Angiotensin I–Converting Enzyme Inhibitors Are Allosteric Enhancers of Kinin B1 and B2 Receptor Function

Ervin G. Erdös, Fulong Tan, Randal A. Skidgel

Abstract—The beneficial effects of angiotensin I-converting enzyme (ACE) inhibitors go beyond the inhibition of ACE to decrease angiotensin (Ang) II or increase kinin levels. ACE inhibitors also affect kinin B1 and B2 receptor (B1R and B2R) signaling, which may underlie some of their therapeutic usefulness. They can indirectly potentiate the actions of bradykinin (BK) and ACE-resistant BK analogs on B2Rs to elevate arachidonic acid and NO release in laboratory experiments. Studies indicate that ACE inhibitors and some Ang metabolites increase B2R functions as allosteric enhancers by inducing a conformational change in ACE. This is transmitted to B2Rs via heterodimerization with ACE on the plasma membrane of cells. ACE inhibitors are also agonists of the B1R, at a Zn-binding sequence on the second extracellular loop that differs from the orthosteric binding site of the des-Arg-kinin peptide ligands. Thus, ACE inhibitors act as direct allosteric B1R agonists. When ACE inhibitors enhance B2R and B1R signaling, they augment NO production. Enhancement of B2R signaling activates endothelial NO synthase, yielding a short burst of NO; activation of B1Rs results in a prolonged high output of NO by inducible NO synthase. These actions, outside inhibiting peptide hydrolysis, may contribute to the pleiotropic therapeutic effects of ACE inhibitors in various cardiovascular disorders. (Hypertension. 2010;55:214-220.)

Key Words: angiotensin I–converting enzyme inhibitors ▪ kininase II ▪ kinins ▪ bradykinin B2 receptor ▪ bradykinin B1 receptor ▪ allosteric regulation ▪ 7-transmembrane G protein–coupled receptor

Millions of patients are treated with angiotensin I–converting enzyme (ACE) inhibitors to combat hypertension, congestive heart failure or diabetic renal diseases.1–4 ACE inhibitors significantly reduce mortality after myocardial infarction5 and are beneficial in other high risk patients. ACE inhibitors block the metabolism of several peptides by ACE, notably the conversion of angiotensin (Ang) I to II,6 and the inactivation of bradykinin (BK)7–8 or the hemoregulatory tetrapeptide Ac-Ser-Asp-Lys-Pro.9 The conversion of Ang I to Ang II was first found to occur in horse plasma6; with kidney and human plasma, one of us reported the identity of ACE and kininase II, which we had discovered previously.6–8,10,11 Consequently, a single peptidyl-dipeptidase not only releases the hypertensive Ang II, but also inactivates the hypotensive BK. How much of the therapeutic effectiveness of ACE inhibitors is attributable to blocking Ang II release12 or to prolonging the short half-life of BK7 and its congener Lys1-BK (kallidin) has been debated. This is further complicated by the existence of 2 kinin receptors. The first characterized, but incongruously named, B2 receptor (B2R) is activated by native BK or kallidin.13 The second, so-called B1 receptor (B1R), does not bind native kinins; its ligands are metabolites of BK and kallidin lacking the C-terminal arginine14 removed by plasma carboxypeptidase (CP)N15–17 or membrane CPM.18–20 Whereas the B2R is widely expressed constitutively, B1R expression is usually induced after noxious stimuli or by inflammatory cytokines,13,14,21–23 although some cells (bovine lung endothelial or human fibroblasts) express B1Rs constitutively.

ACE inhibitors can enhance both B2 and B1R signaling. Blocking kinin inactivation by ACE raises the concentration of intact B2R agonists, which are also the substrates of CPN and -M. This can generate more des-Arg-kinin B1R agonists (Figure). The successful use of antagonists of the Ang II type 1 receptor (AT1R) for many of the same indications as ACE inhibitors does not prove ACE inhibitors work only through reducing Ang II as there are complex interrelationships among Ang II, BK and their receptors. Ang II has 2 receptors, AT1R and AT2R. AT1R is blocked by drugs such as losartan, which can shift Ang II actions to the AT2R. This switching of receptors further counteracts AT1R effects because it leads to the release of mediators such as nitric oxide (NO) and is attributed partially to release of BK to activate B2Rs, a form of “crosstalk.”12,24,25 Intricate Ang II and kinin receptor interrelationships were also indicated in animal experiments where both kinin B1 and B2Rs share in the favorable cardiovascular effects of AT1R blockade.26,27

Despite the established role of Ang and kinin receptors in ACE inhibitor effects, much remains puzzling. For example, BK levels in plasma, even after ACE inhibitors, are lower...
than the effective doses of exogenous peptide. Crosstalk between AT2Rs and B2Rs does not necessarily require the release of BK by kallikrein, because kallikrein and other proteases can directly activate the B2R.28–31 Suggestions that ACE inhibitors lead to an “accumulation” of BK are not plausible.

We suggest that ACE inhibitors have actions that go beyond blocking covalent peptide bond hydrolysis and may explain some of their therapeutic effectiveness. As summarized below, we propose that these drugs are also allosteric effectors of B1Rs and B2Rs, functioning as direct allosteric agonists of B1Rs and as indirect allosteric enhancers of kinin activity on B2Rs via interactions with ACE.

ACE Inhibitors Are Allosteric Enhancers of B2R Signaling Via ACE-B2R Interactions on the Cell Surface

Regarding allosterism, Monod et al32 concluded that in regulatory proteins, indirect interactions between distinct specific binding sites explain their regulatory function. The presentation of Monod and Jacob at a meeting in 1961 was considered revolutionary.33 Proteins could recognize more than one molecular partner at an unrelated second site, forming the basis for allosteric effects.34 Put another way, the 2 sites can “talk” to each other, and, as we understand it, this “talking” is mediated by conformational change. According to the International Union of Pharmacology Committee on Receptor Nomenclature,34 allosteric enhancers are modulators of receptor function that enhance orthosteric ligand affinity and/or agonist efficacy, while having no effect on their own. They can indirectly bias receptor signaling to endogenous agonists by allosteric modification of the receptor.35,36 G protein–coupled receptors, such as the B2R, are prototypes of allosteric proteins that can exist in many different conformational states. Allosteric transition involves isomerization and stabilization of one of the many conformational states of the receptor, each of which may have a different affinity for a ligand and/or preferentially activate different signal transduction pathways. Thus, allosteric enhancers may affect only a subset of the full signaling pathway of a receptor; this “collateral efficacy” can lead to new drug discovery.35

Investigations into allosteric modulators of G protein–coupled receptors have focused on small molecule ligands,37 but we propose that ACE serves this function with the B2R. Thus, ACE inhibitors enhance mediator release (eg, NO,
prostaglandins or endothelium-derived hyperpolarizing factors, not only by protecting BK from degradation but also indirectly by binding to ACE, which allosterically enhances B2R activation by orthostERIC ligand BK (Figure).

The first clinically tested ACE inhibitor, teprotide, came from snake venom peptides that potentiated BK action on isolated organs. Although the Bothrops jararaca venom peptides BPP5a and BPP9a inhibited ACE, with other BK-potentiating peptides or analogs, ACE inhibition, kinin inactivation, and BK potentiation did not correlate. This indicated an additional mechanism(s). For example, adding an ACE inhibitor to the isolated guinea pig ileum when isotonic contraction to BK was, at its maximum, rapidly doubled its magnitude. This was not attributable to blocking BK inactivation because its \( t_{1/2} \) was 12 to 15 minutes in the ileal preparation. On guinea pig atria, enalaprilat enhanced BK-potentiating peptides or analogs, ACE inhibition, kinin inactivation, and BK potentiation did not correlate. This was confirmed with porcine endothelial cells and attributed to inhibitors that ACE inhibitors potentiated BK actions in tissue preparations and not by inhibition of BK degradation. This led to the supposition that ACE inhibitors acted on B2Rs or stimulated a crosstalk between ACE and the B2R.

Experiments with cultured cells led us to conclude that ACE inhibitors induce a conformational change in ACE that is transmitted to the B2R owing to their close contact, resulting in potentiation or reactivation of receptor signaling. For example, enalaprilat preserved the B2R in high affinity form that increased arachidonic acid release. The inhibitor also resensitized B2Rs, which had been desensitized to BK, and reduced its internalization. B2Rs are desensitized after prolonged exposure to kinins and become unresponsive to a second dose of ligand. Thus, resensitization by ACE inhibitors causes the B2R to react again to agonist already present in the medium. The resensitization of B2R by ACE inhibitor was confirmed with porcine endothelial cells and attributed to blocking sequestration of receptor into caveolin-rich membranes. These and numerous additional experiments indicate that ACE inhibitors can act through ACE as indirect allosteric enhancers of BK effects on B2R, resulting in increased mediator release.

More proof of these indirect effects of ACE inhibitors on B2R signaling was obtained with BK analogs such as HT-BK (≈50% resistant to cleavage by ACE) or other almost completely resistant BK analogs. Generally, ACE only cleaves oligopeptides of less than 13 residues efficiently, but BK agonists can be larger molecules. For example, BK coupled at the N terminus to soluble dextran or Lys1-BK dalsylated at the \( \alpha \)-amino groups of Lys1 remained B2R agonists not appreciably cleaved by ACE. Another ACE-resistant B2R agonist has a nonpeptide bond \([\text{Ph}e8\text{(CH}_2)_2\text{NH}]_{\text{Arg9}}\) at the C terminus. Even with these BK analogs, ACE inhibitors still augmented B2R signaling obviously without preventing enzymatic degradation.

ACE inhibitor enhancement of B2R action involves signaling pathways different from those stimulated by B2R agonists alone. For example, B2R activation by BK is not affected by protein kinase C and phosphatase inhibitors, but they blocked the resensitization of the B2R to BK by an ACE inhibitor. ACE inhibitors also decreased B2R phosphorylation, suggesting the involvement of phosphorylation and dephosphorylation of the B2R in this response. The tyrosine kinase inhibitor genistein also blocked B2R resensitization caused by ACE inhibitors or Ang1–7, and Ang1–9 peptides, indicating that these endogenous peptides are also allosteric enhancers of B2R function (see below).

For ACE inhibitors to augment BK responses, ACE and the B2R must be coexpressed on the plasma membrane, close enough to transmit allosteric effects. For example, ACE inhibitors did not potentiate BK effects in cells lacking ACE or when purified soluble ACE was added to the medium. Furthermore, B2R expression can modulate ACE activity. We used several techniques to show that ACE and B2Rs interact. ACE and the B2R coimmunoprecipitated and confocal microscopy revealed that they were colocalized on the membrane. Using B2Rs tagged at C terminus with yellow fluorescent protein (acceptor) and ACE labeled at the C terminus with donor cyan fluorescent protein, fluorescence resonance energy transfer (FRET) indicated that the fluorophores attached to ACE and B2R were within 10 nm on the membrane.

Most of the ACE molecule exists free in the extracellular space (ratio of extracellular to transmembrane and cytosolic residues ≈25:1), quite different from the human B2R, which has only a short free N-terminal domain (ratio of N-domain to transmembrane and cytosolic residues is ≈1:12). To establish whether the extracellular domains are also close enough for FRET, we labeled B2R with an N-terminal yellow fluorescent protein and ACE with an N-terminal cyan fluorescent protein and detected significant FRET, indicating the extracellular portions are also within 10 nm (Z. Chen, R.D. Minshall, F.T., E.G.E., R.A.S., 2009, data to be published).

The formation of ACE/B2R heterodimers on the membrane should be a bimolecular reaction dependent on reactant concentrations. If ACE is in excess, ACE inhibitors could more effectively enhance the activation of B2R by kinins, whereas if cells express many more B2Rs than ACE, ACE inhibitors would not be effective.

The precise interaction sites and orientation of B2Rs and ACE on the membrane are still unknown, but biochemical and structural features provide clues. Somatic ACE has 2 active sites contained in N and C domains, connected by a bridge section. The short transmembrane anchor is followed by a cytosolic tail (Figure). The cytosolic and transmembrane domains of ACE are not required for interaction and potentiation of B2R responses. However, for ACE to interact with the B2Rs, ACE has to be membrane anchored for proper orientation. A chimeric B2R molecule with ACE fused to its N terminus had ACE activity and was a functional B2R, but ACE inhibitors did not potentiate B2R responses. Finally, with an ACE construct containing the C domain active site but lacking most of the N domain, ACE inhibitor still potentiated B2R responses. This suggests that the extracellular C domain interacts with B2Rs (Figure). This is consistent with the ACE crystal structure and modeling indicating 2 possible orientations for N and C domains, placing the N domain either ≈36 Å or 72 Å from the membrane. Based on the crystal structure of the \( \beta \)-adrenergic
receptor, extracellular domains of the B2R could extend maximally $\sim 20$ Å above the membrane. Supportive evidence indicates that binding of ACE inhibitors alters the conformation of ACE, a necessary movement for allosteric modification of the B2R. For example, ACE inhibitors induce phosphorylation of the C-terminal tail of ACE, thereby activating signal transduction pathways to increase expression of proteins such as COX-2. The 2 active domains of ACE have different specificities and exhibit negative cooperativity; binding of ACE inhibitor to one domain alters the conformation of the second domain, rendering it inaccessible to a second inhibitor. The crystal structures of the C- or N-domain revealed a deep active site cleft closed to exterior by a "lid"; consequently, a conformational change would be required for the access of substrate or inhibitor to the enzyme. Modeling of the ACE structure indicates intrinsic flexibility around the active site, suggesting a hinge mechanism to open it for substrate/inhibitor binding. Thus, inhibitor binding to ACE does change its conformation, which can be transmitted to the B2R owing to their close association on the membrane, resulting in allosteric enhancement of B2R signaling (Figure).

ACE Inhibitors and Kinin B1Rs

ACE inhibitors affect the 2 kinin receptors differently, The human B1R is directly activated by ACE inhibitors, even in absence of ACE expression (Figure), ACE inhibitors activate B1Rs to release NO, most consistently in cultured human endothelial cells via inducible NO synthase (iNOS), in the same range as the B1R ligands des-Arg10-kallidin or des-Arg9-BK (10$^{-10}$ to 10$^{-8}$ mol/L). Although des-Arg10-kallidin has higher affinity for the B1R than des-Arg9-BK, the 2 are about equally active on human B1Rs owing to their efficacies. Enalaprilat, quinaprilat, ramiprilat, and captopril are active B1R agonists; lisinopril is not at the same concentrations, probably because of its positively charged $\text{e}^{-}\text{NH}_2$. In ACE, the canonical pentamer sequence (HEXXXH) containing zinc-binding residues in the N- and C-domain active sites are important for inhibitor binding. In metallopeptidases, all 3 zinc-binding residues (2 His and 1 Glu) do not have to be sequential provided they are sterically close (within $\approx 3$ Å) in the 3D structure. The second extracellular loop of the human B1R has the same consensus sequence (HEAWH; residues 195 to 199) required for ACE inhibitor (but not peptide ligands) to activate B1Rs (Figure). Thus, allosteric ACE inhibitor activity is blocked by agents or mutations not affecting orthosteric des-Arg-kinin activity. For example, a synthetic undecapeptide containing the pentamer (LLPHEAWHFAR; residues 192 to 202), blocked B1R activation by enalaprilat but not by des-Arg-kinin. Mutation of H195 to Ala in human B1R did not affect peptide agonist action, but the effect of enalaprilat was much reduced (Ignjatovic et al., F.T., E.G.E., R.A.S., 2009, data to be published).

B1R activation can increase inflammation, pain, and fibrosis in diabetic cardiomyopathy, but it is also beneficial after myocardial infarction in rats or mice. Increased NO synthesis, owing to B1R activation, may also contribute to the therapeutic effects of ACE inhibitors after MI and protect cardiomyocytes. NO release, after ACE inhibitor activation of B1R, inhibited protein kinase $\text{Ce}^{-23}$ that can benefit the failing heart. B1R signaling was recently reported to prevent homing of encephalitogenic T-lymphocytes into the CNS, which was enhanced in B1R$^{-/-}$ mice. CPM, closely associated with myelin centrally and peripherally, should contribute by generating B1R ligands. The report mentioned that ACE inhibitor also suppresses inflammation in the CNS.

More Considerations About B2 and B1Rs

Without carboxypeptidases, endogenous orthosteric B1R ligands could not be generated and B1R signaling would not occur. CPM and B1Rs interact on the cell membrane and based on the crystal structure and modeling of CPM, its active site would be properly oriented along the membrane to deliver agonist effectively to B1R. In bovine or human endothelial cells, B2R agonists cause B1R-dependent release of calcium or generation of NO, which also depended on CPM. Activation of B1 and B2Rs can promote inflammation or intensify pain, but can also improve the functions of the failing heart or kidney. Plasma prekallikrein-mediated stimulation of B2Rs, independent of kinin release, 30% after prekallikrein activation. Plasma prekallikrein may also be allosterically activated by prolylcarboxypeptidase or heat shock protein 90. This could result from induction of a conformational change in prekallikrein, exposing it to another protease or to trace autacolytic activity, yielding activated prekallikrein.

Endogenous B2R Enhancers

Endogenous peptides, such as Ang derivatives Ang1–7 and Ang1–9, can also augment orthosteric BK effects on B2Rs. Ang1–9 is released from Ang I by a carboxypeptidase or by cathepsin A (deamidase). Ang1–9, a relatively stable intermediate, is also liberated by human heart tissue. Ang1–7 is cleaved from Ang I by human nephrilysin and from Ang II by ACE2 and prolylcarboxypeptidase. Ang1–7 counteracts Ang II actions for example by improving baroreceptor reflex and decreasing vascular and smooth muscle growth. Ang1–7 activates the Mas receptor and also potentiates BK effects in vivo. Both Ang1–9 and Ang1–7 can inhibit ACE, but they augment BK effects on B2Rs at orders of magnitude lower concentrations in cultured cells than their IC50 values. Thus, Ang1–7 and Ang1–9 could antagonize Ang II effects in vivo, also as allosteric enhancers of the B2R.

Perspectives

We did not, and could not, aim to complete the history of ACE inhibitors and leave no major questions unanswered, but have sought to summarize some modes of actions that may contribute to the efficacy of these drugs. The complexities...
make it difficult to interpret their effects as attributable only to a single mediator. ACE cleaves other active peptides besides Ang I and BK and ACE inhibitors enhance responses of kinin receptors beyond blocking kinin catabolism. Exogenous ACE inhibitors and endogenous Ang1–7 and Ang1–9 peptides are indirect allosteric enhancers of B2R activation by the orthosteric peptide ligands. They augment collateral efficacy by inducing conformation changes via ACE and B2R complexes on cell plasma membranes. This leads to enhanced release of mediators such as NO, endothelium-derived hyperpolarizing factor, prosstaclynidins, ACE inhibitors are also direct activators of B1Rs at an allosteric site that differs from the orthosteric site of peptide ligands. The consequence is a prolonged high-output NO production by iNOS in human endothelial cells. Finally, ACE inhibitors can potentiate direct actions of kalirein on the B2R in the absence of kinin release.

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**Disclosures**
None.

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