New fACEs to the Renin-Angiotensin System

Inhibition of the angiotensin-converting enzyme (ACE) protects against the progression of several cardiovascular diseases. Recent evidence suggests that some of the beneficial effects of ACE inhibitors can be attributed to the activation of a distinct ACE signaling cascade rather than to the changes in angiotensin II and bradykinin levels. Moreover, at least one other ACE homolog (ACE2) plays a significant role in the regulation of heart and kidney function.

Angiotensin-converting enzyme

Two distinct forms of angiotensin-converting enzyme (ACE) are expressed in humans and are generated from the same gene by alternative transcription initiation sites. The somatic form of ACE is particularly abundant on endothelial cells, whereas a smaller isoenzyme is found exclusively in testis. Somatic and testis ACE exist at the cell surface as ectoenzymes as well as in soluble form derived from the membrane-bound proteins through the action of the ACE secretase (for review, see Ref. 10). All of the ACE enzymes hydrolyze circulating peptides and catalyze the extracellular conversion of the decapeptide angiotensin I to the octapeptide angiotensin II, which is a potent vasopressor (24). ACE also inactivates the vasodilator peptides bradykinin and kallidin, which are derived from kininogen by the action of kallikrein (32). Inhibition of ACE is expected to prevent the formation of angiotensin II and to potentiate the hypotensive response to bradykinin, which would lead to the lowering of blood pressure.

Somatic ACE also plays a role in vascular remodeling, effects best highlighted by the fact that the in vivo gene transfer of ACE into the uninjured rat carotid artery results in the development of vascular hypertrophy independent of systemic factors and hemodynamic effects (21).

Selective overexpression of ACE in the heart also results in morphological changes in the atria, arrhythmia, and sudden death (31). The ACE substrate angiotensin I in such models most probably comes from the plasma, although there is evidence indicating that angiotensinogen, renin, and ACE exist in the heart, implying that some tissues may possess the enzymatic machinery required to generate angiotensin II locally (1, 23). Antisense oligonucleotides against ACE, on the other hand, are reported to prevent neointimal formation after balloon angioplasty (22), and ACE inhibitors decrease vascular hypertrophy in hypertensive animals (4).

The evidence for an ACE signaling pathway

ACE inhibitors, such as ramiprilat, exert beneficial effects on endothelial function and vascular remodeling (19, 25) as well as protect against the progression of atherosclerosis and the occurrence of cardiovascular events in humans (11) (FIGURE 1). Although the beneficial effects of ACE inhibitors are generally attributed to a decrease in the ACE-mediated generation of angiotensin II and the accumulation of bradykinin (30), a number of the effects of this class of compounds cannot be accounted for by inhibition of the enzyme per se. For example, a cross-talk has been proposed to exist between ACE and the bradykinin B2 receptor (2, 20), because ACE inhibitors attenuate and/or partially reverse the bradykinin-induced translocation of the B2 receptor to caveolin-rich membrane sites (a phenomenon that is associated with receptor sequestration and desensitization) and reactivate receptor signaling (increase in intracellular Ca2+ as well as the activa-
tion of extracellular signal-regulated kinases 1/2 in cells desensitized to bradykinin by prior stimulation with high concentrations of the agonist (2). For a cross-talk to exist between ACE and the B₂ receptor, or other signaling cascades, it was reasoned that the binding of an ACE inhibitor to ACE should be able to elicit an intracellular event and that ACE should be capable of outside-in signaling. Moreover, the cytoplasmic tail of ACE should be able to bind soluble intracellular signal molecules and/or adaptor proteins, which initiate a chain of events ultimately linking to effects such as the reactivation of the B₂ kinin receptor. In all of the species studied to date, the short cytoplasmic tail of ACE contains between three and five serine residues (FIGURE 2), one of which (Ser₁²⁷₀ of the human sequence) is located in a highly conserved 13-amino-acid sequence at the extreme COOH-terminal end of the protein. The serine residue in question is phosphorylated by the casein kinase 2 (CK₂), which also physically interacts with the protein (14). The basal phosphorylation of ACE by CK₂ stabilizes its localization in the plasma membrane, and either the mutation of this site or the inhibition of CK₂ both enhance the cleavage/secretion of the enzyme (14).

CK₂ is not the only protein that associates with the cytoplasmic tail of ACE in endothelial cells. Using ACE immunoprecipitated from ACE-overexpressing cells as well as an affinity column composed of a peptide corresponding to the cytoplasmic tail of ACE, a mitogen-activated protein kinase kinase 7 and the c-Jun NH₂-terminal kinase (JNK) were also found to associate with the intracellular domain of the enzyme (FIGURE 3). Moreover, the binding of an ACE inhibitor (ramiprilat or perindoprilat) to ACE enhances the CK₂-dependent phosphorylation of Ser₁²⁷₀ as well as the activity of ACE-associated JNK. Although the binding of the ACE substrate angiotensin I does not increase the activity of ACE-associated JNK, the binding of bradykinin does increase kinase activity, albeit to a lesser extent than an ACE inhibitor.

**FIGURE 2.**

<table>
<thead>
<tr>
<th>Amino acid sequence of the cytoplasmic tail of mature ACE from different species</th>
<th>Homo sapiens</th>
<th>RRLFSRHRSLRHRSHPFQGSEVELRHS 1277</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan troglodytes</td>
<td>RRLFSRHRSLRHRSHPFQGSEVELRHS 1277</td>
<td></td>
</tr>
<tr>
<td>Oryctolagus cuniculus</td>
<td>RLFSPYQSL-RPHHGFQGSEVELRHS 1278</td>
<td></td>
</tr>
<tr>
<td>Bos taurus</td>
<td>RLFSPRHSLRGPRHGFQGSEVELRHS 1277</td>
<td></td>
</tr>
<tr>
<td>Rattus norvegicus</td>
<td>RLYNNHHSVLRHRHPFQGSEVELRHS 1278</td>
<td></td>
</tr>
<tr>
<td>Mus musculus</td>
<td>RLYNNHHSVLRHRHPFQGSEVELRHS 1278</td>
<td></td>
</tr>
</tbody>
</table>

**ACE2**

A zinc metalloproteinase that shares ~42% identity with the catalytic site of somatic ACE is highly expressed in vascular endothelial cells of the heart, kidney, and testis and can be shed from cells by cleavage NH₂-terminal to the transmembrane domain (8, 28). The ACE-related carboxypeptidase or...
ACE2 hydrolyzes a spectrum of peptides, in particular angiotensin II to angiotensin-(1—7) and angiotensin I to angiotensin-(1—9) (FIGURE 4), but the catalytic efficiency of ACE2 is 400-fold higher with angiotensin II as a substrate than with angiotensin I ($K_{cat}/K_m = 1.8 \times 10^6$ vs. $4.9 \times 10^3$ M$^{-1}$s$^{-1}$) (29). The enzyme can also cleave des-Arg$^9$-bradykinin but does not hydrolyze bradykinin and is insensitive to ACE inhibitors (8, 28, 29).

The fact that ACE2 metabolizes angiotensin II to give the vasodilator angiotensin-(1—7) has been interpreted to mean that ACE2 provides a counterbalance, preventing the deleterious consequences of the overactivity of the classical renin-angiotensin system (7). Indeed, the improvement in ventricular contractility observed after coronary artery ligation in rats treated with an angiotensin II type-1 receptor blocker was associated with a significant increase in ACE 2 mRNA expression (12). Modifying the expression of ACE2 has marked consequences on the heart, findings that fit well with the report that an ACE2 homolog (ACER) in Drosophila is required for normal heart development (6).

However, the exact relationship between ACE2 levels and cardiac function remains to be clarified, because the cardiac-specific overexpression of ACE2 was associated with severe, progressive conduction and rhythm disturbances, ventricular tachycardia, and sudden death (9), whereas aging ACE2 knockout mice develop a significant defect in both the speed and the overall percentage of contraction (6). ACE2 levels are also altered in diabetes, but again the situation is rather confusing and the expression of ACE2 seems to alter during the progression of the disease. For example, ACE2 expression is reported to be increased in young mice with type 2 diabetes and was thought to be renoprotective (33), whereas in a rat model of streptozocin-induced diabetes the tubule ACE2 protein levels were reduced compared with the control group, an effect that was prevented by treatment with an ACE inhibitor (27).

Summary

It seems that just when we thought that we knew all there was to know about the renin-angiotensin system, we find that we don’t even know half the story. The majority of the beneficial effects of ACE inhibitors have been attributed to the inhibition of angiotensin II production, but it is probable that the ACE-inhibitor-induced signaling process recently described contributes to the clinical outcome. Currently the best “agonists” of the ACE signaling cascade seem to be ACE inhibitors rather than known ACE substrates, and it will be interesting to determine whether a better endogenous agonist for this signaling pathway exists and whether changes in agonist concentration correlate with the extent of cardiovascular disease.

ACE2 remains a relatively unknown but potentially cardioprotective enzyme, and although current research has concentrated on assess-
ing changes in ACE2 expression and the consequences on angiotensin metabolism, ACE2 may possess other properties. For example, ACE2 was, intriguingly, recently identified as a receptor for the coronavirus that induces the severe acute respiratory syndrome (18). ACE2, like ACE, consists of an extracellular domain containing a single active site, a transmembrane domain, and a short cytoplasmic tail that contains a consensus sequence for phosphorylation by CK2 as well as for tyrosine kinases (28). Future work must clarify whether ACE2 also exhibits properties consistent with a role in endothelial cell signaling and the consequences of such a signaling cascade on cardiovascular homeostasis.

Work performed in our laboratory is supported by the Deutsche Forschungsgemeinschaft (FL 364/1-2), and we belong to the European Vascular Genomics Network, a Network of Excellence supported by the European Community’s sixth Framework Programme (contract no. LSHM-CT-2003-503254).

References


