Angiotensin-Converting Enzyme Is Involved in Outside-In Signaling in Endothelial Cells

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Abstract—Not all of the cardiovascular effects of angiotensin-converting enzyme (ACE) inhibitors can be attributed to changes in angiotensin II and bradykinin levels. Because the cytoplasmic tail of ACE is phosphorylated, we determined whether ACE inhibitors affect the phosphorylation of ACE and whether ACE possesses the characteristics of a signal transduction molecule. The ACE inhibitors ramiprilat and perindoprilat, and the substrate bradykinin (but not angiotensin I), enhanced the activity of ACE-associated CK2 and the phosphorylation of ACE Ser1270 in cultured endothelial cells. Mitogen-activated protein kinase kinase 7 and c-Jun N-terminal kinase (JNK) coprecipitated with ACE, and stimulation of endothelial cells with ACE inhibitors increased the activity of ACE-associated JNK and elicited the accumulation of phosphorylated c-Jun in the nucleus. Ramiprilat was however unable to activate JNK or to stimulate the nuclear accumulation of c-Jun in endothelial cells expressing a S1270A ACE mutant or in ACE-deficient cells. Because the ACE inhibitor–induced increase in ACE expression has been linked to the formation of c-Jun homodimers, we investigated whether ACE signaling via JNK contributes to this response in vitro and in vivo. Prolonged ramiprilat treatment increased ACE expression in primary cultures of human endothelial cells and in vivo (mouse lung), a response that was prevented by pretreatment with the JNK inhibitor SP600125. Thus, ACE is involved in outside-in signaling in endothelial cells and “ACE signaling” may be an important cellular mechanism contributing to the beneficial effects of ACE inhibitors. (Circ Res. 2004;94:60-67.)

Key Words: angiotensin-converting enzyme ■ c-Jun N-terminal kinase ■ CK2 ■ bradykinin ■ angiotensin I

The angiotensin-converting enzyme (ACE) is an ectoenzyme that catalyzes the conversion of angiotensin I to the vasoconstrictor angiotensin II as well as the degradation of the potent vasodilator bradykinin (see review1). Two distinct forms of ACE are expressed in humans, a somatic form that is particularly abundant on the endothelial surface of the lungs, and a smaller isoenzyme that is found exclusively in testis. The activity of somatic ACE is thought to play a crucial role in blood pressure regulation and in processes involved 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. The inhibition of ACE activity is reported to improve endothelial function2 and to stimulate vascular remodeling,3 as well as attenuate the progression of arteriosclerosis4 and the occurrence of cardiovascular events in humans.4–6 The deleterious effects of ACE on the cardiovascular system were initially thought to be a consequence of the formation of angiotensin II, which initiates a cascade of events involving increased free radical production and vascular smooth muscle cell proliferation. However, as bradykinin is much more readily hydrolyzed by ACE than angiotensin I,1,7 the hydrolysis of bradykinin may also contribute to this phenomenon. Indeed, the cardioprotective effects of the ACE inhibitor ramipril are diminished in mice lacking the B 2 kinin receptor gene.8 Over the last 10 years however, there have been a number of reports showing that ACE inhibitors can amplify responses to bradykinin in continuously perfused systems in which the accumulation of the peptide cannot be assumed to occur fast enough to account for the effects observed.9,10 Moreover, ACE inhibitors also potentiate the effects of ACE-resistant analogues of bradykinin,9,11 prevent the sequestration of the B 2 receptor via caveolae,10 and reactivate the desensitized B 2 kinin receptor.10,12 As ACE inhibitors do not directly bind to the B 2 receptor,13 we and others have proposed that some form of cross-talk may occur between ACE and the B 2 kinin receptor.10,13 Because we recently demonstrated that the cytoplasmic tail of ACE is phosphorylated in endothelial cells,14 the aim of the present study was to determine whether ACE is involved in endothelial cell signaling and whether or not the binding of either an ACE inhibitor or an ACE substrate to the enzyme can affect such signaling processes. For such an outside-in signaling to

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take place, we also hypothesized that the cytoplasmic tail of ACE should be able to bind to signal molecules and/or adaptor proteins that initiate a chain of discrete signaling events.

Materials and Methods

Cell Culture

Human umbilical vein endothelial cells or porcine aortic endothelial cells were isolated and cultured as described.13 The human somatic ACE cDNA used was provided by François Alhenc-Gelas (Hôpital Lariboisière, Paris, France) and the point mutants were generated using a PCR-based mutagenesis kit (Stratagene). The mutation of Ser1253, Ser1263, and Ser1270 to alanine was verified by sequencing. Porcine aortic endothelial cells were stably transfected with ACE, S1253A, S1263A, or S1270A using electroporation as described.14

Immunoprecipitation and Immunoblotting

Cells or lung homogenates were lysed in Nonidet lysis buffer, left on ice for 10 minutes, and centrifuged at 10,000g for 10 minutes. Either ACE or JNK was immunoprecipitated from whole cell lysates with a mixture of protein A/G Sepharose or anti-mouse IgG-covered Dynabeads M-450 (DYNAL) as described.17 Proteins in the immunoprecipitate were heated with SDS-PAGE sample buffer and separated by SDS-PAGE. Immunoprecipitated proteins were detected using their respective antibodies. In some experiments, endothelial cells were labeled with 32P for 12 hours, ACE was then immunoprecipitated, and phosphorylation determined by autoradiography as described.14

In Vitro Kinase Assays

ACE-associated CK2 activity was determined using ACE immunoprecipitated from endothelial cells as described.14 To assess JNK activity, JNK was immunoprecipitated as described above and in vitro kinase activity measured using 2 μg GST-c-Jun as substrate, as described.17 In some experiments, JNK activity was measured in ACE immunoprecipitates. The reactions were stopped and the products were resolved by SDS-PAGE (12%). The incorporation of 32P was visualized by autoradiography and quantified by scanning densitometry.

Immunofluorescence

Endothelial cells were grown on glass coverslips and stimulated as described. After fixation with formaldehyde (2% in phosphate buffered saline) and washing with glycerin (2% in PBS) and PBS, cells were permeabilized with Triton X-100 (0.2% v/v). After overnight incubation with the phospho-Jun antibody, followed by a fluorescein-conjugated anti-rabbit IgG (Dako Diagnostika GmbH) for 1 hour, preparations were mounted with ProLong Antifade kit (Molecular Probes) and viewed using a confocal microscope.

Animals

To study the acute effects of ACE inhibition, male mice (C57 black 6, 6 weeks, Charles River, Sulzfeld, Germany) were anesthetized (isoflurane 1.5%) and ramiprilat (250 μg/mouse dissolved in HEPES-modified Tyrode’s solution) or solvent were administered intraperitoneally (i.p., 100 μL bolus). After 15 minutes, animals were euthanized by a transverse cut through the large abdominal vessels and the lungs perfused rapidly with cold phosphate-buffered saline and snap-frozen.

For prolonged administration, ramiprilat (5 mg/kg per day) was given with the drinking water for 5 days. The JNK inhibitor SP600125 (Tocris, Bristol, UK) was dissolved in polyethylene glycol (PEG400) as described18 and applied daily as subcutaneous injection (30 mg/kg per day, 40% in water, 100 μL) for 7 days (ie, beginning 2 days before ACE inhibitor treatment). Experiments conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23) and were approved by the local government (II25.3-19c20/15-F28/02).

Statistical Analysis

Data are expressed as mean±SEM and statistical evaluation was performed using Student’s t test for unpaired data or one-way analysis of variance (ANOVA) followed by a Bonferroni t test where appropriate. Values of P<0.05 were considered statistically significant.

Results

Effect of Ramiprilat on the Phosphorylation of ACE in Endothelial Cells

To assess the effects of ramiprilat on the phosphorylation of ACE, the enzyme was immunoprecipitated from 32P-labeled primary cultures of human umbilical vein endothelial cells (Figure 1A), which also express the angiotensin AT1 receptor and the B2 kinin receptor, or porcine aortic endothelial cells overexpressing human somatic ACE (data not shown) but lacking both G protein–coupled receptors. In both cell types, the ACE inhibitor (100 nmol/L) transiently increased ACE phosphorylation with a maximal effect (a 1.3±0.1-fold increase in 32P incorporation versus control, n=9, P<0.05) evident after 2 minutes. Phosphorylation then decreased over the next 5 minutes (Figure 1A).

The cytoplasmic tail of ACE contains five potentially phosphorylatable serine residues, three of which (Ser1253, Ser1263, and Ser1270) are within recognition sequences for known protein kinases (PKC, PKA, and CK2, respectively). Mutation of either Ser1253 or Ser1263 to alanine failed to affect the basal or ramiprilat-induced phosphorylation of ACE overexpressed in porcine endothelial cells, whereas the S1270A mutant was not phosphorylated in either the absence or presence of ramiprilat (100 nmol/L, 2 minutes; Figure 1B).

Using an antibody that specifically recognizes phosphorylated ACE Ser1270, we assessed the effects of ramiprilat in ACE-overexpressing porcine endothelial cells. As in the primary endothelial cell cultures, ramiprilat elicited the rapid phosphorylation of ACE on Ser1270; phosphorylation was increased by 2.1±0.1-fold over control values (n=7, P<0.05) 2 minutes after the addition of the ACE inhibitor (Figure 1C). ACE phosphorylation decreased over the next 5 minutes (Figure 1C) and reached control levels within 10 minutes, but a secondary increase in phosphorylation was detected after 12 to 24 hours and remained elevated for at least 48 hours (Figure 1D). The acute (2 minutes) effect of ramiprilat on the phosphorylation of ACE Ser1270 was also concentration-dependent with maximal effects being observed using 30 to 100 nmol/L (Figure 1E).
Effect of Ramiprilat on the Activity of ACE-Associated CK2

In ACE-overexpressing endothelial cells, the CK2 inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB; 100 μmol/L, 8 hours) attenuated the basal phosphorylation of ACE, as previously reported, and prevented the ramiprilat-induced phosphorylation of the enzyme (Figure 2A). The amount of ACE recovered from the DRB-treated endothelial cells was slightly lower than that recovered from solvent-treated cells, an effect that can be attributed to the enhanced cleavage/secretion of dephosphorylated ACE. A second CK2 inhibitor, apigenin (20 μmol/L), also prevented the ramiprilat-induced increase in ACE phosphorylation (data not shown).

As CK2 is associated with and phosphorylates ACE in endothelial cells, we determined whether or not ramiprilat affects the association of the kinase with ACE. Exposure of ACE-overexpressing endothelial cells to ramiprilat (100 nmol/L) did not alter the association of the two proteins (Figure 2B) but did increase the activity of ACE-associated CK2, as determined by an in vitro kinase assay (Figure 2C).

Although the S1270A ACE mutant was not phosphorylated, CK2 was associated with the protein and the addition of ramiprilat resulted in the activation of S1270A-associated CK2 (Figure 2D). Thus, the mutation of the CK2 consensus sequence affects the phosphorylation of ACE but not its association with, or the ACE inhibitor-induced activation of, CK2.

A second ACE inhibitor, perindoprilat (100 nmol/L), also elicited the time-dependent phosphorylation of ACE Ser1270 as well as the activation of ACE-associated CK2 (Figure 3). The time course of these effects was slightly different from those of ramiprilat with ACE phosphorylation and CK2 activity peaking between 5 and 7 minutes and a second peak in phosphorylation being detected after 12 to 24 hours. Neither perindoprilat (Figure 3B) nor ramiprilat (data not shown) increased the activity of CK2 in ACE-deficient cells.

Bradykinin but not Angiotensin I Activates CK2 and Increases ACE Phosphorylation in Endothelial Cells

To determine whether the activation of CK2 and the phosphorylation of ACE could be modulated by ACE substrates/products, we assessed the effects of bradykinin, angiotensin I, and angiotensin II on ACE phosphorylation and CK2 activity. To avoid potential complications arising from the cross-talk between ACE and the kinin and angiotensin receptors, these experiments were performed using ACE-overexpressing cells that lack B2 and AT1 receptors. Bradykinin (100 nmol/L) increased the phosphorylation of ACE (Figure 4A) as well as the activity of ACE-associated CK2 (Figure 4B), both of which peaked within 2 to 5 minutes of cell stimulation. Neither angiotensin I (Figure 4C), nor angiotensin II (data not shown), effected the phosphorylation of ACE Ser1270.

Activation of JNK and Phosphorylation of c-Jun by ACE Inhibitors

Analysis of ACE immunoprecipitates revealed that additional proteins of approximately 46 and 55 kDa associated with the cytoplasmic tail of ACE (data not shown). Using immunoprecipitation and Western blot analysis, we identified MAP kinase 7 (MKK7; Figure 5A) and the c-Jun N-terminal kinase (JNK) as ACE-associated kinases (Figure 5B). Identical results were obtained using primary cultures of human umbilical vein endothelial cells and the porcine endothelial cells that overexpress human somatic ACE.

Exposure of ACE-overexpressing endothelial cells to ramiprilat (100 nmol/L, 7 minutes) enhanced the activity of ACE phosphorylation on Ser1270 in porcine endothelial cells stably overexpressing human somatic ACE. Bar graph summarizes results obtained in 12 to 16 different experiments. **P<0.01 vs CTL.

Figure 1. ACE phosphorylation on Ser1270 is enhanced by ramiprilat. A, Autoradiograph and Western blot showing the effect of solvent (CTL) and ramiprilat (100 nmol/L, 2 to 7 minutes) on the phosphorylation of ACE immunoprecipitated from 32P-labeled human umbilical vein endothelial cells. Similar results were obtained in 3 additional experiments. B, Autoradiograph and Western blot showing the effect of solvent (S) and ramiprilat (R, 100 nmol/L, 2 minutes) on the phosphorylation of ACE immunoprecipitated from porcine aortic endothelial cells stably transfected with ACE S1253A, S1263A, or S1270A. Identical results were obtained in 3 additional experiments. C, Representative Western blots showing the time course (2 to 7 minutes) of ramiprilat-induced changes in Ser1270 phosphorylation in human endothelial cells assessed with a phosphospecific antibody. D, Prolonged time course (6 to 48 hours) of the effects of ramiprilat (100 nmol/L) on the phosphorylation of ACE Ser1270 in porcine endothelial cells stably overexpressing human somatic ACE. E, Concentration-dependent effect of ramiprilat (1 to 100 nmol/L, 2 minutes) on the phosphorylation of ACE Ser1270 in porcine endothelial cells stably overexpressing human somatic ACE. Bar graph summarizes results obtained in 3 additional experiments. **P<0.01 vs CTL.
ACE-associated JNK, assessed in an in vitro kinase assay using GST-c-Jun as a substrate (Figure 5C). Although JNK associated with the nonphosphorylatable S1270A ACE mutant, ramiprilat was unable to activate the kinase in S1270A overexpressing cells (Figure 5C). A similar effect was observed when the activity of JNK was assessed in total cell lysates; ramiprilat pretreatment increased the activity of JNK immunoprecipitated from ACE-overexpressing cells whereas no such increase was observed in cells expressing the S1270A mutant or in ACE-deficient cells (Figure 5D). Bradykinin, but not angiotensin I, also increased ACE-associated JNK activity (data not shown).

The activation of JNK is normally associated with the nuclear accumulation of phosphorylated c-Jun. Therefore, ACE-deficient cells and cells overexpressing ACE or the S1270A mutant were stimulated with ramiprilat, and the nuclear accumulation of phosphorylated c-Jun was assessed by immunohistochemistry. Ramiprilat (100 nmol/L) stimulated the nuclear accumulation of phosphorylated c-Jun in cells overexpressing ACE but not in cells overexpressing the S1270A mutant or in ACE-deficient cells (Figure 6). In the latter cell types, anisomycin (1 μmol/L) was used as a positive control for the phosphorylation of c-Jun. Nuclear accumulation of phosphorylated c-Jun was also stimulated by perindoprilat (100 nmol/L).

**Effect of ACE Inhibitors on the Expression of ACE in Cultured Endothelial Cells**

ACE inhibitors are reported to increase the expression of ACE in endothelial cells in vitro and in vivo by a mechanism that is independent of either angiotensin or bradykinin. Because the formation of c-Jun homodimers can affect ACE expression, we assessed whether or not the ramiprilat-induced expression of ACE could be attributed to “ACE signaling” via JNK.

In primary cultures of human umbilical vein endothelial cells, ramiprilat induced a time-dependent increase in ACE expression (Figure 7A), which was prevented by pretreating the cells with the JNK inhibitor, SP600125 (5 μmol/L; Figure 7B).

**Figure 2.** Involvement of CK2 in the ramiprilat-induced phosphorylation of ACE. A, Effect of the CK2 inhibitor DRB (100 nmol/L) on the ramiprilat (100 nmol/L)-induced phosphorylation of ACE in porcine aortic endothelial cells overexpressing human somatic ACE. Similar results were obtained in 3 additional experiments. B, Effect of solvent (CTL) and ramiprilat (100 nmol/L) on the association of CK2 with ACE. C and D, Bar graphs showing the results of in vitro CK2 activity assays performed using the ACE immunoprecipitates from porcine endothelial cells overexpressing either ACE (C) or the S1270A mutant (D). Results are the mean ± SEM of data obtained in 5 to 11 separate experiments. *P<0.05 vs CTL.

**Figure 3.** Effect of perindoprilat on the phosphorylation of ACE Ser^{1270} and the activation of ACE-associated CK2 immunoprecipitated from porcine endothelial cells overexpressing human somatic ACE. A, Western blots showing the short time course (2 to 7 minutes) of ACE Ser^{1270} phosphorylation in response to perindoprilat (100 nmol/L). B, Effect of perindoprilat (hatched bars) on the activity of ACE-associated CK2 in ACE-expressing endothelial cells (left) and on global CK2 activity in ACE-deficient (−ACE) endothelial cells (right). Experiments were performed in the absence and presence of DRB (100 μmol/L, filled bars). Results are the mean ± SEM of data obtained in 3 to 7 separate experiments. *P<0.05 vs control (CTL); #P<0.05 vs absence of DRB. C, Prolonged time course (6 to 24 hours) showing the effect of perindoprilat (100 nmol/L) on the phosphorylation of ACE Ser^{1270} in ACE-overexpressing endothelial cells. Similar results were obtained in 8 additional experiments.
Effect of a JNK Inhibitor on the ACE Inhibitor–Induced Increase in ACE Expression In Vivo

To assess the relevance of this signaling pathway in vivo, we assessed the effects of ramiprilat on JNK activity and ACE expression in the mouse lung. The acute administration of ramiprilat (250 μg/mouse, i.p. 15 minutes) resulted in the activation of JNK immunoprecipitated from lung homogenates (Figure 8A) as well as in the activation of ACE-associated JNK (ramiprilat increased ACE-associated JNK activity by 34±9%, n=4; P<0.05). Prolonged treatment of mice with the prodrug ramipril (5 mg/kg per day for 5 days) markedly increased ACE expression in the lung (Figure 8B). Pretreatment of mice with the JNK inhibitor (30 mg/kg per day, i.p. daily for 7 days, ie, beginning 2 days before ramipril) attenuated JNK activity in lung homogenates (activity was 70±7% of that detected in lung homogenates from vehicle-treated mice, n=5; P<0.05) as well as the ramipril-induced increase in ACE expression (Figure 8B).

Discussion

In the present study, we have shown that the binding of an ACE inhibitor to ACE elicits outside-in signaling in endothelial cells. The pathway activated includes CK2, the phosphorylation of ACE Ser1270, the activation of ACE-associated JNK, and the subsequent nuclear accumulation of phosphorylated c-Jun. This signaling pathway ultimately resulted in an increase in ACE expression in vitro and in vivo.

Because we recently demonstrated that Ser1270 is phosphorylated by CK2 in primary cultures of human endothelial cells as well as porcine aortic endothelial cells overexpressing ACE, we hypothesized that this residue may be the start point for an ACE inhibitor and/or ACE substrate-activated signal transduction cascade in endothelial cells. Although the
cross-talk with the B₂ kinin receptor and in particular the
events controlling receptor sequestration²⁰ may represent the
best known effect of ACE signaling, we performed most of
the experiments in endothelial cells that no longer express the
B₂ receptor to avoid confusion with the effects of classical
bradykinin signaling. However, each step in the ACE inhib-
itator-activated signal transduction cascade was confirmed in
primary cultures of human endothelial cells, ie, in cells
expressing normal levels of ACE as well as receptors for
angiotensin II and bradykinin and, where possible, also in
lungs isolated from ramipril-treated mice.

Our finding that two ACE inhibitors (ramiprilat and per-
indoprilat) as well as the preferred ACE substrate (bradyki-
nin) enhance the activity of ACE-associated CK2, increase
the phosphorylation of ACE, and lead to the activation of
JNK as well as the accumulation of phosphorylated c-Jun in
the endothelial cell nucleus provide conclusive evidence for a
role of ACE as a signal transduction molecule. Moreover,
because the effects described were not observed in cells either
lacking ACE or overexpressing S1270A ACE, it appears that
the CK2-mediated phosphorylation of Ser¹²⁷⁰ is a key event in
the activation of JNK, and the transmission of ACE signaling
from the plasma membrane to other intracellular compart-
ments. Our observation that bradykinin but not angiotensin I
was able to activate ACE-signaling may be the consequence
of the difference in Kᵐ values for angiotensin I and bradyki-
nin,²³ or differential binding to the two catalytic sites.²³

Although we have demonstrated that the binding of an
ACE inhibitor to ACE enhanced the activity of ACE-
associated CK2, the phosphorylation of Ser¹²⁷⁰, and the
activation of JNK, the exact steps that link the binding of the
inhibitor to the extracellular domain of ACE to the activation
of the aforementioned kinases remains to be elucidated. CK2
can phosphorylate JNK on Ser⁴⁰⁷ and Thr⁴⁰⁴,²⁴ and there is
circumstantial evidence that indicates that a CK2-JNK path-
way exists in some cell types.²⁵ However, it is unlikely that

Figure 6. Immunohistochemical staining of phosphorylated c-Jun in porcine aortic
endothelial cells overexpressing human somatic ACE, the S1270A ACE mutant
(S1270A) or in ACE-deficient (−ACE) endothelial cells. Cells were incubated with
solvent (CTL), ramiprilat (Rami, 100 nmol/L), or perindoprilat (Perindo, 100 nmol/L)
for up to 30 minutes. In some experiments, anisomycin (1 μmol/L, 10 min-
utes) was used as a positive control for the activation of JNK and phosphoryla-
tion of c-Jun. Bars represent 10 μm, and the results are representative of data
obtained in 2 further experiments.

Figure 7. Effect of ramiprilat on the expression of ACE in primary cultures of human endothelial
cells. A, Time course of the ramiprilat (Rami, 100
nmol/L)-induced increase in ACE expression and
the effect of the JNK inhibitor, SP600125 (5
μmol/L) on ramiprilat-induced ACE expression (B).
Expression of ACE was normalized to the endo-
thelial cell marker protein PECAM-1. Bar graphs
show data obtained in 4 to 9 independent experi-
ments. *P<0.05, **P<0.01, and *** P<0.001 vs
control (CTL).
the ACE inhibitor–induced activation of CK2 directly results in the phosphorylation of JNK because comparison of the responses obtained in expressing wild-type ACE and cells expressing the S1270A ACE mutant indicates that the activation of JNK is a process strictly dependent on the phosphorylation of ACE Ser1270. The activation of CK2 by the ACE inhibitors was, on the other hand, unaffected by the mutation of Ser1270. Because the JNK protein kinases are activated via phosphorylation on threonine and tyrosine residues by MKK4 and MKK7,24 and MKK7 coprecipitated with ACE, it is tempting to suggest that the activation of MKK7 is involved in ACE signaling. Exactly how the binding of an ACE inhibitor is able to enhance the activity of ACE-associated CK2 also remains to be resolved. However, it is conceivable that the ACE inhibitor–induced signaling cascade we have described involves ACE clustering. Indeed, ACE has been recovered as a dimer and even as an oligomer under certain conditions.26,27

At first sight, ACE seems an unlikely candidate for a signal transduction molecule as it is a zinc metallopeptidase and the cytoplasmic domain of the enzyme is short (only 29 amino acids). However, at least two other ectoenzymes, matrix metalloproteinase-1 (MMP-1)28 and ADAM12,29 have recently been associated with a signaling function. MMPs have well documented effects on intracellular signaling as a consequence of the extracellular cleavage of proteins such as big endothelin-1,30 calcitonin gene–related peptide,31 and thrombin.32 However, a direct role as a signal transduction molecule has recently been attributed to MMP-1 in platelets on the basis of the observation that its activation markedly increases tyrosine phosphorylation as well as the targeting of β1 integrin to areas of cell contact.28 Moreover, in addition to liberating active signaling molecules such as EGF from inactive precursor forms in response to a number of stimuli,33,34 members of the ADAM (A Disintegrin and A Metalloprotease) family may also play an active role in signal transduction. Indeed, ADAM12 is capable of initiating intracellular signaling via the binding of the tyrosine kinase Src to specific domains within its short cytoplasmic tail.29

The data presented in this article clearly demonstrate that the binding of an ACE inhibitor to ACE results in the activation of JNK as well as the phosphorylation of c-Jun and its translocation to the nucleus. This signaling pathway is therefore likely to affect the expression of several proteins and in particular the expression of ACE itself. Indeed, the expression of ACE in phorbol ester–stimulated human endothelial cells can be attributed to the binding of an AP-1 complex containing a c-Jun homodimer to the cAMP-responsive element/12-O-tetradecanoylphorbol 13-acetate responsive element sequence of the ACE promoter.25 Although it is tempting to claim that the signaling via ACE that is initiated by the binding of an ACE inhibitor is protective/beneficial to vascular cell function and or the development of cardiovascular disease, this is currently speculation because the end point identified in the present study, ie, an increase in the expression of ACE itself, could also be expected to have deleterious effects by leading to the enhanced generation of angiotensin II. However, it should be noted that the latter phenomenon has been demonstrated in lung tissue and plasma from ACE inhibitor–treated rats21,35 and in serum from patient populations which distinctly benefit from ACE inhibitor therapy.36,37 Additional investigation is therefore required to identify additional components of the ACE signaling cascade that influence endothelial cell function.

Taken together, the results of the present investigation indicate that ACE can be added to the list of nonreceptor cell surface proteins that play a role in endothelial cell signaling. The identification of ACE as a signaling molecule that can be activated by the binding of ACE inhibitors may account for some of the beneficial effects of this class of compounds on the cardiovascular system.

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