapeptide. Thus the vasodilator effect of Ang-(1–7) on porcine, canine, or human arterial strips was attributed to NO release, very likely mediated by BK.⁹,¹³ This vasodilation and hypotension by Ang-(1–7) were also abolished by the BK B₂ receptor antagonist HOE 140.¹⁴ In addition, Ang-(1–7) inhibits purified canine ACE.⁹ It is hypothesized that Ang-(1–7) is synergistic with BK because it either has a different Ang receptor subtype, is a ligand for the B₂ receptor, or inhibits the enzymatic inactivation of BK.⁹

Ang I and BK are among the physiologically important substrates of Ang I–converting enzyme (kininase II; ACE); thus ACE inhibitors have dual actions in blocking hydrolysis of both peptides.¹⁵-¹⁸ The molecular cloning and sequencing of the complementary DNA for human and animal ACE revealed that ACE (somatic ACE) has two homologous domains,¹⁹-²¹ each containing an active center. According to their position in the N-terminal half or in the C-terminal half of the single chain protein, they are designated N- or C-domain, which will be referred to here as N-ACE and C-ACE. The synthesis of ACE is directed by a single gene in the body. The testicular form of the enzyme (germinal ACE) is shorter than the somatic ACE (732 versus 1306 residues).
and contains only the C-domain, which is attached to cell membranes, thus it lacks the N-domain active site. Because keto-ACE inhibited the hydrolysis of a tripeptide representing the C-terminus of Ang I at an order lower concentration than it inhibited hydrolysis of a tripeptide similar to the C-terminus of BK, it was tested to see whether it would be a relatively specific inhibitor for one of the active centers. We also report here that Ang-(1–7) is cleaved to Ang-(1–5) and His-Pro, mainly by N-ACE, as it is only very slowly hydrolyzed by C-ACE. As such, it is a more potent inhibitor of the C-domain active site than N-ACE. Although Ang-(1–7) is an inhibitor, it potentiates the effects of BK independent of blocking its metabolism. In this regard, it acts similarly to classic ACE inhibitors that induce "crosstalk" between the enzyme and the receptor.

Inhibition Studies
The effects of both keto-ACE and Ang-(1–7) on the hydrolysis of various substrates by N-ACE or C-ACE were determined by preincubating the enzyme for 30 minutes at 4°C with the potential inhibitor before addition of the substrate. A range of inhibitor concentrations was used and IC50 was calculated from the inhibition curve. The Ks was then calculated with kinetic parameters determined for uninhibited enzymes, with the following equation:

\[ v = \frac{V}{1 + \frac{K_s}{i} + i/K_i} \]

Transfection of CHO Cells and Expression of ACE and B2 Receptor

Construction
An EcoRI fragment of B2 receptor cDNA (177 to 1770 bp) containing the whole coding region was cloned into the EcoRI site of pcDNA1. The direction of the insert was determined by restriction enzyme digestion and sequencing. A 4-kb EcoRI fragment of human ACE cDNA was also cloned into the EcoRI site of pcDNA3, and its direction was determined by restriction enzyme digestion.

Transfection
Human ACE-pcDNA3, BK B2 receptor–pcDNA1, and pH5APHr-3pneo containing plasmids were used to transfect CHO cells with Lipofectin Reagent (Gibco BRL) as reported by us. Cell monolayers (approximately 50% confluent in 60- or 100-mm diameter dishes) were washed three times with serum-free Ham’s F-12. Lipofectin (30 to 80 μL) and DNA (5 to 15 μg/μL) were first individually diluted to 200 μL and then mixed together and incubated at room temperature for 15 minutes. An aliquot of the mixture (200 μL) was then diluted to 2 or 4 mL in serum-free Ham’s F-12 and applied to the cell culture monolayers. After 24 hours, the cells were washed twice and fed 5 or 10 mL of media containing 10% FBS. Two days later, the monolayers were subcultured after being treated with 0.5 mL trypsin/EDTA (0.025% trypsin/0.1 mmol/L EDTA) and plated at a fluorometric assay, the dipeptide cleaved from this substrate was coupled to α-phthalic dicarboxaldehyde. A radiometric assay used [3H]Hip-Gly-Gly. The cleavage of longer peptide substrates was assessed by high-pressure liquid chromatography (HPLC).

Analysis of Hydrolysis Products
The hydrolysis of the various peptides by the ACE enzymes was assayed by HPLC. After incubation of enzymes with a peptide, ice-cold 5% trifluoroacetic acid (TFA) was added to 1.7% final concentration to stop the reaction. Peptides and their hydrolysis products were separated on a Waters µBondapak C18 reverse-phase column with an increasing (5% to 40%) linear gradient of acetonitrile/0.05% TFA in H2O/0.05% TFA and detected with a Waters 484 detector at a wavelength of 214 nm. It was established that cleavage of the substrates followed zero-order kinetics to the time point of the assay.

Hydrolysis of Peptides
The rates of hydrolysis of the BK and angiotensin peptides were generally determined at 37°C in 50 mmol/L Tris-maleate buffer, pH 7.4, containing 150 mmol/L NaCl, 100 μmol/L substrate, and 2 mmol/L enzyme. The effect of chloride concentration was assessed in the same buffer containing from 0 to 150 mmol/L NaCl. The kinetics of Ang-(1–7) hydrolysis were established by incubating a 2-nmol/L concentration of either N-ACE, somatic ACE, or a 300-nmol/ml concentration of C-ACE with the peptide in five different concentrations varying from 10 to 200 μmol/L in 50 mmol/L Tris-maleate, pH 7.4, and 150 mmol/L NaCl at 37°C. The amount of Ang-(1–5) formed was calculated from the peak area by comparison to a known standard. Kinetic parameters were calculated from Lineweaver-Burk plots.

Methods
Materials
Hippuryl-glycyl-glycine (Hip-Gly-Gly), BK, angiotensin, and their metabolites, tissue culture medium, buffers, and reagents were purchased from Sigma Chemical Co. Phenyl-4-(n)-[3H]-hippuryl-glycyl-glycine ([1H]-Hip-Gly-Gly) and 5,6,8,9,11,12,14,15-[3H]-arachidonic acid ([1H]-AA) were purchased from Amersham and hippuryl-histidyl-leucine (Hip-His-Leu) from Bachem. The keto-ACE, functional C-domain active site (ACE K361, K365), was a gift of Dr R.G. Ahlquist at the Stanford Research Institute (Stanford, Calif). CHO cells were obtained from the American Type Culture Collection. The more ACE-resistant BK derivative HT-BK was from Atlanta Biologicals. The cDNAs of ACE and B2 receptor–pcDNA1, and pH5APHr-3pneo containing plasmids were used to transfect CHO cells with Lipofectin Reagent (Gibco BRL) as reported by us. Cell monolayers (approximately 50% confluent in 60- or 100-mm diameter dishes) were washed three times with serum-free Ham’s F-12. Lipofectin (30 to 80 μL) and DNA (5 to 15 μg/μL) were first individually diluted to 200 μL and then mixed together and incubated at room temperature for 15 minutes. An aliquot of the mixture (200 μL) was then diluted to 2 or 4 mL in serum-free Ham’s F-12 and applied to the cell culture monolayers. After 24 hours, the cells were washed twice and fed 5 or 10 mL of media containing 10% FBS. Two days later, the monolayers were subcultured after being treated with 0.5 mL trypsin/EDTA (0.025% trypsin/0.1 mmol/L EDTA) and plated at

Enzyme Assays
ACE activity was determined by several techniques. In a recording spectrophotometric assay, Hip-His-Leu was the substrate. In a spectrophotometric assay, the dipeptide cleaved from this substrate was coupled to α-phthalic dicarboxaldehyde. A radiometric assay used [3H]Hip-Gly-Gly. The cleavage of longer peptide substrates was assessed by high-pressure liquid chromatography (HPLC).

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\[ v = \frac{V}{1 + \frac{K_s}{i} + i/K_i} \]
low density in media containing 10% FBS and 600 μg/mL geneticin. Clonal colonies that grew in the presence of the selection medium were isolated with glass cloning rings, fed every 48 to 72 hours, and subcultured as needed. Cells that expressed B2 receptor only were designated as CHO-3B cells. Cotransfected cells expressing both active ACE and B2 receptor were the CHO-15AB cells.

**Effect of Ang-(1–7) on Arachidonic Acid Release by HT-BK**

Cells were grown on six-well plates in Ham’s F-12 medium containing 10% FBS. After reaching 60% to 80% confluence, the culture medium was replaced with Ham’s F-12 medium containing 0.5% FBS and 1 μCi/mL of [3H] arachidonic acid (loading medium). Cells were incubated in this medium for 24 hours. Monolayers were then washed three times with the release buffer (RB) (25 mmol/L HEPES, 150 mmol/L NaCl, 5 mmol/L KCl, 5.5 mmol/L dextrose, 0.8 mmol/L MgSO4, 1 mmol/L CaCl2, and 0.1% fatty acid-free BSA, pH 7.4). Triplicate wells were then incubated at 37°C for 30 minutes with one of the following treatments: (1) 1 mL of RB alone to measure basal [3H] arachidonic acid release (this value was later subtracted from each triplicate); (2) 1 μmol/L HT-BK in 1 mL of RB; (3) 1 μmol/L HT-BK plus 1 μmol/L of Ang-(1–7) in 1 mL of RB; (4) 1 μmol/L HT-BK plus 10 μmol/L of Ang-(1–7) in 1 mL of RB; (5) 1 μmol/L HT-BK plus 10 μmol/L of Ang II in 1 mL of RB; or (6) 10 μmol/L of Ang-(1–7) in 1 mL of RB without added HT-BK. After this time period, RB from each well was simply transferred to scintillation vials (released [3H] arachidonic acid is bound to fatty acid–free BSA and counted).

**Results**

**Hydrolysis of Ang-(1–7) and BK by N-ACE and C-ACE**

The decrease in the amount of unhydrolyzed substrate and the increase in hydrolysis products [Ang-(1–5), BK-(1–7), and Hip-His-Leu (data not shown)] was not tried because of the limited amount of available material.

**Effect of Cl– Concentration on Ang-(1–7) Hydrolysis**

To determine this, the substrate was exposed to the enzymes at Cl– concentrations ranging from 0 to 300 mmol/L. The hydrolysis of Ang-(1–7) by N-ACE was stimulated less than twofold when the Cl– concentration was raised to 300 mmol/L from 0. (Because the N-ACE was not dialyzed, the activity at 0 Cl– may in fact be due to a trace of Cl– present.) There was still no detectable hydrolysis of Ang-(1–7) by C-ACE at any chloride concentration used.

**Kinetics of Ang-(1–7) Hydrolysis**

Because Ang-(1–7) was not hydrolyzed when the peptide was incubated with 2 nmol/L C-ACE for up to 3 hours, a much higher concentration of the enzyme was tried. Measurable activity was observed when 100 μmol/L Ang-(1–7) was incubated with 300 mmol/L C-ACE for 3 hours. The product was Ang-(1–5), and the reaction was completely blocked by 1 μmol/L enalaprilat, indicating that the hydrolysis was due to ACE (data not shown). Therefore, the kinetics of hydrolysis of Ang-(1–7) were determined with 300 mmol/L C-ACE, 2 nmol/L N-ACE, and 2 nmol/L somatic ACE with various substrate concentrations (Table 2). The Km of Ang-(1–7) with three representative forms of ACE was 4.3, 6.8, and 6.6 μmol/L for N-ACE, C-ACE, and somatic ACE, respectively. The kcat value for N-ACE (27 min–1) was 75 times higher than the kcat for C-ACE (0.36 min–1) and quite similar to the kcat for somatic ACE (23.7 min–1). These values yield a specificity constant (kcat/Km) for N-ACE (6.1) that is 100 times larger than that for C-ACE (0.06) and 1.6 times greater than that for somatic ACE (3.7).

Ang-(1–7) was incubated with another source of C-ACE, somatic ACE, 23,24 where His in positions 361 and 365 was mutated to Lys, thereby rendering the N-ACE domain inactive. Ang-(1–7) in these experiments was not hydrolyzed by the C-ACE (10 mmol/L), although the enzyme cleaved Hip-His-Leu (data not shown). Higher concentration of enzyme was not tried because of the limited amount of available material.

**Inhibition of C-ACE and N-ACE by Ang-(1–7)**

The low Km with both active sites (lower than that of Ang I)21 and the very low kcat of Ang-(1–7) with C-ACE indicated that Ang-(1–7) may be an inhibitor of the enzyme. This idea was tested by determining the IC50 values and KKi of Ang-(1–7) with Hip-His-Leu, Ang I, and BK as substrates of C-ACE and N-ACE. Table 3 shows that Ang-(1–7) inhibited C-ACE in μmol/L concentrations. The IC50 values of Ang-(1–7) with Ang I, BK, and Hip-His-Leu hydrolysis were 1.2, 3.9, and 8.2 μmol/L, respectively. With N-ACE, the IC50 values were 9 to 23 times higher (28, 46, and 71 μmol/L for inhibition of Ang I, BK, and Hip-His-Leu hydrolysis, respectively). The KKi values are also indicated in Table 3.
Inhibition of ACE by Keto-ACE

The reported pattern of keto-ACE inhibition of hydrolysis of various substrates by somatic ACE indicated that it may be a relatively specific inhibitor for one of the ACE active sites. We therefore studied the inhibition of the hydrolysis of BK and Ang I by both N-ACE and C-ACE. Table 4 shows that keto-ACE is indeed relatively actively site-specific. With equal concentrations of N-ACE and C-ACE, the IC\textsubscript{50} of keto-ACE was 47 times lower for C-ACE (0.51 m\textsubscript{mol/L}) than for N-ACE (24 m\textsubscript{mol/L}) with BK as the substrate. When Ang I was the substrate, the IC\textsubscript{50} was 38 times lower for C-ACE (0.04 m\textsubscript{mol/L}) than for N-ACE (1.5 m\textsubscript{mol/L}). Thus keto-ACE is a more potent inhibitor of the C-domain active site of ACE.

Effect of Ang-(1–7) on BK-Induced Arachidonic Acid Release From Transfected CHO Cells

We reported that ACE inhibitors potentiate the effect of BK and analogues on B\textsubscript{2} receptors, and this potentiation in the systems used is not due to inhibition of BK hydrolysis by ACE.\textsuperscript{29,30} Therefore we tested the effect of Ang-(1–7) on the response of CHO cells grown in culture and transfected to express both ACE and B\textsubscript{2} receptor (CHO-15AB cells) or B\textsubscript{2} receptor only (CHO-3B). As ligand, the BK analogue HT-BK,\textsuperscript{29,30} which is more resistant to hydrolysis by ACE than BK, was used (Figure). Arachidonic acid released in response to HT-BK in the absence or presence of Ang-(1–7) was measured. Values were normalized to the amount of arachidonic acid released by HT-BK stimulation alone. The addition of either 1 m\textsubscript{mol/L} or 10 m\textsubscript{mol/L} of Ang-(1–7) to CHO-15AB cells increased the arachidonic acid release by HT-BK approximately twofold or threefold but only from cells that contained both ACE and B\textsubscript{2} receptor (CHO-15AB).

Table 3. Inhibition of C-ACE and N-ACE by Ang-(1-7)

<table>
<thead>
<tr>
<th>Substrate, Concentration</th>
<th>C-ACE\textsuperscript{*} IC\textsubscript{50} and K\textsubscript{i} Ang-(1-7), m\textsubscript{mol/L}</th>
<th>N-ACE\textsuperscript{†} IC\textsubscript{50} and K\textsubscript{i} Ang-(1-7), m\textsubscript{mol/L}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hip-His-Leu, 1 m\textsubscript{mol/L}</td>
<td>8.2±1.2 0.16±0.03</td>
<td>71 (68–74) 4.2 (4.0–4.4)</td>
</tr>
<tr>
<td>Ang I, 100 m\textsubscript{mol/L}</td>
<td>1.2±0.4 0.13±0.05</td>
<td>28 (22–33) 3.4 (2.7–4.1)</td>
</tr>
<tr>
<td>BK, 10 m\textsubscript{mol/L}</td>
<td>3.9±0.6 0.05±0.01</td>
<td>46 (43–48) 1.7 (1.6–1.8)</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Expressed as mean±SEM; n=3 for each substrate. \textsuperscript{†}Values are the average (and range) of two experiments.

Table 4. Inhibition of Hydrolysis of BK and Ang I by Keto-ACE

<table>
<thead>
<tr>
<th>Substrate\textsuperscript{*}</th>
<th>Keto-ACE IC\textsubscript{50}† N-ACE, m\textsubscript{mol/L}</th>
<th>C-ACE, m\textsubscript{mol/L}</th>
</tr>
</thead>
<tbody>
<tr>
<td>BK</td>
<td>24±5</td>
<td>0.51±0.4</td>
</tr>
<tr>
<td>Ang I</td>
<td>1.5±0.3</td>
<td>0.04±.005</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Substrate concentration, 100 m\textsubscript{mol/L}. \textsuperscript{†}Enzyme concentration, 2 nmol/L. All values expressed as mean±SEM (n=3).

Discussion

We report here that Ang-(1–7) potentiates BK activity on its B\textsubscript{2} receptor. However, Ang-(1–7) does not act directly on the B\textsubscript{2} receptor, since it was inactive in CHO-3B cells expressing the receptor but no ACE. Ang-(1–7) stimulated arachidonic acid release in CHO-15AB cells in response to BK and HT-BK, but not in CHO-3B cells. This suggests that the effect of Ang-(1–7) on BK activity requires the presence of both ACE and B\textsubscript{2} receptor.
acid release by the ligand of the B2 receptor in CHO-15AB cells when both human ACE and B2 receptor were coexpressed, and this effect was not due to inhibition of BK inactivation. The parent peptide Ang II was inactive, since it is neither a substrate or inhibitor of ACE, possibly because its C-terminus is Pro\(^2\)-Phe\(^8\) and not Pro\(^2\) as in Ang-(1–7). C-terminal proline is an important component of ACE inhibitors for interaction with the enzyme.\(^{15,17}\)

In isolated heart tissue,\(^{30}\) guinea pig ileum (R.D. Minshall, S.F. Rabito, R. Igic, E.G. Erdös, unpublished observations, 1997), or cultured cells cotransfected with B2 receptor and ACE,\(^{29}\) the activation of B2 receptor by ACE inhibitors was not primarily due to blocking kinin breakdown because ACE-resistant B2 ligands were also potentiated. Addition of an active ACE inhibitor immediately enhanced BK activity as much as severalfold on all preparations tested.\(^{26,30}\) This immediate response cannot be correlated with the much slower hydrolysis of kinins in the same preparations. Ang-(1–7) acted similarly to other ACE inhibitors, demonstrated in more extensive studies.\(^{29,30}\) In these investigations,\(^{29}\) ACE inhibitors enhanced the number of BK binding sites, protected high-affinity sites, abolished the desensitization of the receptor, and delayed endocytosis. That Ang-(1–7) inhibits ACE was also shown by others.\(^9\) However, the inhibition, because of the differences in activity of the two domains, is more complex. Ang-(1–7) is a substrate of N-ACE but it also inhibits N-ACE, although at higher concentration than C-ACE. C-ACE cleaves Ang-(1–7) approximately 100 times slower than N-ACE, but Ang-(1–7) inhibits it at an order of magnitude lower concentration.

In ACE, the active site on the N-domain may more readily be exposed to bloodborne substrates and inhibitors; the enzyme is inserted into the plasma membrane by a transmembrane anchor peptide of the C-domain.\(^{15,22–25,35,36}\) ACE inhibitors bind to both active sites, but depending on their structure, they may differ in their affinities, primarily because of differences in dissociation rates from the two active sites.\(^{32,37,38}\) We discovered in human ileal fluid collected after surgery a naturally occurring, short form of ACE having only the N-domain active site; the molecular mass of this deglycosylated ACE is 68 kD. This enzyme was the source of N-ACE in these experiments. For C-ACE, we used rabbit testicular ACE, which has 87% identity with the human C-domain. In the regions containing the active residues (426–469 rabbit, 989–1032 human somatic ACE), the identity is 100%.\(^{23,24}\) Nevertheless, we carried out control experiments with mutant recombinant human somatic ACE in which only the C-domain was functional (not shown) because two His residues were mutated to Lys.\(^{23,24}\) The results were similar to those obtained with rabbit testicular ACE, and this human C-ACE also did not cleave Ang-(1–7).

It appears that vasodilation and probably NO release by Ang-(1–7)\(^{9,13,14}\) can be due to indirect potentiation of BK as an agonist of the B2 receptor. This explains why Ang II receptor blockers were inactive, but a B2 receptor blocker abolished Ang-(1–7) effects.\(^{3,14}\) It also follows that ACE inhibitors may block some of the activity of Ang-(1–7) by similar effects and competing for ACE.\(^{9,29,30}\)

Because of the differences in the cleavage of biological substrates by the two domains, it would be important to develop a second generation of ACE inhibitors that react mainly with one of the active sites of ACE.\(^{39,40}\) Ang-(1–7) appears to be a relatively specific substrate of N-ACE but an inhibitor of C-ACE. The other inhibitor we used, keto-ACE, also inhibits C-ACE at much lower concentration than N-ACE. Keto-ACE is a ketomethylene derivative of a blocked tripeptide substrate, Bz-Phe-Gly-Pro.\(^{41}\) The IC\(_50\) for N-ACE was 38 to 47 times higher than for C-ACE with Ang I and BK substrates. The reported inhibition of short substrates by keto-ACE can be interpreted now as caused by the different affinities of substrates and inhibitors for the two active sites.

In conclusion, we have shown that Ang-(1–7) is both a substrate and an inhibitor of ACE. Some of its pharmacological effects may be attributed to an indirect potentiation of BK action on its B2 receptor by binding to the active site of ACE. This potentiation is not due primarily to inhibition of BK inactivation by ACE but to an induction of “crosstalk” between B2 receptor and ACE on plasma membranes, which is triggered by ACE inhibitors.\(^{29}\)

Acknowledgments

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