The Relevance of Tissue Angiotensin-Converting Enzyme: Manifestations in Mechanistic and Endpoint Data

Victor J. Dzau, MD, Kenneth Bernstein, MD, David Celermajer, MBBS, PhD, Jerome Cohen MD, Björn Dahlöf, MD, PhD, John Deanfield, MD, Javier Diez, MD, PhD, Helmut Drexler, MD, Roberto Ferrari, MD, Wiek van Gilst, PhD, Lennart Hansson, MD, Burkhard Hornig, MD, Ahsan Husain, PhD, Colin Johnston, MD, Harold Lazar, MD, Eva Lonn, MD, Thomas Lüscher, MD, John Mancini, MD, Albert Mimran, MD, Carl Pepine, MD, Ton Rabelink, MD, PhD, Willem Remme, MD, PhD, Luis Ruilope, MD, Marcel Ruzicka, MD, Heribert Schunkert, MD, Karl Swedberg, MD, Thomas Unger, MD, Douglas Vaughan, MD, and Michael Weber, MD

Angiotensin-converting enzyme (ACE) is primarily localized (>90%) in various tissues and organs, most notably on the endothelium but also within parenchyma and inflammatory cells. Tissue ACE is now recognized as a key factor in cardiovascular and renal diseases. Endothelial dysfunction, in response to a number of risk factors or injury such as hypertension, diabetes mellitus, hypercholesteremia, and cigarette smoking, disrupts the balance of vasodilation and vasoconstriction, vascular smooth muscle cell growth, the inflammatory and oxidative state of the vessel wall, and is associated with activation of tissue ACE. Pathologic activation of local ACE can have deleterious effects on the heart, vasculature, and the kidneys. The imbalance resulting from increased local formation of angiotensin II and increased bradykinin degradation favors cardiovascular disease. Indeed, ACE inhibitors effectively reduce high blood pressure and exert cardio- and renoprotective actions. Recent evidence suggests that a principal target of ACE inhibitor action is at the tissue sites. Pharmacokinetic properties of various ACE inhibitors indicate that there are differences in their binding characteristics for tissue ACE. Clinical studies comparing the effects of antihypertensives (especially ACE inhibitors) on endothelial function suggest differences. More comparative experimental and clinical studies should address the significance of these drug differences and their impact on clinical events.

Am J Cardiol 2001;88(suppl):1L–20L

From the Brigham Women's Hospital, Department of Medicine, Boston, Massachusetts, USA (VJD); Emory University School of Medicine, Department of Pathology and Laboratory Medicine, Atlanta, Georgia, USA (KB); University of Sydney, Department of Medicine, c/o Department of Cardiology, Royal Prince Alfred Hospital, Sydney, Australia (DC); Saint Louis University School of Medicine, Department of Internal Medicine, Saint Louis, Missouri, USA (JC); Sahlgrenska University Hospital/Ostra, Department of Medicine, Nilssonsberg, Sweden (BD); Great Ormond Street Hospital for Children, Vascular Physiology Unit, London, United Kingdom (J Deanfield); Universidad de Navarra, Unidad de Fisiopatologia Vascular, Edificio de Ciencias, Calle Iruniarrea, Pampalona, Spain (J Diez); Medizinische Hochschule Hannover, Department of Cardiology, Hannover, Germany (HD, BH); Nuove Cliniche, Department of Clinical and Experimental Medicine, Corso Ferrara, Italy (RF); University Hospital Groningen, Department of Clinical Pharmacology, Groningen, the Netherlands (WVG); University of Uppsala, Clinical Hypertension Research, Uppsala, Sweden (LH); Victor Chang Cardiac Research Institute, Darlinghurst, Sydney, Australia (AH); Baker Institute, Melbourne, Australia (CJ); Boston Medical Center, Department of Cardiothoracic Surgery, Boston, Massachusetts, USA (HL); Hamilton General Hospital–McMaster Clinic, Division of Cardiology, Hamilton, Ontario, Canada (EL); University Hospital Zurich, Division of Cardiology, Zurich, Switzerland (TL); Vancouver General Hospital, Department of Medicine, Vancouver, British Columbia, Canada (JM); Hospital Lapeyronie, Medecine Interne et Hypertension Arterielle, Centre Hospitalier, Universitaire, Montpellier, France (AM); University of Florida Gainesville, Division of Cardiovascular Medicine, Gainesville, Florida, USA (CP); University Hospital Utrecht, Department of Internal Medicine, Utrecht, the Netherlands (TR); Sticares Cardiovascular Research Foundation, Rhoon, The Netherlands (WR); Unidad de Hypertension, Hospital, Madrid, Spain (LR); University of Ottawa Heart Institute, Ottawa, Ontario, Canada (MR); Klinik und Poliklinik fur Innere Medizin II, Franz-Josef-Strauss-Allee, Regensburg, Germany (HS); Sahlgrenska University Hospital/Ostra, Göteberg, Sweden (KS); Christian-Albrechts-University Kiel, Kiel, Germany (TU); Vanderbilt University School of Medicine, Division of Nashville, Tennessee, USA (DV); Brookdale Hospital, Department of Medicine, Brooklyn, New York, USA (MW).

This work was supported by a grant from Parke-Davis (now Pfizer Inc.).

Address for reprints: Victor J. Dzau, MD, Brigham Women's Hospital, 75 Francis Street, Suite 210, Tower 1, Boston, Massachusetts 02115.

INTRODUCTION

Our awareness and appreciation of the role of tissue angiotensin-converting enzyme (ACE) in endothelial function and vascular health has begun to influence the treatment of cardiovascular and renal disorders. The results of experimental and clinical research have provided the rationale for intervening in the underlying pathophysiologic processes associated with activated tissue ACE in conditions such as congestive heart failure, coronary artery disease, hypertension, and nephrosclerosis. Extensive evidence indicates that ACE inhibition favorably affects the heart, the vasculature, and the kidney, the results of which are associated with improved patient outcomes. This consensus report will provide an extensive review of the biology and function of tissue ACE, its role in the pathophysiology of cardiovascular disease, the importance of tissue ACE as a therapeutic target, and evidence from clinical trials for the beneficial effects of tissue-ACE inhibition. The article will also examine the pharmacologic properties of ACE inhibitors and explore the potential clinical effects related to differences in binding for tissue ACE.

TISSUE ANGIOTENSIN-CONVERTING ENZYME: BIOLOGY, FUNCTION, AND PATHOPHYSIOLOGY

The structure of ACE is well known, and the enzyme's predominant localization in tissue, rather than plasma, was established nearly 30 years ago. Despite this knowledge and an abundance of recent experimental data, the role of genetic variability in ACE activity has yet to be fully resolved.

BIOCHEMISTRY AND GENETICS OF ACE

ACE: ACE is a zinc metallopeptidase that catalyzes 1 of the main steps in the renin cascade—the conversion of angiotensin I (Ang I) to angiotensin II (Ang II), a potent vasoconstrictor. ACE is also involved in the inactivation of the vasodilator hormones, bradykinin and substance P.[1] The ACE enzyme exists in 2 forms, a high molecular weight form (170 kDa) found in endothelial, epithelial, and neuronal cells, and a low molecular weight form (90 kDa) found in germinal cells. The 2 forms are encoded by 2 different messenger RNAs corresponding to molecular sizes of 2.0 kilobase (kb) and 4.3 kb. ACE is found in the plasma and in a number of tissues including blood vessels, heart, kidney, brain, and the adrenal gland.[2] Somatic ACE (the form of ACE made by endothelium and other somatic tissues) is a single polypeptide chain that contains 2 homologous protein domains. Each domain is independently catalytic with roughly equivalent affinities for Ang I. ACE is synthesized with an amino terminal signal sequence. This leads to export of both catalytic domains from the cell, but the last carboxyl-terminal portion of the molecule is hydrophobic and anchors the protein within the cell membrane. Thus, ACE is an ectoenzyme with both catalytic domains outside of the cell (Figure 1).

Plasma versus tissue ACE: Biochemical measurements of ACE activity illustrate that ACE is a tissue-based enzyme.[3] Indeed, $<10\%$ of ACE is found circulating in the plasma.[3] The functional importance of tissue-based ACE has been demonstrated in genetically altered mice devoid of tissue ACE but having substantial plasma ACE levels. These mice have demonstrated an inability to activate their renin–angiotensin system and consequently develop marked hypotension.[4] The precise function of plasma ACE is unclear. However, because it represents only a small proportion of the body's total ACE activity, its role is thought to be minimal.

Role of the genetic variations of ACE: The chromosomal locus of the ACE gene has been linked to the variability of ACE activity and arterial hypertension, as well as left ventricular mass (independent of blood pressure) in several rodent breeding experiments.[5,6] In addition, genetic factors may also regulate vasculature ACE expression and production in humans. In 1990, Rigat et al[7] described an insertion/deletion polymorphism of the ACE gene that accounted for 40% of the interindividual variation in serum and cardiac ACE activity.[7–9] ACE levels are highest in individuals who are homozygous for the D allele, lowest in those homozygous for the I allele, and intermediate in I/D heterozygous individuals. Since 1990, the ACE D allele has been associated with a number of disease states for which activation of the renin–angiotensin system has been implicated in playing a role, including acute myocardial infarction (MI) in low-risk patients,[10] left ventricular hypertrophy,[11–13] and progressive diabetic nephropathy.[14] This association has been attributed to increased formation of Ang II in individuals who carry the ACE D allele. These results have not been duplicated by other investigators.[15,16] It has been suggested that in healthy subjects, negative feedback inhibition may neutralize the genetically enhanced expression of singular components in the Ang II synthetic cascade.[17] By contrast, the ACE DD genotype may play a substantial role in the development of left ventricular hypertrophy when the cardiac growth machinery is activated. This hypothesis is il-

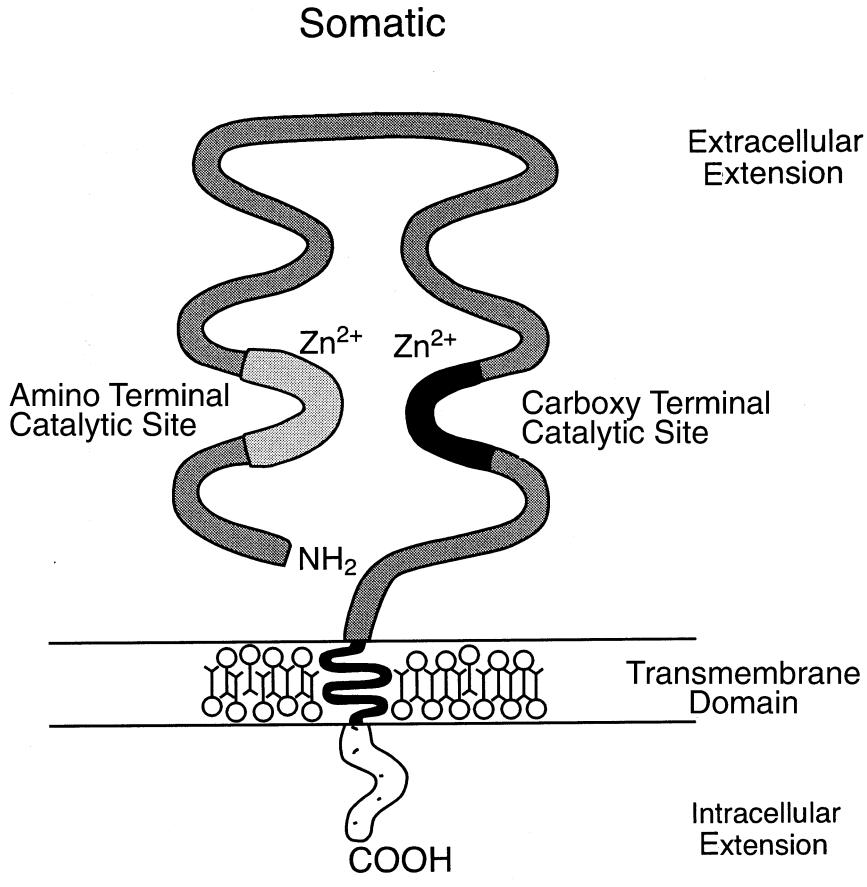

FIGURE 1. Angiotensin-converting enzyme (ACE; EC 3.4.15.1) Schematic drawing shows the structure of ACE. There is a catalytic site on each extracellular lobe, each of which binds a zinc (Zn^{2+}) atom. (Adapted with permission from *Hypertension*).[159]

lustrated by recent data from Montgomery et al[18] in which young healthy subjects were studied before and after a rigorous exercise protocol. Only those participants who carried the ACE deletion allele displayed an increase in left ventricular mass. Thus, the ACE genotype may act only under specific conditions, suggesting an interaction between altered hemodynamics, ACE, or other genetic cofactors in the modulation of left ventricular mass. In agreement with this notion are the observations of Pinto et al[19] and Ohmichi et al[20] who both found that pathologic remodeling early after MI occurs predominantly in those subjects with the ACE DD genotype. Furthermore, transgenic rats with high levels of cardiac ACE expression have normal (or even smaller) hearts, as long as these animals are housed under physiologic conditions. However, cardiac growth and diastolic dysfunction were augmented in the same ACE transgenic rats when the animals were stressed by abdominal aortic banding and subsequent cardiac pressure overload.[21] However, the ACE gene polymorphism has not been consistently associated with hypertension or the prevalence or extent of coronary artery disease or MI.[22,23] Thus, the role of the genetic variability of ACE remains to be fully elucidated.

TISSUE ACE, THE CARDIOVASCULAR SYSTEM, AND THE KIDNEYS

The importance of tissue ACE in the pathophysiology of cardiovascular disease is reflected by findings that, despite the existence of alternative Ang II pathways, marked ACE induction occurs in almost all models of cardiac injury. Within the vasculature, tissue ACE plays a critical role in endothelial function through the direct pleiotropic actions of Ang II and also through a bradykinin-dependent mechanism. There is also substantial evidence that in atherosclerosis, plaque represents an important target of ACE inhibitor action. Finally, the kidneys are especially susceptible to the toxic effects of chronically elevated levels of Ang II; thus, the exuberant response to injury may ultimately lead to renal failure.

Tissue ACE and the heart: TISSUE SITES OF ACE EXPRESSION: ACE activity is distributed in a tissue and cell-type specific fashion.[3] Very high levels are found in the capillary bed of the lungs.[24] Because of its high ACE levels, the pulmonary vasculature—albeit a tissue site—is considered an integral part of the classic circulating renin–angiotensin system.[24] In contrast, some tissues, including the heart, express relatively low levels of ACE, at least under physiologic condi-

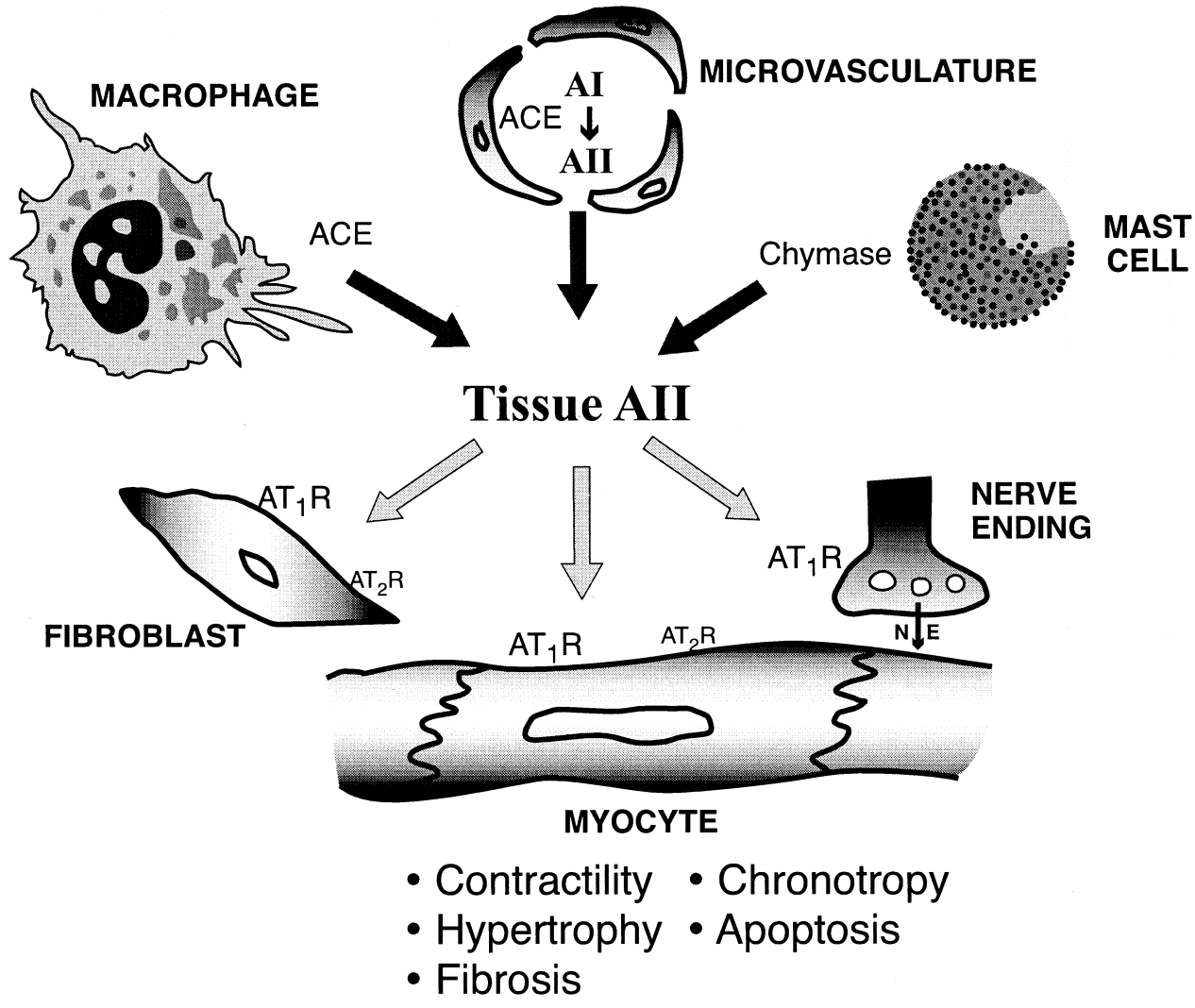

FIGURE 2. Origins and actions of myocardial tissue angiotensin-converting enzyme (ACE)–angiotensin II (Ang II). AT_1R = angiotensin II type 1 receptor; AT_2R = angiotensin II type 2 receptor; NE = norephrine.

tions.[3,25] Within the normal heart, the right atrium elaborates a moderate density of ACE, which is higher than that of the left atrium and the ventricles.[26] The vast majority of immunohistochemical ACE staining is found in the endothelium of large and small cardiac arteries and arterioles, whereas only half the capillaries are immunoreactive, and venous vessels are almost completely devoid of the enzyme.[26,27] Other sites of cardiac tissue ACE expression include the endocardial layer and the cardiac valves.[26] Very little, if any, ACE is found in normal adult cardiac myocytes in situ.

LOCALIZATION AND REGULATION OF ACE IN HEART DISEASE: After our initial observation of ACE upregulation in pressure-overloaded, hypertrophied hearts,[25] marked ACE induction has been found in virtually all models of cardiac injury including volume overload,[28,29] MI,[30,31] and heart failure.[32] Additionally, increased cardiac ACE levels have been correlated with the aging process.[33] Elevated wall stress is believed to be a critical factor for cardiac ACE induction, because elevated enzyme levels were found exclusively in the affected ventricle.[34] Interestingly, ACE upregulation is not restricted to the vasculature,[27] because fibroblasts and myocytes are also recruited for ACE expression in injured hearts.[27,31,32] Likewise, cardiac myocytes in cell culture have been reported to express ACE and are able to generate Ang II locally, especially in response to mechanical stretch.[35] Moreover, macrophages invade injured myocardium and carry high levels of ACE activity to interstitial sites where Ang II, the product of ACE, accumulates.[27,36,37] In addition, mast cells in cardiac tissue are another source of tissue Ang II through the action of chymase.[38] The role of tissue ACE in the heart is summarized in Figure 2.

Whereas cardiac ACE increases in the failing heart, pulmonary ACE tends to decrease when pulmonary congestion complicates the condition.[39] These opposing regulatory steps may protect Ang I from conversion/degradation in the lung and increase ACE substrate in the heart.[39]

KINETICS AND MECHANISMS OF CARDIAC ANG II FORMATION: During a single passage through the coronary system, approximately 3% to 10% of Ang I is con-

verted to Ang II.[25,40] However, these measurements may only reflect vascular conversion. More precise insights on the intracardiac events leading to Ang II generation were revealed by experiments that used intracoronary infusions of minute concentrations of radiolabeled (exogenous) Ang I or Ang II followed by measurements of native (endogenous) as well as labeled angiotensins in the interstitial fluid, the cellular compartment, and the coronary effluent.[8,41] These experiments revealed that angiotensinogen and renin are extracted from the coronary circulation.[42,43] Indeed, cardiac concentrations of renin may substantially exceed renin levels in the plasma, suggesting an active mechanism for cardiac renin accumulation.[42,43] In addition, there appears to be local generation of angiotensinogen and renin, at least during disease conditions.[44,45]

These kinetic studies document that >80% of Ang I found in the cardiac interstitium is formed locally by renin (which is largely taken up from the circulation) cleaving angiotensinogen (which is both locally formed and taken up from the circulation).[40,41] Likewise, most of the Ang II found in the heart is synthesized in situ. Specifically, the conversion of Ang I to Ang II appears to be mediated by tissue ACE rather than blood-derived enzymes.[46] Consequently, the tissue levels of Ang II are several times higher than the circulating levels.[36] It is conceivable, therefore, that the local levels of ACE activity reflect the cardiac Ang II concentrations.

In experimental models and in humans, the cardiac conversion of Ang I to Ang II is largely blocked by ACE inhibitors.[37,46–49] By contrast, in ex vivo membrane preparations of cardiac tissue (human and rat), the conversion of Ang I to Ang II occurs largely independently of ACE.[50,51] Chymase, a mast cell enzyme with high affinity for Ang I, has been shown to catalyze this reaction and chymase inhibitors were effective in its inhibition.[38] This apparent discrepancy between in vivo and in vitro data[51] may have been resolved by the findings of Kokkonen et al,[52] who demonstrated that interstitial fluid completely inhibits chymase activity, whereas ACE remains active under these same conditions. Despite this finding, chymase may still be important, and further investigation is necessary to define its role in the formation of Ang II in humans.

FUNCTIONAL ROLE OF ACE IN THE NORMAL AND FAILING HEART: The normal development of the heart does not require the functional integrity of the cardiac renin–angiotensin system. Thus, genetically altered mice lacking cardiac ACE do not experience cardiac pathology.[53] Furthermore, Ang II is not required for the maintenance of normal cardiac function.[54] In this regard, the role of the cardiac renin–angiotensin system differs from that of the renal renin–angiotensin system, which requires Ang II for normal kidney development.[55]

In the failing heart, however, activation of the renin–angiotensin system may have a series of functional implications. Ang II has been shown to enhance protein synthesis independently of load in the intact heart as well as in the isolated myocyte.[56,57] Ang II, then, is considered to be an important factor contributing to the development of cardiac hypertrophy. ACE appears to be involved in this process because, on the one hand, the activity of the enzyme is enhanced in hypertrophied hearts and on the other hand, inhibition of the enzyme may cause regression of left ventricular hypertrophy, even when the pressure or volume overload persists.[58,59] Even more strikingly, the inhibition of cardiac ACE with a high tissue-affinity ACE inhibitor (quinapril) prevented the development of volume overload hypertrophy more efficiently than an ACE inhibitor (enalapril) with low affinity for tissue ACE.[28,60] Moreover, tissue-ACE activity is involved in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis.[61]

Ang II not only induces hypertrophy of cardiac myocytes but also hyperplasia of cardiac fibroblasts.[62] Accordingly, the activation of the cardiac renin–angiotensin system and specifically, cardiac ACE, may contribute to the development of cardiac fibrosis.[62] It has been demonstrated that fibrotic areas of the heart display the highest levels of cardiac ACE activity[31,32] and that fibroblasts themselves generate Ang II.[63] ACE inhibitors, on the other hand, may prevent the accumulation of extracellular matrix proteins and the development of fibrosis of the heart (Figure 3), even when pressure overload persists.[64,65] The intimate communication between cardiac fibroblasts and myocytes was elegantly demonstrated in chimeric mice that had Ang II receptor type 1A gene null mutant cells and Ang II receptor type 1A gene intact cells expressing the lacZ gene. Proliferating cardiac fibroblasts were present predominantly in areas of Agtr1a intact cardiomyocytes. Therefore, an intact cardiac renin–angiotensin system appears to be a requirement for local proliferation of fibroblasts and the consequent development of fibrosis.[66]

Ang II has also been shown to induce apoptosis of cardiac myocytes, whereas cardiac fibroblasts are fairly resistant to the effects of Ang II on cell death.[67] Specifically, the enhanced local renin–angiotensin system decreases the *bcl-2*-to-BAX protein ratio in cardiomyocytes, thus decreasing the resistance to undergo programmed cell death.[67] There appears to be a vicious circle, given that an apoptosis-related protein, p53, induces the local renin–angiotensin system.[68] In fact, the induction of cardiac ACE parallels the appearance of apoptosis in the pressure-overloaded heart.[69] Again, use of ACE inhibitors has been shown to prevent apoptosis of cardiac myocytes in pressure-overloaded hearts.[69]

The activation of tissue ACE in cardiac remodeling has direct functional consequences. Ang II causes a depression of diastolic function in the hypertrophied heart.[25,47] Likewise, perfusion of isolated hearts with Ang I, followed by intracardiac conversion to Ang II, causes an increase in left ventricular end-diastolic pressure, suggesting that local ACE may facilitate this response.[47] ACE inhibitors infused into the coronary arteries of isolated experimental hearts or hearts of

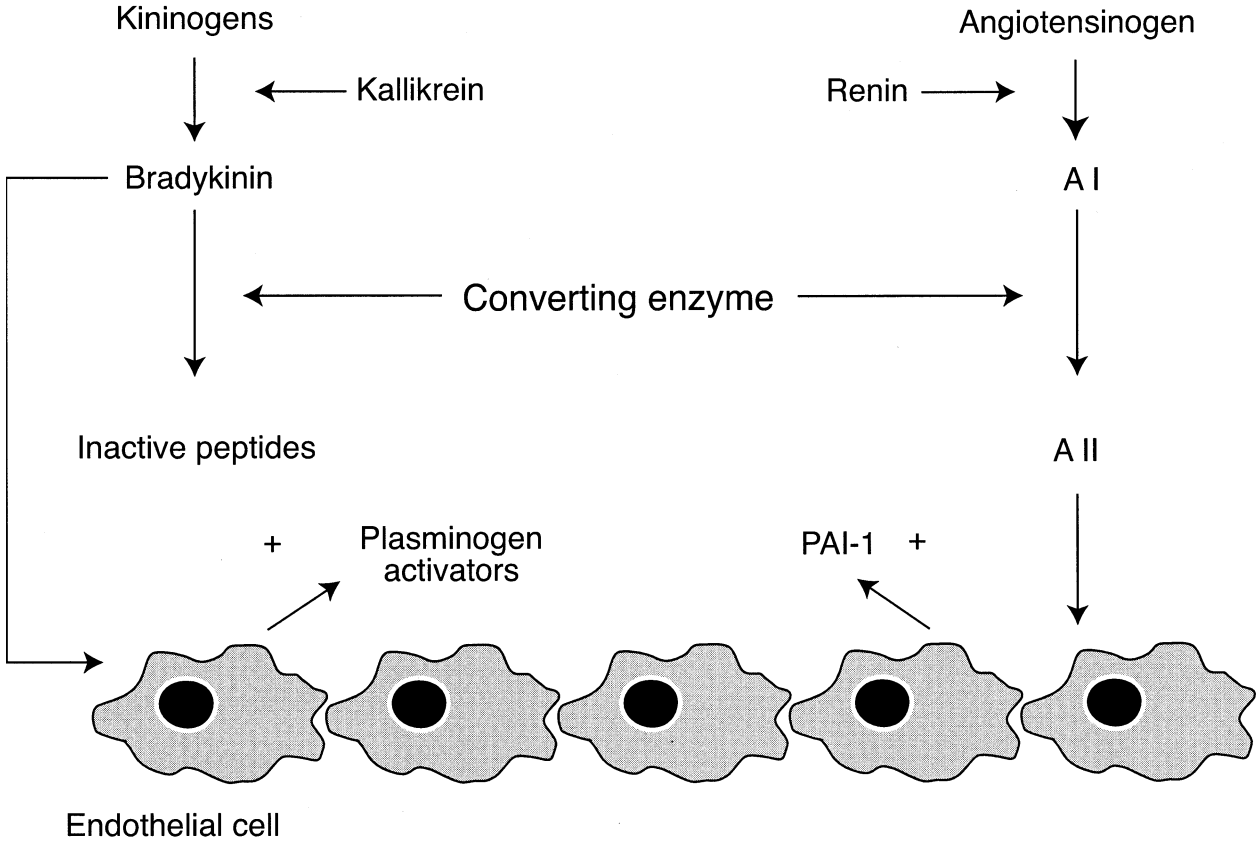

FIGURE 3. Angiotensin-converting enzyme (ACE) inhibition and fibrinolysis. Inhibition of ACE prevents the degradation of bradykinin and the formation of angiotensin II (Ang II), thus preserving fibrinolytic balance. Ang I = angiotensin I; PAI-1 = plasminogen activator inhibitor type 1. (Adapted with permission from *Circulation*.[160])

patients with aortic stenosis caused a significant improvement in diastolic function.[47,70,71] This response is even amplified in hearts exposed to an ischemia/reperfusion injury.[72] Conversely, the effects of Ang II on systolic function in the failing ventricles are minimal.[54]

Local generation of Ang II may also increase the vascular tone of the coronary bed. Specifically, in patients with dilated cardiomyopathy, intracoronary enalaprilat induced a significant coronary vasodilation,[73] and in an animal model of cardiomyopathy, long-term treatment with quinapril resulted in a significant cardioprotective effect.[74] These data provide strong evidence for the functional significance of the cardiac renin–angiotensin system in both patients with heart disease and in controlled experimental situations.

In 2 studies, the effects of ACE inhibition (ramipril or fosinopril) on survival in experimental aortic stenosis were examined.[59,71] This model allows the separation of peripheral and cardiac drug effects, because afterload reduction is prevented by a clip at the ascending aorta. Both studies demonstrated a survival benefit in animals receiving the ACE inhibitor, thus suggesting that the inhibition of cardiac ACE contributes to the prognostic relevance of these agents in patients with heart failure.[75,76]

Role of tissue ACE in the vasculature: REGULATION OF VASCULAR ACE: ACE is the most important enzyme controlling the activation of angiotensin and the degradation of bradykinin.[77] Although ACE is widely distributed through the tissues, it appears that ACE expression is regulated by a number of different mechanisms. In cultured endothelial cells, the expression of ACE is modulated by steroids, calcium ionophores, and growth factors.[78] The expression of ACE in endothelial cells and culture is also a function of confluence, as ACE enzyme levels increase exponentially after confluence is obtained.[79] Thus, the regulation of endothelial ACE is a determinant of vascular function in both health and disease.

Studies of Ang I infusion into human forearm or coronary arteries have shown that Ang I is converted to Ang II. This conversion is blocked by ACE inhibitor treatment. The primary vasodilatory action of ACE inhibitors is the blockade of Ang II formation. The contribution of bradykinin to the action of ACE inhibitors has been debated. With long-term administration, ACE inhibitors lower blood pressure, even in patients with low renin hypertension, suggesting an effect that is independent of a decrease in Ang II. Bradykinin is a potent vasodilator, acting through the release of prostacyclin, nitric oxide, and endothelial-derived hyperpolarization factor. Accurate measure-

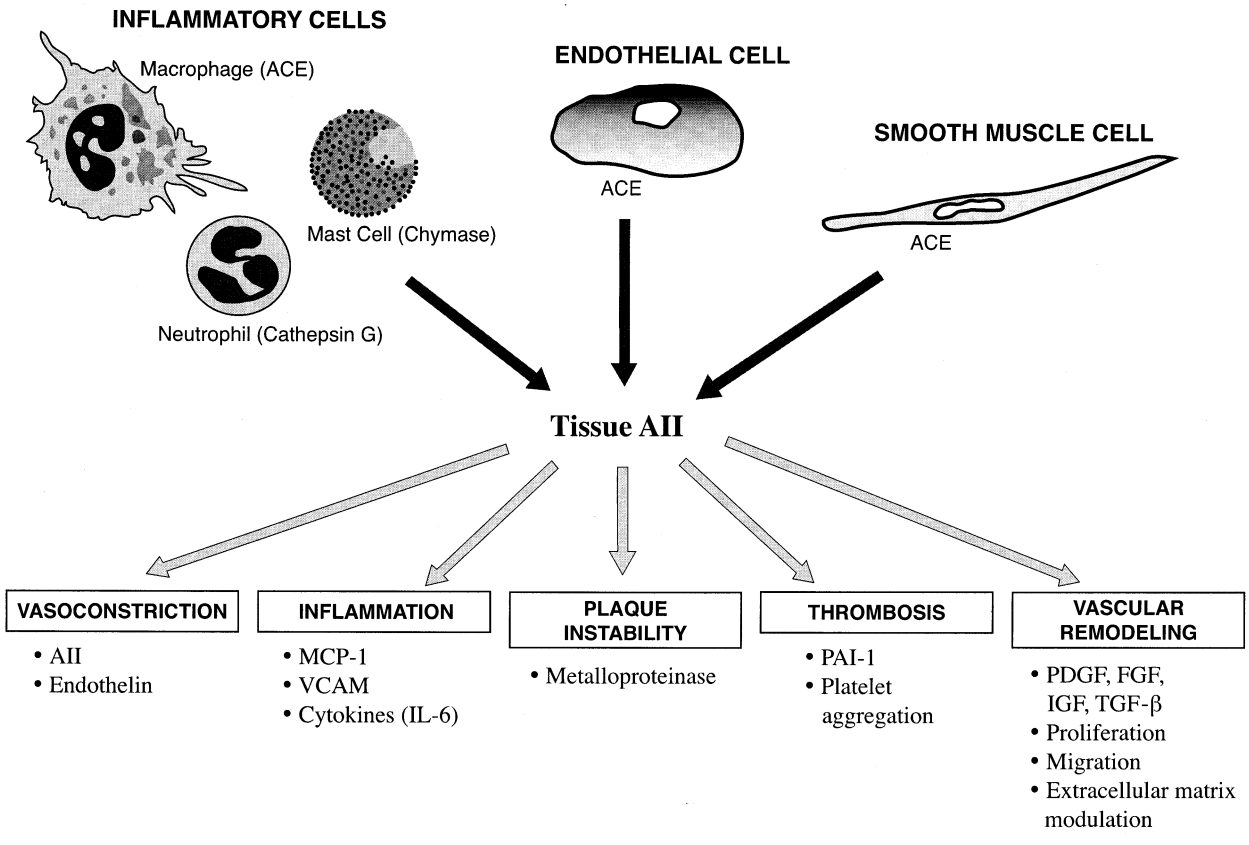

FIGURE 4. Origins and actions of vascular tissue angiotensin-converting enzyme (ACE)-angiotensin II (Ang II). FGF = fibroblast growth factor; IGF = insulinlike growth factor; IL-6 = interleukin-6; MCP-1 = monocyte chemoattractant protein-1; PAI-1 = plasminogen activator inhibitor type 1; PDGF = platelet-derived growth factor; TGF-β = transforming growth factor–β.

ment of bradykinin concentrations in plasma has been technically challenging; these concentrations have been shown to be increased or unchanged after ACE inhibition. Although it is clear that ACE inhibition potentiates the hemodynamic effects of exogenous bradykinin, this observation does not address whether endogenous bradykinin plays a part in the action of ACE inhibitors. Recent studies performed by Gainer et al[80] indicate that the coadministration of the bradykinin receptor antagonist, icatibant acetate (HOE 140), significantly attenuates the hypotensive effect of captopril. Although HOE 140 does not alter the renal hemodynamic response to captopril, it does significantly alter the change of plasma renin activity in response to ACE inhibition. These effects appear to be similar in both normotensive and hypertensive subjects. These data confirm that bradykinin contributes to the short-term effects of ACE inhibition in blood pressure in normotensive and hypotensive persons and suggest that bradykinin also contributes to the short-term effects of ACE inhibition on the renin–angiotensin system. Similar results have been seen in the effects of ACE inhibitors on endothelial vasodilator function. Studies performed by Hornig et al[81] have shown that ACE inhibitors augment flow-dependent, endothelial-mediated dilation in humans by a bradykinin-dependent mechanism.

ACE regulates other important vascular functions (Figure 3). Studies in healthy human volunteers have provided additional support for ACE in regulating vascular fibrinolytic balance. Specifically, examination of the effect of activation of the renin–angiotensin system by low salt intake (10 mEq vs 200 mEq sodium per day) on plasma fibrinolytic parameters demonstrated that low salt intake was associated with a significant increase in morning plasminogen activator inhibitor type 1 (PAI-1) levels, and plasma PAI-1 correlated dramatically with serum aldosterone levels ($R = 0.56$, $p < 0.10^{-7}$). Treatment with quinapril significantly lowered PAI-1 concentrations and the molar ratio of PAI-1 to tissue plasminogen activator throughout the day.[82]

PATHOPHYSIOLOGY OF VASCULAR ACE: The endothelium plays a crucial role in the maintenance of normal vascular tone and structure, local hemostasis, and vascular-wall proliferation processes (Figure 4).[83,84] These processes are mediated by the reactive release of vasoactive substances (thromboxane A_2, free radicals, endothelin, prostacyclin) among which nitric oxide is perhaps the most important. Nitric oxide (1) relaxes vascular smooth muscle through a cyclic guanosine monophosphate–mediated decrease in cytosolic calcium, resulting in vasodilation; (2) mediates coagulation by the inhibition of platelet aggregation and the expression of adhesion molecules for both monocytes and neutrophils; and (3) prevents structural

changes by inhibiting the growth and migration of smooth muscle cells. These regulatory processes are all subject to disruption by Ang II.

Ang II, elaborated by activated endothelial ACE, impairs nitric oxide bioactivity, mainly because of oxidative stress through the Ang II–induced production of superoxide radicals (O_2-) that can scavenge nitric oxide and reduce endothelium-dependent vasodilation.[85] This action is independent of the effects of ACE in degrading bradykinin and modulating the endothelial-dependent vasodilation in response to activation of the β_2-receptor.

There is evidence that ACE expression is increased in atherosclerosis and that Ang II may contribute to disease progression by increasing oxidative stress and attenuating chemoattractant and adhesion molecule expression, leading to inflammation. As discussed, tissue Ang II can also exert proproliferative and prothrombotic actions (Figure 4). Diet et al[86] reported that tissue ACE in human atherosclerotic plaque localizes to regions of inflammatory cells, especially areas of clustered macrophages and microvessel endothelial cells. The accumulation of ACE and metalloproteinase in the shoulder region of the vulnerable plaque may contribute to increased local circumferential stress and plaque instability. Thus, ACE accumulation within the vascular lesions may be a factor in the pathophysiology of coronary artery disease.

This hypothesis is underscored by the findings of an elegant experiment in which endothelial nitric oxide synthetase gene knockout mice developed atherosclerotic lesions in response to adventitial vessel-wall injury.[87] Wild-type mice with normal endothelial function were able to produce nitric oxide and were therefore protected from this effect. The evidence cited above indicates that plaque ACE may be an important target of ACE inhibitor action.

Tissue ACE and the kidney: The prominent role of Ang II in renal physiology, as briefly outlined below, renders the kidneys highly susceptible to injury caused by the de novo production of Ang II. The kidneys, under the regulation of Ang II and aldosterone, maintain the electrolyte balance in the body. Sodium homeostasis, in particular, is maintained by the local action of Ang II on both the proximal and distal tubules. The filtration function of the kidneys is also preserved during changes in systemic blood pressure by local Ang II, which acts to constrict the afferent and efferent glomerular arterioles. The efferent arterioles are very sensitive to Ang II, and the resulting vasoconstriction, together with prostaglandin-induced vasodilation of the afferent arterioles, regulates intraglomerular pressure, thereby maintaining the glomerular filtration rate.

Because Ang II is essential in normal kidney function, increases in the level of locally elaborated Ang II frequently result in pathophysiologic conditions. In renovascular hypertension, for example, filter function of the ischemic kidney is compensated with afferent vasodilation and efferent Ang II–induced vasoconstriction. Renin production is greatly increased as well. This response to injury increases blood pressure, exposing the contralateral kidney to the sequelae of systemic hypertension. Ang II also maintains the glomerular filtration rate in chronic renal failure regardless of the cause of tissue damage. Despite this compensatory response, there is a progressive loss of renal function that results in further Ang II–generated increases in glomerular blood pressure and, therefore, continuing injury to the remaining nephrons.[88] Ang II–induced glomerular hypertrophy[89] and renal fibrosis[90,91] escalate the response to injury into a destructive cycle, which ultimately concludes with complete renal failure.

CLINICAL CONSEQUENCES OF TISSUE ANGIOTENSIN-CONVERTING ENZYME INHIBITION

Based on experimental data, hypertension may be associated with increased local Ang II production, which may play an important role in vasoconstriction and direct tissue pathology. Consequently, antihypertensive therapy with ACE inhibitors not only controls hypertension by interrupting the renin–angiotensin system, but it has the added benefit of reducing the risk associated with Ang II–induced disease processes, including cardiovascular disease and renal failure. Thus, our evolving understanding of the role of tissue ACE in cardiovascular and renal disease culminates with the therapeutic application of this knowledge. In this context, the beneficial consequences of tissue ACE inhibition may occur independently of changes in blood pressure (ie, overt renin–angiotensin system activation); therefore, the value of inhibiting tissue ACE may extend to a broader range of patients than are currently being treated.

TISSUE ACE INHIBITION AND HYPERTENSION, DIABETES, AND RENAL DISEASE

The hallmark of essential hypertension is nephrosclerosis, the first clinical sign of which is protein (chiefly albumin) in the urine. Proteinuria is a principal predictor of cardiovascular disease in patients without diabetes mellitus and with type 2 diabetes,[92] as well as in progressive renal disease in type 1 diabetes, and in patients with overt diabetic nephropathy.[93] Treatment with ACE inhibitors has been shown to consistently reduce proteinuria in these patients, as compared with other antihypertensive agents that appear to have milder effects.[94–96]

The lack of an antiproteinuric effect by other antihypertensive agents that effectively reduce blood pressure suggests that renal protection afforded by ACE inhibitors may occur through a blood pressure–independent mechanism. Support for this hypothesis can be derived from examining large clinical trials and evaluation of the high-risk groups that were treated for hypertension.

Aggressive antihypertensive treatment in patients with type 2 diabetes mellitus was assessed in a subgroup analysis (n = 1,148) of the United Kingdom Prospective Diabetes study in which 758 patients (tight control group, blood pressure <150/85 mm Hg) were randomized to either an ACE inhibitor or a β-blocker (captopril or atenolol, respectively) as the main treatment.[97] A total of 390 patients were treated less aggressively (blood pressure <180/105 mm Hg) with the same antihypertensive drugs. This study demonstrated that aggressively treated patients had clinically important reductions in the risks of death or complications associated with diabetes compared with patients who were treated less aggressively regardless of the antihypertensive agent used. In light of these data, could further evaluation of high-risk patients with modest reductions in blood pressure uncover additional beneficial effects attributable to a specific class of antihypertensive agent?

The Captopril Prevention Project (CAPPP) was designed to compare the effects of ACE inhibition and conventional therapy (diuretics and β blockers) on cardiovascular morbidity and mortality in hypertensive patients.[98] A subgroup of >700 CAPPP patients were at increased risk for cardiovascular complications caused by diabetes mellitus. A total of 337 of these patients were randomized to captopril and 380 to conventional therapy. Although those patients treated with conventional therapy had significantly lower blood pressure than did the patients who received captopril, conventional therapy did not result in any additional benefit for diabetes-related risk. Those patients treated with the ACE inhibitor had a 66% reduction in fatal and nonfatal MIs and a reduced frequency of all cardiac events and total mortality. Moreover, within the entire study population (N = 10,085), the incidence of diabetes was lower in the captopril-treated patients than in those who received conventional therapy (relative risk 0.86, confidence interval 0.74 to 0.99, p = 0.039).

Evidence from the Appropriate Blood Pressure Control in Diabetes (ABCD) trial further supports the advantage of ACE inhibitor therapy in high-risk patients.[99] ABCD was a prospective, randomized, blinded trial comparing the effects of moderate blood-pressure control (80 to 89 mm Hg, target diastolic blood pressure) with intensive control (75 mm Hg, target diastolic blood pressure) on the incidence and progression of diabetic vascular complications in hypertensive patients. First-line antihypertensive therapy with a dihydropyridine calcium antagonist (nisoldipine) or enalapril was also evaluated. A clinically important and highly statistically significant difference in the cardiovascular event rate was observed after 67 months of treatment in the hypertensive cohort. Patients treated with ACE inhibitor therapy had fewer nonfatal MIs (chi-square test: p = 0.001), all MIs (chi-square test: p = 0.001), and overall cardiovascular events (chi-square test: p = 0.002) than patients treated with the calcium antagonist. This relation was maintained in both the moderate and intensive blood-pressure control groups.[99] Because of ethical considerations, there was no placebo control group in this study. Therefore, the difference between the ACE inhibitor group and the calcium antagonist group cannot be definitively ascribed to a beneficial effect of ACE inhibition. It is possible that calcium antagonists exerted a deleterious effect on this study population. Comparisons with other studies,[100–102] however, suggest that the rate of MIs in the calcium antagonist group is not different from these historical controls; therefore, the results of the ABCD trial may be attributed to a protective effect of ACE inhibition rather than to a deleterious effect of calcium antagonists.[99]

The blood pressure–independent renoprotective effects of ACE inhibition have been clearly established in 2 large placebo-controlled clinical trials. The first study was conducted to determine whether captopril has kidney-protecting properties independent of its effect on blood pressure in patients with diabetic nephropathy.[103] All patients had type 1 diabetes mellitus, proteinuria ≥500 mg/day, and serum creatinine concentration ≥2.5 mg/dL. Patients already on conventional antihypertensive therapy were randomized to captopril (n = 207) or placebo (n = 202) and were observed for 4 years. Doubling of baseline serum creatinine concentration—the primary study endpoint—occurred in 43 patients who received placebo and in only 25 ACE inhibitor–treated patients (p = 0.007), representing a risk reduction of 48%. Risk associated with the combined secondary endpoints (death, dialysis, and kidney transplantation) was reduced by 50%, and an aggregate analysis revealed significantly less proteinuria in the captopril-treated patients than in those patients who received placebo (p = 0.001). Over the course of the study, there was no difference in blood pressure in those patients with pre-existing hypertension who were randomized to ACE inhibitor therapy (n = 155) or placebo (n = 153, p = 0.16). Among patients who were normotensive at study entry, blood pressure was only marginally higher in the placebo group (p <0.001). Because blood pressure was not different between the groups with hypertension, and 85% of the patients who reached the primary endpoint were hypertensive, the decreased progression of diabetic nephropathy most likely occurred through a mechanism that is not dependent on blood pressure reduction.

Most recently, treatment with ramipril was found to result in vasculoprotective and renoprotective effects in patients with diabetes who had a previous cardiovascular event and at least 1 other cardiovascular risk factor.[104] A total of 3,577 patients were randomized to ramipril (10 mg/day) or placebo, and vitamin E or placebo (2×2 factorial design). Treatment with ramipril reduced the risk of overt nephropathy by

24% (95% confidence interval 3 to 40, p = 0.027), and that of the combined primary outcome measure (MI, stroke, or cardiovascular death), even after adjusting for changes in both systolic and diastolic blood pressure, by 25% (confidence interval 12 to 36, p = 0.004).

These results extend to patients whose renal insufficiency stems from causes other than diabetic nephropathy. The role of ACE inhibition in the preservation of renal function in patients with mild-to-moderate renal insufficiency because of diverse causes (eg, nephrosclerosis, glomerular disease, diabetic nephropathy) was evaluated using benazepril, an ACE inhibitor with high tissue-ACE affinity.[105] A total of 583 patients were randomized to ACE inhibitor therapy (n = 300) or placebo (n = 283). Renal insufficiency was classified according to baseline creatinine clearance as either mild or moderate (46 to 60 or 30 to 45 mL/min). The primary study endpoint was a doubling of the baseline creatinine concentration or the need for dialysis. At 3 years, the primary endpoint was reached by 57 patients who received placebo and by 31 benazepril-treated patients ($p < 0.001$), yielding an extraordinary overall risk reduction of >50%. Patients with mild or moderate renal insufficiency had risk reductions of 71% and 46%, respectively. ACE inhibition most effectively slowed the progressive deterioration of renal function in patients with glomerular diseases; however (and not unexpectedly), patients with polycystic disease (who also do not respond to low-protein diets) benefited the least.

Statistical adjustment for changes in blood pressure among the benazepril-treated patients and those who received placebo revealed that the risk reduction could not be completely attributed to the antihypertensive action of the ACE inhibitor. Additionally, the renoprotective effect of benazepril, as reflected by reduced urinary-protein excretion, was also found to occur independently of changes in blood pressure.

CLINICAL ASPECTS OF TISSUE ACE AND ITS RELEVANCE TO CORONARY ARTERY DISEASE

ACE inhibitors as first-line therapy in patients with heart failure, asymptomatic left ventricular dysfunction, and in post-MI patients with a low ejection fraction: More than 2 decades of experience have demonstrated that ACE inhibitors save lives and decrease the number of hospitalizations in patients with heart failure, asymptomatic left ventricular dysfunction, and those post-MI patients with a low left ventricular ejection fraction (Table 1).[106–108] Consequently, ACE inhibitors are now considered first-line therapy for these patients.[109] Benefits have been observed with different ACE inhibitors, including captopril, enalapril, zofenopril, ramipril, and trandolapril, thus suggesting a class effect.

The Cooperative Northern Scandinavian Enalapril Survival Study (CONSENSUS) demonstrated a significant 40% reduction in 6-month mortality in enalapril-treated patients with severe heart failure versus those patients who received placebo.[75] Enalapril was also found to reduce mortality in patients with less-severe congestive heart failure. In the treatment arm of the Studies On Left Ventricular Dysfunction (SOLVD), enalapril significantly reduced overall mortality by 16% versus placebo in patients with a left ventricular ejection fraction of <0.35 and New York Heart Association (NYHA) functional class II and III.[76] Whereas no mortality benefit was demonstrated in the prevention arm of the SOLVD trials, which enrolled asymptomatic patients with a left ventricular ejection fraction <0.35, there was a significant reduction in hospitalizations for heart failure. The benefits of ACE inhibitor therapy in heart failure are also substantiated by a systematic overview of randomized trials of ACE inhibitors in patients with heart failure.[110] This meta-analysis of 32 trials, including 3,870 patients with symptomatic heart failure randomized to ACE inhibitor therapy and 3,235 control patients, reveals a 23% reduction in total mortality and a 35% reduction in congestive heart failure in the ACE inhibitor group. Similar benefits were noted in this meta-analysis across various subgroups defined by age, sex, etiology of heart failure, and NYHA class.

Trials in patients with recent MI and moderate reductions in the left ventricular ejection fraction including the Acute Infarction Ramipril Efficacy (AIRE) study,[111] the Survival and Ventricular Enlargement (SAVE) trial,[112] and the Trandolapril Cardiac Evaluation (TRACE) trial,[113] also demonstrate significant mortality benefits for patients treated with ACE inhibitors. The AIRE study evaluated ramipril treatment in MI patients who had any sign of heart failure subsequent to the MI.[111] The risk of mortality was decreased in the ramipril-treated patients by 27% versus placebo. In a similar trial (SAVE), patients who received captopril had a 19% reduction in mortality.[112] In the TRACE study, patients who had an MI with echocardiographic evidence of left ventricular dysfunction and who were treated with trandolapril had a 27% increase in life expectancy as compared with patients given placebo.[113]

A recent systematic overview of long-term ACE inhibitor therapy in patients with heart failure or left ventricular dysfunction used pooled data from 12,763 patients randomly assigned to ACE inhibitor treatment or placebo for an average of 35 months.[109] In the 3 postinfarction trials included in this meta-analysis (SAVE, AIRE, and TRACE), patients treated with an ACE inhibitor had a 26% lower mortality, a 27% lower rate of hospital admission for heart failure, and a 20% lower reinfarction rate. Similarly, when, in addition to the trials of patients with recent MI, trials of patients with chronic heart failure or left ventricular dysfunction were considered, significant reductions in death, reinfarction, and heart failure rates were observed in patients treated with an ACE inhibitor. These benefits were observed early after the start of therapy and persisted long term. Moreover, the benefits of ACE inhibitor treatment were independent of age, sex, and baseline use of diuretics, aspirin, and β-blockers.

TABLE 1 Angiotensin-Converting Enzyme (ACE) Inhibitor Clinical Trials Summary

Trial	ACE Inhibitor	Patient Group	Outcome
CONSENSUS (N = 253)	Enalapril vs placebo	NYHA IV, CHF	↓ Overall mortality
SOLVD, treatment arm (N = 2,569)	Enalapril vs placebo	NYHA II & III, CHF	↓ Overall mortality
V-HeFT II[107] (N = 804)	Enalapril vs hydralazine-isosorbide	NYHA II & III, CHF	→ Overall mortality
SAVE (N = 2,231)	Captopril vs placebo	Recent MI with asymptomatic LVD	→ Overall mortality
SOLVD, prevention arm (N = 4,228)	Enalapril vs placebo	Asymptomatic LVD	↓ Death and hospitalization due to CHF
AIRE (N = 2,006)	Ramipril vs placebo	Recent MI with overt CHF	↓ Overall mortality
ISIS-4 (N >50,000)	Captopril vs placebo	Acute MI	→ Overall mortality
GISSI-3 (N = 19,394)	Lisinopril vs open control	Acute MI	→ Overall mortality
TRACE (N = 1,749)	Trandolapril vs placebo	Recent MI with LVD	→ Overall mortality
SMILE[108] (N = 1,556)	Zofenopril vs placebo	Acute MI	→ Overall mortality

AIRE = Acute Infarction Ramipril Efficacy trial; CHF = congestive heart failure; CONSENSUS = Cooperative New Scandinavian Enalapril Survival Study; GISSI-3 = Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardica III; ISIS-4 = International Study of Infarct Survival 4; LVD = left ventricular dysfunction; MI = myocardial infarction; NYHA = New York Heart Association; SAVE = Survival and Ventricular Enlargement trial; SOLVD = Studies on Left Ventricular Dysfunction; SMILE = Survival of *Myocardial Infarction Long-Term Evaluation trial*; TRACE = Trandolapril Cardiac Evaluation trial; V-HeFTII = Vasodilator–Heart Failure Trial II.

Adapted from *The Pharmacological Basis of Therapeutics*, New York: McGraw-Hill.[106]

Finally, the results of 2 large studies and a comprehensive meta-analysis[114] have firmly established the benefits of ACE inhibition in acute MI patients. The Fourth International Study of Infarct Survival (ISIS-4) evaluated nearly 60,000 patients who were randomized to oral mononitrate, intravenous magnesium sulphate, or captopril. Only captopril significantly reduced mortality.[115] The Third Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI-3) study randomized almost 19,000 patients to lisinopril or transdermal glycerin trinitrate. Once again, only the ACE inhibitor was effective, resulting in a 12% risk reduction in mortality.[116]

The effect of ACE inhibitors on MI and coronary events: ACE inhibitors may also have the potential to prevent major acute ischemic events, perhaps through a mechanism that is independent of their ability to lower blood pressure. The SOLVD and SAVE trials have suggested that, in addition to reducing mortality and hospitalizations for heart failure, ACE inhibitors can also prevent major acute ischemic events when administered long term in patients with a low left ventricular ejection fraction.[117,118] The reductions in major acute ischemic events in these studies could not be clearly explained by the acute hemodynamic effects of these agents. Furthermore, the reductions were more pronounced than expected based on the attained blood pressure lowering in these trials, thus suggesting a direct tissue effect of ACE inhibitors to account for the reductions in MI and unstable coronary syndromes. Extending beyond this well-recognized class effect, those ACE inhibitors with a high affinity for tissue ACE may be especially beneficial in patients whose conditions are not characterized by overt renin–angiotensin system activation. In this regard, the tissue effects of ACE inhibitors have been demonstrated in both experimental models and human studies (1) to restore endothelial function; (2) to have antiproliferative and antimigratory effects on smooth muscle cells, neutrophils, and mononuclear lymphocytes; (3) to decrease oxidative stress; (4) to enhance endogenous fibrinolysis; (5) to have antiplatelet effects; and (6) in animals, to be antiatherogenic and capable of stabilizing plaque.[119]

Effects of ACE inhibition in high-risk patients with coronary artery disease with preserved left ventricular function: The Quinapril Ischemic Event Trial (QUIET) trial enrolled >1,750 patients who had coronary artery disease but normal blood pressure and no hyperlipidemia.[120] Patients were randomized to quinapril 20 mg/day or placebo for 3 years. Those receiving quinapril had 13% fewer major vascular events, which, although encouraging, did not achieve statistical significance. This trial, however, was hampered by limitations, including patients who, overall, were at low risk for major cardiovascular events at study entry, and a high rate of drop-ins and dropouts. Nonetheless, a post hoc analysis determined that patients with low-density lipoprotein cholesterol elevated above the study population's median cholesterol level

(130 mg/dL) had a statistically significant reduction in the progression of coronary artery disease.[121]

The therapeutic implications of tissue ACE inhibition have been realized with the publication of the Heart Outcomes Prevention Evaluation (HOPE) study results.[122] The HOPE study was a 2×2 factorial design trial, which randomized 9,541 high-risk patients, ≥55 years of age with evidence of vascular disease or diabetes plus at least 1 additional cardiovascular risk factor, in the absence of known heart failure or a low left ventricular ejection fraction, to treatment with the high tissue-affinity ACE inhibitor ramipril or vitamin E 400 IU or placebo. The duration of therapy extended over a mean period of 4.5 years and the primary study outcome was the composite of MI, stroke, or death from cardiovascular causes. Additionally, the HOPE trial evaluated the effects of ramipril on each of the components of the primary endpoint, namely MI, stroke, and cardiovascular death as well as total mortality, and it also evaluated the effects of therapy on development of heart failure, the need for revascularization procedures, and diabetes-related complications.

The study documented a highly statistically significant 22% reduction in the composite primary endpoint. Treatment with ramipril also reduced the rates of death from cardiovascular causes by 25%, the risk of MI by 20%, and the risk of stroke by a very significant 31%. The risk for all-cause death was also significantly reduced by 16%. Additionally, the study demonstrated a reduction in heart failure, in revascularization procedures, and in macro- and microvascular complications related to diabetes. Notably, there was a 31% reduction in the diagnosis of new diabetes.

The dramatic reduction in major cardiovascular events in the HOPE study was attained with only a modest reduction in blood pressure in a patient population already treated with a variety of antihypertensive medications, and in which most patients did not have a history of hypertension. Thus, the mean reduction in systolic blood pressure was only 3.3 mm Hg and in diastolic blood pressure, 2 mm Hg.

These modest reductions in blood pressure can obviously not explain the large impact of therapy on cardiovascular endpoints. Furthermore, similar benefits were noted in patients with various levels of systolic and diastolic pressure at baseline and throughout the study. These findings suggest that ramipril has benefits over and above blood pressure lowering alone, which may potentially be related to direct tissue effects of the drug. Importantly, the beneficial effect of treatment with ramipril on the composite outcome was consistently observed among all predefined subgroups, including (1) patients with and without diabetes, (2) women and men, (3) those with and without evidence of cardiovascular disease, (4) those <65 years of age and those ≥65 years of age, (5) those with hypertension at baseline and those without a history of hypertension, (6) those with and without microalbuminuria at baseline, (7) those with a history of coronary artery disease and those with no such history, and (8) in those patients with prior MI and those without a history of MI. Although the relative risk reductions among these subgroups were comparable, the largest absolute benefit was derived in individuals with the highest baseline risk, including (1) those with a history of diabetes, (2) those ≥65 years of age, (3) those with a history of hypertension, and (4) those with a history of prior peripheral vascular disease or coronary artery disease. The benefits of ramipril were observed among patients already taking a number of effective treatments, including aspirin, β-blockers, and lipid-lowering agents, thus indicating that the inhibition of ACE offers an additional approach to the prevention of atherothrombotic complications. Results of this landmark study strongly support the use of ACE inhibitor therapy in a broad range of patients at high risk for adverse cardiovascular events, independent of their left ventricular ejection fraction and whether or not they had clinical manifestations of heart failure. Therefore, all individuals with a history of vascular disease affecting the coronary, cerebrovascular, or peripheral vascular trees, and diabetic patients with additional risk factors should be strongly considered for long-term ACE inhibitor therapy. Among these patients, the greatest benefits may be expected in those individuals with the highest baseline risk for adverse cardiovascular outcomes.

The clear benefits demonstrated in the HOPE trial in patients who usually do not have an activated renin–angiotensin system, the uniformity of benefit among different subgroups, and the magnitude of the treatment effect—much larger than expected based on the observed reductions in blood pressure—suggest that the results of this study may indeed be explained by inhibition of tissue ACE–mediated processes that are related to atherosclerotic and ischemic complications. These findings are concordant with the results of numerous laboratory investigations and clinical studies, such as the Trial on Reversing Endothelial Dysfunction (TREND),[123] the Brachial Artery Normalization of Forearm Function (BANFF),[124] the Healing and Early Afterload Reducing Therapy (HEART) trial,[125] and the effects of Quinapril on Vascular Ace and Determinants of Ischemia (QUO VADIS) study,[126] and support the use of ACE inhibitors that effectively inhibit tissue ACE in a wide range of patients.

The effect of ACE inhibitor therapy on cardiovascular outcomes in patients without heart failure and with preserved left ventricular systolic function is also being evaluated in large ongoing clinical trials. The Prevention of Events with ACE Inhibition (PEACE)[127] and European Trial of Reduction of Cardiac Events with Perindopril in Stable Coronary Artery Disease (EUROPA)[128,129] trials, using the high tissue-affinity ACE inhibitors trandolapril and perindopril, and the Ischemia Management with Accupril Post Bypass Graft via Inhibition of Converting Enzyme (IMAGINE) study in patients with recent coronary bypass graft surgery will provide further evidence for the role of ACE inhibitor therapy in different patient subsets. If these trials confirm the large benefits noted in the HOPE study, this will further

TABLE 2 Long-Term Trials Examining the Effects of Angiotensin-Converting Enzyme (ACE) Inhibitors on Atherosclerotic Disease Progression or Ischemic Events in Patients without Heart Failure or Low Ejection Fraction

Trial	ACE Inhibitor	Primary Outcome	Sample Size (n)	Duration (yr)
QUIET[120]	Quinapril	1. Quantitative coronary angiographic measures of CAD progression 2. Cardiac ischemic endpoints*	1,775	3
SCAT[131]	Enalapril	Quantitative coronary angiographic measures of CAD progression	468	5
PART-2[132]	Ramipril	B-mode ultrasound measures of carotid atherosclerosis	600	4
HOPE[133]	Ramipril	Composite of myocardial infarction, stroke, or death from cardiovascular causes	9,297	5

CAD = coronary artery disease; HOPE = Heart Outcomes Prevention Study; PART = Prevention of Atherosclerosis with Ramipril Therapy; QUIET = Quinapril Ischemic Event Trial; SCAT = Simvastatin and Enalapril Coronary Atherosclerosis Trial.

*Composite endpoint including cardiovascular death, nonfatal myocardial infarction, coronary revascularization procedures (coronary artery bypass graft surgery, angioplasty, atherectomy), and hospitalization for unstable angina pectoris.

Adapted from *Circulation*.[119]

support the use of long-term ACE inhibitor therapy in a wide range of patients with atherosclerotic disease but without systemic activation of the renin–angiotensin system.

MECHANISTIC STUDIES WITH POSITIVE OUTCOMES: Mechanistic studies using angiographic measurements have yielded considerable evidence that endothelial dysfunction can be altered or improved with various ACE inhibitors and that there may be differences in effects between these agents. With regard to tissue ACE and its relation to coronary artery disease, the most intriguing mechanistic studies include TREND,[123] BANFF,[124] HEART trial,[125] and the QUO VADIS study.[126]

The TREND study was the first to show improved endothelial function in coronary artery disease patients who were normotensive but did not have severe hyperlipidemia or evidence of heart failure.[123] A total of 105 secondary prevention patients were randomized to quinapril 40 mg/day or placebo and observed for 6 months. Using quantitative coronary angiography, luminal diameter changes in response to acetylcholine were measured in both cohorts at baseline and at study completion. After 6 months, patients in the quinapril group showed significant improvement in endothelial response over the placebo group ($p = 0.002$), suggesting that ACE inhibition attenuates the vasoconstrictive and superoxide-generating effects of Ang II while promoting endothelial cell release of nitric oxide consequent to the accumulation of bradykinin.

The BANFF study[124] compared the effects of quinapril 20 mg, enalapril 10 mg, amlodipine 5 mg, and losartan 50 mg on blood flow and dilation of the brachial artery. These doses were considered equal in antihypertensive efficacy. Results were assessed by measuring flow-mediated vasodilation of the brachial artery in response to hyperemia through high-resolution intravascular ultrasound. Patients, who all had evidence of coronary artery disease confirmed by angiography, were randomized in a crossover design to 3 drugs for 8 weeks each, with a 2-week washout period in between. Although all of the agents improved blood pressure, they differed in their ability to improve endothelial function. Quinapril was the only agent that produced a significant improvement ($p < 0.02$) in endothelial function versus baseline.

The HEART study,[125] in which 120 patients were randomized to ramipril (relatively high affinity for tissue ACE) or placebo within 24 hours of the onset of symptoms of MI, observed a significant decrease in PAI-1 activity levels with the administration of the ACE inhibitor. This finding supports an earlier supposition that the renin–angiotensin system plays an important role in regulating endogenous fibrinolysis and that ACE inhibition may decrease the increase in PAI-1, yielding a clinical benefit.[125] Similar results were also reported with the use of captopril post MI.[130]

STUDIES EVALUATING THE EFFECTS OF ACE INHIBITION ON THE ANATOMIC PROGRESSION OF ATHEROSCLEROSIS: The effects of long-term ACE inhibition on the anatomic progression of atherosclerotic lesions of the coronary and carotid arteries were evaluated (Table 2) in the QUIET study,[120] the Simvastatin Coronary Atherosclerosis Trial (SCAT),[131] the Prevention of Atherosclerosis with Ramipril Therapy-2 (PART-2),[132] and the Study to Evaluate Carotid Ultrasound Changes in Patients Treated with Ramipril and Vitamin E (SECURE).[133] In the PART-2 trial, atherosclerotic progression was measured by B-mode carotid ultrasound of the extracranial carotid arteries; quantitative coronary angiography was used in SCAT. These studies were considered "neutral" because they did not provide clear evidence that ACE inhibitors can delay or reverse atherosclerotic lesions. Both PART-2 and SCAT, however, which together observed >1,000 patients, demonstrated significant improvements in rates of cardiovascular deaths, MI, and stroke. The angiographic substudy of QUIET demonstrated lesser progression of coronary atherosclerosis in patients with elevated cholesterol concentration (low-density lipoprotein cholesterol >3.2 mmol/L) treated with quinapril, but no clear benefit in those with lower low-density lipoprotein cholesterol levels. Finally, the SECURE trial (a substudy of HOPE) showed that ramipril 10 mg/day was effective in retarding the progression of atherosclerosis as evaluated by B-mode carotid ultrasound.[134] These differences are likely related to diversity in the patients studied, the ACE inhibitors used, and most importantly, the methods used to assess the progression of the anatomic extent of atherosclerosis.

PHARMACOLOGY OF ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

The ACE inhibitors currently number more than a dozen different agents worldwide and have long been represented by captopril and enalapril, the first ACE inhibitors to be approved. Because the mechanism of action of the ACE inhibitors is the same (ie, competitive inhibition of ACE), the documented beneficial effects of captopril and enalapril, among others, are attributed to the class as a whole. Nevertheless, individual ACE inhibitors have unique pharmacokinetic properties that may result in differential clinical effects. The most important property, perhaps, is the strength of binding affinity to tissue ACE.

The active catalytic sites of ACE consist of hydrophobic pockets of amino and carboxyterminal side chains on the enzyme's surface. The binding strength of ACE inhibitors to ACE is dependent on the binding of the sulfhydryl-, carboxyl-, or phosphinyl-containing group at the N-terminus of the ACE inhibitor with the coordinated Zn^{2+} as well as the binding of the negatively charged C-terminus of the ACE inhibitor with the postulated positively charged carboxylate dock residue (believed to be an arginine side chain) of ACE.[135] The affinity of ACE inhibitors to ACE is also dependent on the number of auxiliary binding sites, the most important of which are the S'1 and S'2 subsites.[136]

RELATIVE TISSUE AFFINITY OF ACE INHIBITORS

The degree of functional in vivo inhibition of tissue ACE produced by an ACE inhibitor is directly dependent on the binding affinity of the inhibitor and the concentration of the free inhibitor in the tissue. The concentration of the free inhibitor in the tissue, in turn, is dependent on the dynamic equilibrium between the rate of delivery of ACE inhibitor to the tissue and its subsequent washout into the blood. Key factors affecting the concentration of free inhibitor in tissues are dose, bioavailability, half-life in blood, tissue penetration, and tissue retention (or depot effect). Bioavailability and half-life in blood can readily be determined and are important for decision making in initially choosing the correct dose of ACE inhibitor. When blood levels of the ACE inhibitor are consistently high—normally in the first half of the dosing period—tissue retention of the inhibitor is not likely to have a significant effect on functional ACE inhibition. However, toward the end of the dosing period, as the levels of the ACE inhibitor in blood decreases, 2 factors appear to be key in producing functional tissue ACE inhibition: (1) inhibitor binding affinity, and (2) tissue retention (which will directly influence the concentration of the free inhibitor in the tissue).

The rank order of potency of several different ACE inhibitors has been determined by investigators using competition analyses[31,137–139] and by direct binding of tritium-labeled ACE inhibitors to tissue ACE.[140] The potency is: quinaprilat = benazeprilat > ramiprilat > perindoprilat > lisinopril > enalaprilat > fosinopril > captopril. The potency of ACE inhibitors in tissue may also be ranked accordingly (Table 3).[31,106,138,141,142]

Tissue retention of ACE inhibitor has also been examined. Isolated organ bath studies examining the duration of ACE inhibition after the removal of ACE inhibitor from the external milieu shows that functional inhibition of ACE lasts well beyond (2- to 5-fold longer) the time predicted solely on the basis of inhibitor dissociation rates or binding affinity.[140] Indeed, inhibitors with similar dissociation rates from tissue ACE show markedly different degrees of functional inhibition or retentiveness after washout. The rank order of tissue retentiveness is quinaprilat > lisinopril > enalaprilat > captopril and reflects both the binding affinity and lipophilicity of these inhibitors.

CAN ANGIOTENSIN-CONVERTING ENZYME INHIBITORS BE DIFFERENTIATED?

The physiochemical differences among ACE inhibitors that are responsible for their distinct pharmacologic properties—binding affinity, potency, lipophilicity, and depot effect—reveal that a divergent trend allows the arbitrary classification of ACE inhibitors as agents according to tissue-ACE affinity (Table 3). Thus, the recognition that tissue ACE, the endothelium, and the natural history of cardiovascular disease are interrelated leads to the question of whether the degree of tissue-ACE inhibition may extend to differences in efficacy. Clearly, a reduction of Ang II and increased nitric oxide bioavailability may represent the mechanism by which ACE inhibitors confer vascular protection. As a consequence, endothelial function may be regarded as a surrogate marker for vascular protection. The effects of ACE inhibitors on endothelium-dependent relaxation appear to differ among several reports and appear to be dependent on the agents used and the experimental designs (Table 4).[123,124,126,143–148] It is intriguing that consistent im-

TABLE 3 Pharmacological Properties of Various Angiotensin-Converting Enzyme (ACE) Inhibitors in Plasma and Tissue

Tissue Potency*	ACE Inhibitor Potencies (mmol/L $\times 10^{-9}$, ID_{50})†	Enzymatic Inhibition (IC_{50})‡	Radioligand Displacement (DD_{50})‡	Plasma Half-Life§	Relative Lipid Solubility‖
High					
Quinaprilat	0.07	5.5×10^{-11}	4.5×10^{-11}	25	++
Benazeprilat	NA	1.3×10^{-9}	4.8×10^{-11}	11	+
Ramiprilat	0.08	1.9×10^{-9}	7.0×10^{-11}	>50	++
Perindoprilat	0.40	NA	NA	10	++
Lisinopril	NA	4.5×10^{-9}	1.7×10^{-10}	12	NA
Enalaprilat	1.00	4.5×10^{-9}	1.1×10^{-9}	11	+
Fosinoprilat	NA	1.6×10^{-8}	5.1×10^{-10}	11.5	+++
Low					
Captopril	15.00	NA	NA	2	+

NA = not available.

*Radioligand binding studies using the active drug moiety.[31,138,141]

†ID_{50} is the inhibitor concentration required to displace 50% of [^{125}I]531A bound to human plasma.[142]

‡Comparison of 50% inhibition of enzymatic activity (IC_{50}) with 50% displacement of ^{125}I-351A (DD_{50}) from human plasma ACE.[142]

§Values cited for quinaprilat and ramiprilat are for dissociation from tissue ACE, ie, terminal half-life.[106]

‖Lipid solubility based on log P logarithm of the octanol/water partition coefficient of the active drug moiety, except for captopril; + signs represent increased lipid solubility.[142]

provement in endothelial function is reported with those ACE inhibitors with higher tissue-ACE affinity, such as quinapril and ramipril.

Ramipril has been shown to improve endothelial dysfunction by attenuating the toxic effects of oxidized low-density lipoprotein in vitro.[149] More recently, ramiprilat was found to prevent the development of coronary endothelial dysfunction in a canine model. In this model, scanning electron micrographs of subepicardial arterioles from control dogs revealed endothelial leukocyte adhesion and crater formation. These markers of endothelial dysfunction were not observed in ramiprilat-treated dogs.[150] Likewise, perindopril prevented chronic heart failure–induced endothelial dysfunction and reduced media cross-sectional area and collagen density in rats.[151] Perindopril has also been shown to accelerate endothelial regrowth after balloon denudation in rabbits.[152] In humans, long-term treatment with perindopril inhibits both endothelial and adventitial ACE in the internal mammary arteries from patients with ischemic heart disease.[153]

Several studies further extend these lines of evidence, including the TREND[123] and the BANFF[124] studies (see above), which have established that tissue-ACE inhibition improves endothelial function in humans. Interestingly, the BANFF study showed that enalapril and antihypertensive agents from other classes have no effect on endothelial function.

These results are strengthened by those from QUO VADIS,[126] a 2-phase, parallel-arm, phase 3 study of ACE inhibition in coronary artery disease patients scheduled to undergo coronary artery bypass graft surgery. Patients were randomized to a double-blind, placebo-controlled treatment with quinapril (40 mg/day), or a single-blind treatment with captopril 50 mg, 3 times a day (phase 1, before coronary bypass graft surgery). Overall, 75 patients received quinapril, 37 received captopril, and 74 patients received placebo, with treatment beginning, on average, 27 days before coronary bypass graft surgery.

Phase 1 of QUO VADIS was designed (1) to determine the effects of ACE inhibition with quinapril and captopril on vascular tissue ACE, independent of the circulating renin–angiotensin system and the formation of Ang II; and (2) to determine whether functional differences existed between the 2 ACE inhibitors.[126] During coronary bypass graft surgery, segments of internal mammary arteries were harvested for in vitro measurements of tissue-ACE activity. Both quinapril and captopril reduced the production of Ang II. However, only the reduction in Ang II formation in quinapril-treated patients was significant ($p < 0.05$) versus placebo. This result suggests that there is a functional difference in the respective abilities of quinapril and captopril to inhibit endothelial ACE and the local production of Ang II. Phase 2 of the QUO VADIS study[126] evaluated the effect of chronic ACE inhibition (quinapril, 40 mg/day for 1 year) versus placebo, on the incidence of ischemia. Treatment with quinapril significantly ($p = 0.02$) reduced clinical ischemic events during the 1-year period after coronary bypass graft surgery.

The potential importance of tissue-ACE inhibition was further demonstrated in a study of patients with chronic heart failure by quantitating impaired flow-dependent dilation as a measure of endothelial dysfunction.[147] The effects of quinaprilat (high affinity to tissue ACE) were compared with those of enalaprilat. High-resolution ultrasound and Doppler were used to measure radial-artery diameter and blood flow in patients who received intra-arterial infusions of quinaprilat (1.6 μg/min, n = 15) and enalaprilat (5.0 μg/min, n = 15) while at rest and during reactive hyperemia. Measurements were made both before and after N-monomethly-L-arginine was used to inhibit endothelial nitric oxide synthetase and, hence, the production of nitric oxide. Quinaprilat improved flow-dependent dilation by >40%, whereas enalaprilat had no effect. Moreover, although endothelial nitric oxide synthetase was inhibited by N-monomethyl-L-arginine (the part of flow-dependent dilation mediated by nitric

TABLE 4 Tissue Angiotensin-Converting Enzyme (ACE) Inhibition and Endothelial Function

Study	ACE Inhibitor	Population	Outcome
Saris et al[144]	Enalaprilat	Normotensive male volunteers	Inhibition of contractile effects of AI; reduction in fractional conversion of AI to AII
Lyons et al[148]	Quinapril, enalapril	Normotensive male volunteers	Quinapril, but not enalapril, significantly inhibited AII-induced vasoconstriction
Padmanabhan et al[145]	Enalaprilat	Normotensive male volunteers	Enalaprilat failed to inhibit the contractile response to AI
Hornig et al[147]	Quinaprilat, enalaprilat	CHF patients	Endothelial-dependent dilation was improved with quinaprilat, but not with enalaprilat
Prasad et al[146]	Enalaprilat	CAD patients	Enalaprilat significantly potentiated bradykinin-mediated femoral vasodilation
Mancini et al[123] (TREND)	Quinapril	CAD patients with preserved LVF	Increased coronary artery dilation; increased endothelial function in smokers and those with elevated LDL-C
Anderson et al[124] (BANFF)	Quinapril, enalapril, losartan, amlodipine	CAD patients with preserved LVF	Only quinapril significantly improved endothelial function
Oosterga et al[126] (QUO VADIS-1)	Quinapril, captopril	CAD patients with preserved LVF	Quinapril, but not captopril, blocks AI conversion to AII in vascular preparations

CAD = coronary artery disease; CHF = chronic heart failure; LVF = left ventricular function.
Adapted from Vascular Biology Working Group website.[143]

oxide), quinaprilat increased flow-dependent dilation by >100%. Enalaprilat, even when infused twice, had no effect. Similar results have been obtained with oral administration of quinapril and enalapril.[148] Thus, the tissue affinity of quinaprilat may be a key to that agent's ability to improve endothelial-mediated dilation.

This study also sheds light on the potential mechanism by which high tissue-affinity ACE inhibitors improve endothelial-mediated relaxation. The mechanism of increased nitric oxide activity may be the result of enhanced bradykinin-mediated nitric oxide release or reduced nitric oxide degradation by Ang II–induced production of reactive oxygen species.[85] Indeed, the latter mechanism has been demonstrated by Harrison[154] and Warnholtz et al,[155] who also reported that Ang II type 1 receptor blockade can reduce superoxide anion production.

The supposition that high tissue-affinity ACE inhibitors may protect nitric oxide is authenticated by Koh et al,[156] who investigated the effect of quinapril on brachial artery dilator responsiveness to increased shear stress after ischemia induced in the forearm. The study, essentially a bioassay for endothelial nitric oxide available to vascular smooth muscle, measured dilation by ultrasonography in 9 men with coronary artery disease. Patients received quinapril, 20 to 40 mg/day, for 8 weeks. When compared with baseline measurements, quinapril significantly increased flow-mediated dilation ($p < 0.001$), an effect that persisted 1 week after the discontinuation of therapy. Serum nitrogen oxide levels (a measure of endothelial nitric oxide release) were reduced nearly 20% ($p < 0.01$), suggesting that quinapril selectively improves endothelium-dependent vasodilator responsiveness by increased nitric oxide bioactivity in relation to vascular smooth muscle in coronary artery disease patients. Furthermore, this effect was achieved at a reduced rate of nitric oxide release from the endothelium. Similar results have been observed in patients with diabetes who had received enalapril; however, the improvement in endothelial function was not demonstrated beyond 4 hours after dose.[157] Therefore, high tissue-affinity ACE inhibitors such as quinapril, perindopril, and ramipril may increase bradykinin accumulation and thus enhance nitric oxide release or reduce Ang II–induced oxidant stress within the vessel wall, and as a result, protect nitric oxide from superoxide anion inactivation.[156]

Tissue ACE inhibition has also been shown to promote angiogenesis in an ischemic hind-limb animal model by a process thought to involve the endothelium. Ischemia was produced in 1 hind limb in New Zealand White rabbits, which then received a single intra-arterial injection of quinaprilat, captopril, recombinant human vascular endothelial growth factor (positive control), or no treatment (negative control). Both functional and morphologic assessments revealed augmented angiogenesis in quinaprilat-treated rabbits, which was similar to that seen in animals that received recombinant human vascular endothelial growth factor and greater than that observed in captopril-treated rabbits or the negative controls. Residual ACE activity after quinaprilat and cap-

topril was equivalent when measured in plasma but was significantly reduced by quinaprilat in tissue when compared with captopril ($p < 0.01$).[158]

These results and those from HOPE[122] support the importance of tissue-ACE inhibition. Studies such as IMAGINE, PEACE, and EUROPA, using quinapril, trandolapril, and perindopril, respectively, may confirm the findings of the HOPE study and thus validate the use of tissue-ACE inhibitors in clinical practice among high-risk patient populations.

CONCLUSION

The experimental and clinical evidence presented here validates earlier suppositions that long-term tissue-ACE inhibition would provide important clinical benefits to a broad population of patients with coronary artery disease. The cardio- and renoprotective benefits of this drug class appear to extend beyond the therapeutic effects of blood pressure reduction and may distinguish the ACE inhibitors from other antihypertensive agents. The next significant step is to determine if pharmacologic differences among the ACE inhibitors, such as the affinity for tissue ACE and the clinical effect on endothelial dysfunction, are a differentiating factor within this class of important cardiovascular drugs.

1. Linz W, Wiemer G, Gohlke P, Unger T, Scholkens BA. Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacol Rev* 1995;47:25–49.

2. Nash DT. Comparative properties of angiotensin-converting enzyme inhibitors: relations with inhibition of tissue angiotensin-converting enzyme and potential clinical implications. *Am J Cardiol* 1992;69(suppl):26C–32C.

3. Cushman DW, Cheung HS. Concentrations of angiotensin-converting enzyme in tissues of the rat. *Biochim Biophys Acta* 1971;250:261–265.

4. Esther CR, Marino EM, Howard TE, Machaud A, Corvol P, Capecchi MR, Bernstein KE. The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J Clin Invest* 1997;99:2375–2385.

5. Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzau VJ, Lander ES. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell* 1991;67:213–224.

6. Harris EL, Phelan EL, Thompson CM, Millar JA, Grigor MR. Heart mass and blood pressure have separate genetic determinants in the New Zealand genetically hypertensive (GH) rat. *J Hypertens* 1995;13:397–404.

7. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990;86:1343–1346.

8. Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, Schunkert H. Angiotensin-converting enzyme in the human heart: effect of the deletion/insertion polymorphism. *Circulation* 1995;92:1387–1388.

9. Schunkert H. Polymorphism of the angiotensin-converting enzyme gene and cardiovascular disease. *J Mol Med* 1997;75:867–875.

10. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992;359:641–644.

11. Schunkert H, Hense HW, Holmer SR, Stender M, Perz S, Keil U, Lorell BH, Riegger GA. Association between a deletion polymorphism of the angiotensin-converting-enzyme gene and left ventricular hypertrophy. *N Engl J Med* 1994;330:1634–1638.

12. Mayer B, Schunkert H. ACE gene polymorphism and cardiovascular diseases. *Herz* 2000;25:1–6.

13. Iwai N, Ohmichi N, Nakamura Y, Kinoshita M. DD genotype of the angiotensin-converting enzyme gene is a risk factor for left ventricular hypertrophy. *Circulation* 1994;90:2622–2628.

14. Ravid M, Savin H, Jutrin I, Bental T, Lang R, Lishner M. Long-term effect of ACE inhibition on development of nephropathy in diabetes mellitus type II. *Kidney Int Suppl* 1994;45:S161–S164.

15. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, Buring J, Hennekens CH. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. *N Engl J Med* 1995;332:706–711.

16. Lindpaintner K, Lee M, Larson MG, Rao VS, Pfeffer MA, Ordovas JM, Schaefer EJ, Wilson AF, Wilson PW, Vasan RS, Myers RH, Levy D. Absence of association or genetic linkage between the angiotensin-converting-enzyme gene and left ventricular mass. *N Engl J Med* 1996;334:1023–1028.

17. Schunkert H. Controversial association of left ventricular hypertrophy and the ACE I/D polymorphism: is the mist clearing up? *Nephrol Dial Transplant* 1998;13:1109–1112.

18. Montgomery HE, Clarkson P, Dollery CM, Prasad K, Losi MA, Hemingway H, Statters D, Jubb M, Girvain M, Varnava A, et al. Association of angiotensin-converting enzyme gene I/D polymorphism with change in left ventricular mass in response to physical training. *Circulation* 1997;96:741–747.

19. Pinto YM, van Gilst WH, Kingma JH, Schunkert H. Deletion-type allele of the angiotensin-converting enzyme gene is associated with progressive ventricular dilation after anterior myocardial infarction: Captopril and Thrombolysis Study Investigators. *J Am Coll Cardiol* 1995;25:1622–1626.

20. Ohmichi N, Iwai N, Maeda K, Shimoike H, Nakamura Y, Izumi M, Sugimoto Y, Kinoshita M. Genetic basis of left ventricular remodeling after myocardial infarction. *Int J Cardiol* 1996;53:265–272.

21. Pinto YM, Buikema H, van Gilst WH, Scholtens E, van Geel PP, de Graeff PA, Wagner J, Paul M. Cardiovascular end-organ damage in Ren-2 transgenic rats compared to spontaneously hypertensive rats. *J Mol Med* 1997;75:371–377.

22. Turner ST, Boerwinkle E, Sing CF. Context-dependent associations of the ACE I/D polymorphism with blood pressure. *Hypertension* 1999;34:773–778.

23. Pfohl M, Koch M, Prescod S, Haase KK, Haring HU, Karsch KR. Angiotensin I-converting enzyme gene polymorphism, coronary artery disease and myocardial infarction: an angiographically controlled study. *Eur Heart J* 1999;20:1318–1325.

24. Ng KK, Vane JR. Fate of angiotensin I in the circulation. *Nature* 1968;218:144–150.

25. Schunkert H, Dzau VJ, Tang SS, Hirsch AT, Apstein CS, Lorell BH. Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy: effects on coronary resistance, contractility, and relaxation. *J Clin Invest* 1990;86:1913–1920.

26. Yamada H, Fabris B, Allen AM, Jackson B, Johnston CI, Mendelsohn AO. Localization of angiotensin converting enzyme in rat heart. *Circ Res* 1991;68:141–149.

27. Falkenhahn M, Franke F, Bohle RM, Zhu YC, Stauss HM, Bachmann S, Danilov S, Unger T. Cellular distribution of angiotensin-converting enzyme after myocardial infarction. *Hypertension* 1995;25:219–226.

28. Ruzicka M, Skarda V, Leenen FH. Effects of ACE inhibitors on circulating versus cardiac angiotensin II in volume overload-induced cardiac hypertrophy in rats. *Circulation* 1995;92:3568–3573.

29. Pieruzzi F, Abassi ZA, Keiser HR. Expression of renin-angiotensin system components in the heart, kidneys, and lungs of rats with experimental heart failure. *Circulation* 1995;92:3105–3112.

30. Hirsch AT, Talsness CE, Schunkert H, Paul M, Dzau VJ. Tissue-specific activation of cardiac angiotensin converting enzyme in experimental heart failure. *Circ Res* 1991;69:475–482.

31. Fabris B, Jackson B, Kohzuki M, Perich R, Johnston CI. Increased cardiac angiotensin-converting enzyme in rats with chronic heart failure. *Clin Exp Pharmacol Physiol* 1990;17:309–314.

32. Hokimoto S, Yasue H, Fujimoto K, Yamamoto H, Nakao K, Kaikita K, Sakata R, Miyamoto E. Expression of angiotensin-converting enzyme in remaining viable myocytes of human ventricles after myocardial infarction. *Circulation* 1996;94:1513–1518.

33. Heymes C, Swynghedauw B, Chevalier B. Activation of angiotensinogen and angiotensin-converting enzyme gene expression in the left ventricle of senescent rats. *Circulation* 1994;90:1328–1333.

34. Lee YA, Liang CS, Lee MA, Lindpaintner K. Local stress, not systemic factors, regulate gene expression of the cardiac renin-angiotensin system in vivo: a comprehensive study of all its components in the dog. *Proc Natl Acad Sci U S A* 1996;93:11035–11040.

35. Sadoshima J, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* 1993;75:977–984.

36. Danser AH, Schalekamp MA. Is there an internal cardiac renin-angiotensin system? *Heart* 1996;76:28–32.

37. Danser AH, de Lannoy LM, Saxena P, Schalekamp MD. Chymase does not

contribute to angiotensin I-II conversion in the interstitial fluid. *Circulation* 1998;98:I-793.
38. Urata H, Kinoshita A, Misono KS, Bumpus FM, Husain A. Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart. *J Biol Chem* 1990;265:22348–22357.
39. Pfeifer M, Bruckschlegel G, Holmer SR, Paul M, Riegger AJ, Schunkert H. Reciprocal regulation of pulmonary and cardiac angiotensin-converting enzyme in rats with severe left ventricular hypertrophy. *Cardiovasc Res* 1998;38:125–132.
40. Lindpaintner K, Jin M, Wilhelm M, Toth M, Ganten D. Aspects of molecular biology and biochemistry of the cardiac renin-angiotensin system. *Br J Clin Pharmacol* 1989;27:159S–165S.
41. van Kats JP, Danser AH, van Meegen JR, Sassen LM, Verdouw PD, Schalekamp MA. Angiotensin production by the heart: a quantitative study in pigs with the use of radiolabeled angiotensin infusions. *Circulation* 1998;98:73–81.
42. Danser AH, van Kesteren CA, Bax WA, Tavenier M, Derkx FH, Saxena PR, Schalekamp MA. Prorenin, renin, angiotensinogen, and angiotensin-converting enzyme in normal and failing human hearts: evidence for renin binding [abstract]. *Circulation* 1997;96:220–226.
43. Danser AH, de Lannoy LM, Schalekamp MD. Uptake of renin and prorenin by the isolated rat heart. *Circulation* 1997:96(suppl):I-280.
44. Passier RC, Smits JF, Verluyten MJ, Daemen MJ. Expression and localization of renin and angiotensinogen in rat heart after myocardial infarction. *Am J Physiol* 1996;271:H1040–H1048.
45. Lindpaintner K, Lu W, Neidermajer N, Schieffer B, Just H, Ganten D, Drexler H. Selective activation of cardiac angiotensinogen gene expression in post-infarction ventricular remodeling in the rat. *J Mol Cell Cardiol* 1993;25:133–143.
46. MaassenVanDenBrink A, de Vries R, Saxena PR, Danser AH. ACE, but not chymase, generates angiotensin II in close proximity to the AT_1 receptor in the human isolated coronary artery. *Circulation* 1998;98:I-606.
47. Schunkert H, Jackson B, Tang SS, Schoen FJ, Smits JF, Apstein CS, Lorell BH. Distribution and functional significance of cardiac angiotensin converting enzyme in hypertrophied rat hearts. *Circulation* 1993;87:1328–1339.
48. Zisman LS, Abraham WT, Meixell GE, Vamvakias BN, Quaife RA, Lowes BD, Roden RL, Peacock SJ, Groves BM, Raynolds MV, Bristow MR, Perryman MB. Angiotensin II formation in the intact human heart: predominance of the angiotensin-converting enzyme pathway. *J Clin Invest* 1995;96:1490–1498.
49. Muller DN, Bohlender J, Hilgers KF, Dragun D, Costerousse O, Menard J, Luft FC. Vascular angiotensin-converting enzyme expression regulates local angiotensin II. *Hypertension* 1997;29:98–104.
50. Urata H, Healy B, Stewart RW, Bumpus FM, Husain A. Angiotensin II-forming pathways in normal and failing human hearts. *Circ Res* 1990;66:883–890.
51. Muller DN, Fischli W, Clozel JP, Hilgers KF, Bohlender J, Menard J, Busjahn A, Ganten D, Luft FC. Local angiotensin II generation in the rat heart: role of renin uptake. *Circ Res* 1998;82:13–20.
52. Kokkonen JO, Saarinen J, Kovanen PT. Regulation of local angiotensin II formation in the human heart in the presence of interstitial fluid: inhibition of chymase by protease inhibitors of interstitial fluid and of angiotensin-converting enzyme by Ang-(1-9) formed by heart carboxypeptidase A-like activity. *Circulation* 1997;95:1455–1463.
53. Bernstein KE. ACE knockout mice: lessons for adult nephrology. *Nephrol Dial Transplant* 1998;13:2991–2994.
54. Holubarsch C, Hasenfuss G, Schmidt-Schweda S, Knorr A, Pieske B, Ruf T, Fasol R, Just H. Angiotensin I and II exert inotropic effects in atrial but not in ventricular human myocardium: an in vitro study under physiological experimental conditions. *Circulation* 1993;88:1228–1237.
55. Esther CR Jr, Howard TE, Marino EM, Goddard JM, Capecchi MR, Bernstein KE. Mice lacking angiotensin-converting enzyme have low blood pressure, renal pathology, and reduced male fertility. *Lab Invest* 1996;74:953–965.
56. Baker KM, Aceto JF. Angiotensin II stimulation of protein synthesis and cell growth in chick heart cells. *Am J Physiol* 1990;259:H610–H618.
57. Schunkert H, Sadoshima J, Cornelius T, Kagaya Y, Weinberg EO, Izumo S, Riegger G, Lorell BH. Angiotensin II-induced growth responses in isolated adult rat hearts: evidence for load-independent induction of cardiac protein synthesis by angiotensin II. *Circ Res* 1995;76:489–497.
58. Kromer EP, Riegger GA. Effects of long-term angiotensin converting enzyme inhibition on myocardial hypertrophy in experimental aortic stenosis in the rat. *Am J Cardiol* 1988;62:161–163.
59. Bruckschlegel G, Holmer SR, Jandeleit K, Grimm D, Muders F, Kromer EP, Riegger GA, Schunkert H. Blockade of the renin-angiotensin system in cardiac pressure-overload hypertrophy in rats. *Hypertension* 1995;25:250–259.
60. Lear W, Ruzicka M, Leenen FH. ACE inhibitors and cardiac ACE mRNA in volume overload-induced cardiac hypertrophy. *Am J Physiol* 1997;273:H641–H646.
61. Takemoto M, Egashira K, Usui M, Numaguchi K, Tomita H, Tsutsui H, Shimokawa H, Sueishi K, Takeshita A. Important role of tissue angiotensin-converting enzyme activity in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis in rats. *J Clin Invest* 1997;99:278–287.
62. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium: fibrosis and renin-angiotensin-aldosterone system. *Circulation* 1991;83:1849–1865.
63. Katwa LC, Campbell SE, Tyagi SC, Lee SJ, Cicila GT, Weber KT. Cultured myofibroblasts generate angiotensin peptides de novo. *J Mol Cell Cardiol* 1997;29:1375–1386.
64. Grimm D, Kromer EP, Bocker W, Bruckschlegel G, Holmer SR, Riegger GA, Schunkert H. Regulation of extracellular matrix proteins in pressure-overload cardiac hypertrophy: effects of angiotensin converting enzyme inhibition. *J Hypertens* 1998;16:1345–1355.
65. Panizo A, Pardo J, Hernandez M, Galindo MF, Cenarruzabeitia E, Diez J. Quinapril decreases myocardial accumulation of extracellular matrix components in spontaneously hypertensive rats. *Am J Hypertens* 1995;8:815–822.
66. Matsusaka T, Katori H, Inagami T, Fogo A, Ichikawa I. Communication between myocytes and fibroblasts in cardiac remodeling in angiotensin chimeric mice. *J Clin Invest* 1999;103:1451–1458.
67. Leri A, Claudio PP, Li Q, Wang X, Reiss K, Wang S, Malhotra A, Kajstura J, Anversa P. Stretch-mediated release of angiotensin II induces myocyte apoptosis by activating p53 that enhances the local renin-angiotensin system and decreases the Bcl-2-to-Bax protein ratio in the cell. *J Clin Invest* 1998;101:1326–1342.
68. Pierzchalski P, Reiss K, Cheng W, Cirielli C, Kajstura J, Nitahara JA, Rizk M, Capogrossi MC, Anversa P. p53 Induces myocyte apoptosis via the activation of the renin-angiotensin system. *Exp Cell Res* 1997;234:57–65.
69. Diez J, Panizo A, Hernandez M, Vega F, Sola I, Fortuno MA, Pardo J. Cardiomyocyte apoptosis and cardiac angiotensin-converting enzyme in spontaneously hypertensive rats. *Hypertension* 1997;30:1029–1034.
70. Friedrich SP, Lorell BH, Rousseau MF, Hayashida W, Hess OM, Douglas PS, Gordon S, Keighley CS, Benedict C, Krayenbuehl HP, Grossman W, Pouleur H. Intracardiac angiotensin-converting enzyme inhibition improves diastolic function in patients with left ventricular hypertrophy due to aortic stenosis. *Circulation* 1994;90:2761–2771.
71. Weinberg EO, Schoen FJ, George D, Kagaya Y, Douglas PS, Litwin SE, Schunkert H, Benedict CR, Lorell BH. Angiotensin-converting enzyme inhibition prolongs survival and modifies the transition to heart failure in rats with pressure overload hypertrophy due to ascending aortic stenosis. *Circulation* 1994;90:1410–1422.
72. Eberli FR, Apstein CS, Ngoy S, Lorell BH. Exacerbation of left ventricular ischemic diastolic dysfunction by pressure-overload hypertrophy: modification by specific inhibition of cardiac angiotensin converting enzyme. *Circ Res* 1992;70:931–943.
73. Foult JM, Tavolaro O, Antony I, Nitenberg A. Direct myocardial and coronary effects of enalaprilat in patients with dilated cardiomyopathy: assessment by a bilateral intracoronary infusion technique. *Circulation* 1988;77:337–344.
74. Haleen SJ, Weishaar RE, Overhiser RW, Bousley RF, Keiser JA, Rapundalo SR, Taylor DG. Effects of quinapril, a new angiotensin converting enzyme inhibitor, on left ventricular failure and survival in the cardiomyopathic hamster: hemodynamic, morphological, and biochemical correlates. *Circ Res* 1991;68:1302–1312.
75. The CONSENSUS Trial Study Group. Effects of enalapril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). *N Engl J Med* 1987;316:1429–1435.
76. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 1991;325:293–302.
77. Erdos EG, Skidgel RA. The angiotensin I-converting enzyme. *Lab Invest* 1987;56:345–348.
78. Fishel RS, Eisenberg S, Shai SY, Redden RA, Bernstein KE, Berk BC. Glucocorticoids induce angiotensin-converting enzyme expression in vascular smooth muscle. *Hypertension* 1995;25:343–349.
79. Shai SY, Fishel RS, Martin BM, Berk BC, Bernstein KE. Bovine angiotensin converting enzyme cDNA cloning and regulation. Increased expression during endothelial cell growth arrest. *Circ Res* 1992;70:1274–1281.
80. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin-converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med* 1998;339:1285–1292.
81. Hornig B, Kohler C, Drexler H. Role of bradykinin in mediating vascular effects of angiotensin-converting enzyme inhibitors in humans. *Circulation* 1997;95:1115–1118.
82. Brown NJ, Agirbasli MA, Williams GH, Litchfield WR, Vaughan DE. Effect of activation and inhibition of the renin-angiotensin system on plasma PAI-1. *Hypertension* 1998;32:965–971.
83. Britten MB, Zeiher AM, Schachinger V. Clinical importance of coronary endothelial vasodilator dysfunction and therapeutic options. *J Intern Med* 1999;245:315–327.
84. Drexler H, Hornig B. Endothelial dysfunction in human disease. *J Mol Cell Cardiol* 1999;31:51–60.
85. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation: contribution to alterations of vasomotor tone. *J Clin Invest* 1996;97:1916–1923.
86. Diet F, Pratt RE, Berry GJ, Momose N, Gibbons GH, Dzau VJ. Increased accumulation of tissue ACE in human atherosclerotic coronary artery disease. *Circulation* 1996;94:2756–2767.
87. Moroi M, Zhang L, Yasuda T, Virmani R, Gold HK, Fishman MC, Huang PL. Interaction of genetic deficiency of endothelial nitric oxide, gender, and

pregnancy in vascular response to injury in mice. *J Clin Invest* 1998;101:1225–1232.
88. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* 1982;307:652–659.
89. Yoshida Y, Fogo A, Ichikawa I. Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int* 1989;35:654–660.
90. Kagami S, Kuhara T, Okada K, Kuroda Y, Border WA, Noble NA. Dual effects of angiotensin II on the plasminogen/plasmin system in rat mesangial cells. *Kidney Int* 1997;51:664–671.
91. Border WA, Noble NA. Interactions of transforming growth factor-beta and angiotensin II in renal fibrosis. *Hypertension* 1998;31:181–188.
92. Miettinen H, Haffner SM, Lehto S, Ronnemaa T, Pyörälä K, Laakso M. Proteinuria predicts stroke and other atherosclerotic vascular disease events in nondiabetic and non-insulin-dependent diabetic subjects. *Stroke* 1996;27:2033–2039.
93. Breyer JA, Bain RP, Evans JK, Nahman NS Jr, Lewis EJ, Cooper M, McGill J, Berl T. Predictors of the progression of renal insufficiency in patients with insulin-dependent diabetes and overt diabetic nephropathy. The Collaborative Study Group. *Kidney Int* 1996;50:1651–1658.
94. Ruilope LM, Miranda B, Oliet A, Millet VG, Rodicio JL, Romero JC, Raij L. Control of hypertension with the angiotensin converting enzyme inhibitor captopril reduces glomerular proteinuria. *J Hypertens Suppl* 1988;6:S467–S469.
95. Kasiske BL, Kalil RS, Ma JZ, Liao M, Keane WF. Effect of antihypertensive therapy on the kidney in patients with diabetes: a meta-regression analysis. *Ann Intern Med* 1993;118:129–138.
96. Gansevoort RT, de Zeeuw D, de Jong PE. Additive antiproteinuric effect of ACE inhibition and a low-protein diet in human renal disease. *Nephrol Dial Transplant* 1995;10:497–504.
97. UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *Br Med J* 1998;317:703–713.
98. Hansson L, Lindholm LH, Niskanen L, Lanke J, Hedner T, Niklason A, Luomanmaki K, Dahlof B, de Faire U, Morlin C, Karlberg BE, Wester PO, Bjorck JE. Effect of angiotensin-converting-enzyme inhibition compared with conventional therapy on cardiovascular morbidity and mortality in hypertension: the Captopril Prevention Project (CAPPP) randomised trial. *Lancet* 1999;353:611–616.
99. Estacio RO, Schrier RW. Antihypertensive therapy in type 2 diabetes: implications of the appropriate blood pressure control in diabetes (ABCD) trial. *Am J Cardiol* 1998;82(suppl):9R–14R.
100. Koskinen P, Manttari M, Manninen V, Huttunen JK, Heinonen OP, Frick MH. Coronary heart disease incidence in NIDDM patients in the Helsinki Heart Study. *Diabetes Care* 1992;15:820–825.
101. Janka HU, Dirschedl P. Systolic blood pressure as a predictor for cardiovascular disease in diabetes: a 5-year longitudinal study. *Hypertension* 1985;7:II90–II94.
102. Kuusisto J, Mykkanen L, Pyörälä K, Laakso M. NIDDM and its metabolic control predict coronary heart disease in elderly subjects. *Diabetes* 1994;43:960–967.
103. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy: the Collaborative Study Group. *N Engl J Med* 1993;329:1456–1462.
104. HOPE Investigators. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. *Lancet* 2000;355:253–259.
105. Maschio G, Alberti D, Janin G, Locatelli F, Mann JFE, Motolese M, Ponticelli C, Ritz E, Zucchelli P. Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. *N Engl J Med* 1996;334:939–945.
106. Jackson EK, Garrison JC. Renin and angiotensin. In: Hardman JG, Limbird L, eds. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill, 1999:743–746.
107. Cohn JN, Johnson G, Ziesche S, Cobb F, Francis G, Tristani F, Smith R, Dunkman WB, Loeb H, Wong M, et al. A comparison of enalapril with hydralazine-isosorbide dinitrate in the treatment of chronic congestive heart failure. *N Engl J Med* 1991;325:303–310.
108. Ambrosioni E, Borghi C, Magnani B. The effect of the angiotensin-converting-enzyme inhibitor zofenopril on mortality and morbidity after anterior myocardial infarction: the Survival of Myocardial Infarction Long-Term Evaluation (SMILE) Study Investigators. *N Engl J Med* 1995;332:80–85.
109. Flather MD, Yusuf S, Kober L, Pfeffer M, Hall A, Murray G, Torp-Pedersen C, Ball S, Pogue J, Moyé L, Braunwald E. Long-term ACE-inhibitor therapy in patients with heart failure or left-ventricular dysfunction: a systematic overview of data from individual patients. ACE-Inhibitor Myocardial Infarction Collaborative Group. *Lancet* 2000;355:1575–1581.
110. Garg R, Yusuf S. Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure: Collaborative Group on ACE Inhibitor Trials. *JAMA* 1995;273:1450–1456.
111. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators. *Lancet* 1993;342:821–828.
112. Pfeffer MA, Braunwald E, Moyé LA, Basta L, Brown EJ Jr, Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the survival and ventricular enlargement trial: the SAVE Investigators. *N Engl J Med* 1992;327:669–677.
113. Torp-Pedersen C, Kober L. Effect of ACE inhibitor trandolapril on life expectancy of patients with reduced left-ventricular function after acute myocardial infarction: TRACE Study Group. Trandolapril Cardiac Evaluation. *Lancet* 1999;354:9–12.
114. ACE Inhibitor Myocardial Infarction Collaborative Group. Indications for ACE inhibitors in the early treatment of acute myocardial infarction: systematic overview of individual data from 100,000 patients in randomized trials. *Circulation* 1998;97:2202–2212.
115. ISIS-4 (Fourth International Study of Infarct Survival) Collaborative Group. ISIS-4: a randomised factorial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulphate in 58,050 patients with suspected acute myocardial infarction. ISIS-4. *Lancet* 1995;345:669–685.
116. Gruppo Italiano per lo Studio della Sopravvivenza nell'infarto Miocardico. GISSI-3: effects of lisinopril and transdermal glyceryl trinitrate singly and together on 6-week mortality and ventricular function after acute myocardial infarction. *Lancet* 1994;343:1115–1122.
117. Yusuf S, Pepine CJ, Garces C, Pouleur H, Salem D, Kostis J, Benedict C, Rousseau M, Bourassa M, Pitt B. Effect of enalapril on myocardial infarction and unstable angina in patients with low ejection fractions. *Lancet* 1992;340:1173–1178.
118. Rutherford JD, Pfeffer MA, Moyé LA, Davis BR, Flaker GC, Kowey PR, Lamas GA, Miller HS, Packer M, Rouleau JL, Braunwald E. Effects of captopril on ischemic events after myocardial infarction: results of the Survival and Ventricular Enlargement trial. SAVE Investigators. *Circulation* 1994;90:1731–1738.
119. Lonn EM, Yusuf S, Jha P, Montague TJ, Teo KK, Benedict CR, Pitt B. Emerging role of angiotensin-converting enzyme inhibitors in cardiac and vascular protection. *Circulation* 1994;90:2056–2069.
120. Texter M, Lees RS, Pitt B, Dinsmore RE, Uprichard AC. The QUinapril Ischemic Event Trial (QUIET) design and methods: evaluation of chronic ACE inhibitor therapy after coronary artery intervention. *Cardiovasc Drugs Ther* 1993;7:273–282.
121. Cashin-Hemphill L, Holmvang G, Chan RC, Pitt B, Dinsmore RE, Lees RS. Angiotensin-converting enzyme inhibition as antiatherosclerotic therapy: no answer yet. QUIET Investigators. QUinapril Ischemic Event Trial. *Am J Cardiol* 1999;83:43–47.
122. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 2000;342:145–153.
123. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Luscher TF, Klibaner MI, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease: the TREND (Trial on Reversing Endothelial Dysfunction) Study. *Circulation* 1996;94:258–265.
124. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). *J Am Coll Cardiol* 2000;35:60–66.
125. Vaughan DE, Rouleau JL, Ridker PM, Arnold JM, Menapace FJ, Pfeffer MA. Effects of ramipril on plasma fibrinolytic balance in patients with acute anterior myocardial infarction: HEART Study Investigators. *Circulation* 1997;96:442–447.
126. Oosterga M, Voors AA, Buikema H, Pinto YM, Haber HE, Ebels T, Morshuis WJ, Kingma JH, Crijns HJ, van Gilst WH. Angiotensin II formation in human vasculature after chronic ACE inhibition: a prospective, randomized, placebo-controlled study. QUO VADIS Investigators. *Cardiovasc Drugs Ther* 2000;14:55–60.
127. Pfeffer MA, Domanski M, Rosenberg Y, Verter J, Geller N, Albert P, Hsia J, Braunwald E. Prevention of events with angiotensin-converting enzyme inhibition (the PEACE study design): Prevention of Events with Angiotensin-Converting Enzyme Inhibition. *Am J Cardiol* 1998;82(suppl):25H–30H.
128. Fox KM, Henderson JR, Bertrand ME, Ferrari R, Remme WJ, Simoons ML. The European trial on reduction of cardiac events with perindopril in stable coronary artery disease (EUROPA). *Eur Heart J* 1998;19(suppl J):J52–J55.
129. Simoons ML, Vos J, de Feyter PJ, Bots ML, Remme WJ, Grobbee DE, Kluft C, de Maat MP, Fox KM, Deckers JW. EUROPA substudies, confirmation of pathophysiological concepts: European trial on reduction of cardiac events with perindopril in stable coronary artery disease. *Eur Heart J* 1998;19(suppl J):J56–J60.
130. Wright RA, Flapan AD, Alberti KG, Ludlam CA, Fox KA. Effects of captopril therapy on endogenous fibrinolysis in men with recent, uncomplicated myocardial infarction. *J Am Coll Cardiol* 1994;24:67–73.
131. Teo KK, Burton JR, Buller CE, Plante S, Catellier D, Tymchak W, Dzavik V, Taylor D, Yokoyama S, Montague TJ. Long-term effects of cholesterol lowering and angiotensin-converting enzyme inhibition on coronary atherosclerosis: the Simvastatin/Enalapril coronary atherosclerosis trial (SCAT). *Circulation* 2000;102:1748–1754.
132. MacMahon S, Sharpe N, Gamble G, Clague A, Mhurchu CN, Clark T, Hart H, Scott J, White H. Randomized, placebo-controlled trial of the angiotensin-converting enzyme inhibitor, ramipril, in patients with coronary or other occlu-

sive arterial disease. PART-2 Collaborative Research Group. Prevention of Atherosclerosis with Ramipril. *J Am Coll Cardiol* 2000;36:438–443.
133. Lonn EM, Yusuf S, Dzavik V, Doris CI, Yi Q, Smith S, Moore-Cox A, Bosch J, Riley WA, Teo KK. Effects of ramipril and vitamin E on atherosclerosis: the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). *Circulation* 2001;103:919–925.
134. Lonn E, Dzavik V, Yusuf S. Results of the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). *Circulation* 1999;100:I-185.
135. Unger T, Gohlke P. Converting enzyme inhibitors in cardiovascular therapy: current status and future potential. *Cardiovasc Res* 1994;28:146–158.
136. Perich RB, Jackson B, Johnston CI. Structural constraints of inhibitors for binding at two active sites on somatic angiotensin converting enzyme. *Eur J Pharmacol* 1994;266:201–211.
137. Fabris B, Yamada H, Cubela R, Jackson B, Mendelsohn FA, Johnston CI. Characterization of cardiac angiotensin converting enzyme (ACE) and in vivo inhibition following oral quinapril to rats. *Br J Pharmacol* 1990;100:651–655.
138. Johnston CI, Fabris B, Yamada H, Mendelsohn FA, Cubela R, Sivell D, Jackson B. Comparative studies of tissue inhibition by angiotensin converting enzyme inhibitors. *J Hypertens Suppl* 1989;7:S11–S16.
139. Johnston CI, Fabris B, Yoshida K. The cardiac renin-angiotensin system in heart failure. *Am Heart J* 1993;126:756–760.
140. Kinoshita A, Urata H, Bumpus FM, Husain A. Measurement of angiotensin I converting enzyme inhibition in the heart. *Circ Res* 1993;73:51–60.
141. Fabris B, Chen BZ, Pupic V, Perich R, Johnston CI. Inhibition of angiotensin-converting enzyme (ACE) in plasma and tissue. *J Cardiovasc Pharmacol* 1990;15:S6–S13.
142. Opie LH. ACE Inhibitors: Specific agents and pharmacokinetics. In: Opie LH, ed. *Angiotensin-Converting Enzyme Inhibitors: Scientific Basis for Clinical Use*. New York: Authors' Publishing House, 1994:171–247.
143. Pepine CJ. Clinical trials update: tissue ACE inhibition offers new hope for treating cardiovascular disease. Vascular Biology Working Group Web site. Available at: http://www.vbwg.org. Accessed April 20, 2000.
144. Saris JJ, van Dijk MA, Kroon I, Schalekamp MA, Danser AH. Functional importance of angiotensin-converting enzyme-dependent in situ angiotensin II generation in the human forearm. *Hypertension* 2000;35:764–768.
145. Padmanabhan N, Jardine AG, McGrath JC, Connell JM. Angiotensin-converting enzyme-independent contraction to angiotensin I in human resistance arteries. *Circulation* 1999;99:2914–2920.
146. Prasad A, Husain S, Quyyumi AA. Effect of enalaprilat on nitric oxide activity in coronary artery disease. *Am J Cardiol* 1999;84:1–6.
147. Hornig B, Arakawa N, Haussmann D, Drexler H. Differential effects of quinaprilat and enalaprilat on endothelial function of conduit arteries in patients with chronic heart failure. *Circulation* 1998;98:2842–2848.
148. Lyons D, Webster J, Benjamin N. Effect of enalapril and quinapril on forearm vascular ACE in man. *Eur J Clin Pharmacol* 1997;51:373–378.
149. Berkenboom G, Langer I, Carpentier Y, Grosfils K, Fontaine J. Ramipril prevents endothelial dysfunction induced by oxidized low-density lipoproteins: a bradykinin-dependent mechanism. *Hypertension* 1997;30:371–376.
150. Martorana PA, Ruetten H, Goebel B, Koehl D, Roegner B, Schoelkens BA, Keil M. Ramiprilat prevents the development of acute coronary endothelial dysfunction in the dog. *Basic Res Cardiol* 1999;94:238–245.
151. Mulder P, Elfertak L, Richard V, Compagnon P, Devaux B, Henry JP, Scalbert E, Desche P, Mace B, Thuille ZC. Peripheral artery structure and endothelial function in heart failure: effect of ACE inhibition. *Am J Physiol* 1996;271:H469–H477.
152. Van Belle E, Meurice T, Tio FO, Corseaux D, Dupuis B, McFadden EP, Lablanche JM, Bauters C, Bertrand ME. ACE inhibition accelerates endothelial regrowth in vivo: a possible explanation for the benefit observed with ACE inhibitors following arterial injury. *Biochem Biophys Res Commun* 1997;231:577–581.
153. Zhuo JL, Froomes P, Casley D, Liu JJ, Murone C, Chai SY, Buxton B, Mendelsohn FA. Perindopril chronically inhibits angiotensin-converting enzyme in both the endothelium and adventitia of the internal mammary artery in patients with ischemic heart disease. *Circulation* 1997;96:174–182.
154. Harrison DG. Endothelial function and oxidant stress. *Clin Cardiol* 1997;20:II-11–II-17.
155. Warnholtz A, Nickenig G, Schulz E, Macharzina R, Brasen JH, Skatchkov M, Heitzer T, Stasch JP, Griendling KK, Harrison DG, et al. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis: evidence for involvement of the renin-angiotensin system. *Circulation* 1999;99:2027–2033.
156. Koh KK, Bui MN, Hathaway L, Csako G, Waclawiw MA, Panza JA, Cannon RO III. Mechanism by which quinapril improves vascular function in coronary artery disease. *Am J Cardiol* 1999;83:327–331.
157. O'Driscoll G, Green D, Rankin J, Stanton K, Taylor R. Improvement in endothelial function by angiotensin converting enzyme inhibition in insulin-dependent diabetes mellitus. *J Clin Invest* 1997;100:678–684.
158. Fabre JE, Rivard A, Magner M, Silver M, Isner JM. Tissue inhibition of angiotensin-converting enzyme activity stimulates angiogenesis in vivo. *Circulation* 1999;99:3043–3049.
159. Johnston CI. Tissue angiotensin converting enzyme in cardiac and vascular hypertrophy, repair, and remodeling. *Hypertension* 1994;23:258–268.
160. Brown NJ, Vaughan DE. Angiotensin-converting enzyme inhibitors. *Circulation* 1998;97:1411–1420.