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Vasculoprotective and Cardioprotective Mechanisms of Angiotensin-Converting Enzyme Inhibition: The Homeostatic Balance Between Angiotensin II and Nitric Oxide

GARY H. GIBBONS, M.D.

Molecular and Cellular Vascular Biology Research Laboratory, Brigham and Women's Hospital, Boston, Massachusetts, USA

Summary: The ability of the vasculature to modify its geometry in accordance with conditions of its microenvironment—the process of vascular remodeling—is an important pathobiologic process common to vascular disorders such as atherosclerosis, restenosis after angioplasty, and hypertension. Vascular remodeling characterizes the natural history of atherosclerosis, contributes to increased vascular resistance, and may contribute to the clinical complications of hypertension. A growing body of evidence indicates that locally generated vasoactive substances such as angiotensin II and nitric oxide are important determinants of the natural history of vascular disease. In particular, angiotensin II may promote vascular lesion formation by increasing vascular cell population via increased cell growth and decreased programmed cell death, and it may also alter extracellular matrix composition. Thus, angiotensin II is a pleiotropic local mediator capable of modulating cell growth, programmed cell death, migration of vascular smooth muscle cells, and extracellular matrix modulation, all of which are biologic mechanisms of vascular remodeling and intimal formation. This is proposed to occur via a local tissue angiotensin system. Angiotensin II may also promote chronic hypertension by modulating the vascular redox state and promoting the catabolism of the endothelium–derived nitric oxide, an endogenous inhibitory vasodilator. Because angiotensin–converting enzyme (ACE) is strategically positioned to influence the activity of at least three local vasoactive systems—angiotensin II, nitric oxide, and bradykinin—blocking ACE with ACE inhibition may have profound effects on ventricular and vascular structure and function, and have particular efficacy in preventing the morbidity and mortality of vascular diseases such as hypertension and atherosclerosis.

Key words: angiotensin II, angiotensin-converting enzyme, atherosclerosis, hypertension, nitric oxide, vascular remodeling

Introduction

Advances in cardiovascular medicine have improved our capacity to prolong the lives of patients who have suffered myocardial infarctions or congestive heart failure. However, the current challenge is to develop pharmacotherapies that move beyond the treatment of symptoms toward an agenda in which interventions prevent the development of end-stage coronary heart disease. To alter the natural history of cardiovascular disease, it is important to understand the fundamental pathobiologic mechanisms that promote the morbidity and mortality associated with these disorders. This review focuses on the emerging evidence indicating that locally generated vasoactive substances such as angiotensin II (ang II) and nitric oxide (NO) are important determinants of the natural history of vascular disease. Insights into these pathobiologic processes suggest that therapeutic interventions that alter the expression of these vasoactive mediators [such as angiotensin-converting enzyme (ACE) inhibitors] may have particular efficacy in preventing the morbidity and mortality of diseases such as hypertension and atherosclerosis.

Vascular Remodeling: Clinical Implications

The vasculature is a complex, integrated organ capable of modulating its tone and structure in accordance with the conditions of its microenvironment. This ability of the vasculature to modify its geometry—the process of vascular remodeling—is now recognized as an important pathobiologic process common to various vascular disorders such as atherosclerosis, restenosis after angioplasty, and hypertension.[1] Typically, vascular remodeling involves changes in the relative dimensions

Address for reprints:

Dr. Gary H. Gibbons
Molecular and Cellular Vascular Biology Research Laboratory
Thorn–12
Brigham and Women's Hospital
75 Francis Street
Boston, MA 02115, USA

of vessel components such as the outer circumference, the lumen, the wall thickness:lumen ratio, or the intima:media ratio. These alterations in vessel structure are now considered important determinants of vascular resistance and the pathogenesis of hypertension.[1–4] Morphometric studies of hypertensive vessels in animal models and humans have documented several forms of vascular remodeling, including: (1) medial layer hypertrophy, (2) decreased vessel and/or lumen diameter, (3) expansion and/or alteration of the extracellular matrix, and (4) vessel rarefaction (microvessel occlusion). Vascular remodeling is postulated to be a critical, self–perpetuating mechanism that promotes the chronic maintenance of the hypertensive state in the setting of normal levels of vasoconstrictors and vasodilators.

In addition to contributing to increased vascular resistance, the process of vascular remodeling may also participate in the vicious cycle of events that promotes the clinical complications of hypertension. For example, changes in the vasculature of hypertensive patients observed during routine fundoscopic examination (e.g., arteriovenous nicking due to arteriolar hypertrophy) are clinical manifestations of vascular remodeling, and an association has been shown between these structural changes and the natural history of progressive hypertension.[5, 6] In addition, rarefaction in skeletal muscle beds may promote the phenomenon of insulin resistance in hypertension by compromising the delivery of insulin and glucose to skeletal muscle. Likewise, the association among lacunar infarction, subcortical white matter disease, and hypertension may relate to vascular remodeling in the cerebral microvasculature.[7] A similar process appears to occur in the coronary microcirculation and may provide a mechanism for the increased cardiac mortality noted in hypertensive patients without severe epicardial atherosclerotic disease.[8, 9] Furthermore, structural changes in the renal microcirculation may predispose to the development of nephrosclerosis in hypertension and eventual renal failure.[10, 11] Finally, vascular hypertrophy and fibrosis within the structures of conduit vessels such as the aorta may contribute to reduced vascular compliance and predispose to systolic hypertension and left ventricular hypertrophy.[12] Thus, many of the clinical sequelae of hypertension (i.e., myocardial infarction, heart failure, stroke, and renal failure) may result from vascular remodeling within the microcirculation and conduit vessels.

The natural history of atherosclerosis is also characterized by a process of vascular remodeling. The development of clinically significant vessel stenosis depends on changes in overall vessel dimensions as well as expansion of intimal lesions. Studies using intravascular ultrasound have documented the significance of vascular remodeling in the clinical progression of restenosis after angioplasty and in transplant coronary disease and atherosclerosis.[13–16] These recent studies confirm the classic morphometric studies by Glagov and others, demonstrating that the vasculature undergoes a process of compensatory enlargement to mitigate the effect of plaque expansion on lumen dimensions.[15] The locally generated factors that determine whether a vessel undergoes vascular hypertrophy, shrinkage remodeling, or enlargement remodeling are poorly characterized but may have important clinical implications in the treatment of patients with hypertension and atherosclerosis.

Vascular Homeostatic Balance

Although the histopathology of hypertensive vessels is distinct from atherosclerotic lesions, it is intriguing that the pathogenesis of vascular diseases such as hypertension and atherosclerosis share many pathobiologic mechanisms. Epidemiologic studies have established that hypertension is a potent risk factor for the development of coronary heart disease, and it is well known that the superimposition of hypertension potentiates the process of atherosclerotic lesion formation in animal models.[17] However, the mechanisms by which hypertension-promoting factors contribute to atherosclerotic vascular disease are not well defined. Both forms of vascular disease involve alterations in the regulation of vascular cell growth, programmed cell death, migration, and matrix modification. The locally generated, autocrine-paracrine mediators that regulate these processes within vessels during the pathogenesis of vascular disease remain to be further defined.

The homeostatic regulatory mechanisms governing vascular tone involve a "yin-yang" balance in which the interplay between vasoconstrictors and vasodilators modulates vessel resistance. During the pathogenesis of hypertension, this homeostatic balance becomes perturbed, so that the influence of vasoconstrictors such as ang II predominates over the influence of vasodilators such as NO. Moreover, many vasoactive substances that were originally defined as regulators of vessel tone are now recognized as pleiotropic factors that can modulate the critical cellular processes involved in vascular remodeling and lesion formation, that is, vascular cell growth, migration, and changes in extracellular matrix composition.

Mechanisms of Vascular Remodeling and Intimal Lesion Formation: Role of Angiotensin II

Much as the homeostatic regulation of vascular tone is governed by a balance between vasoconstrictors and vasodilators, the balance of growth-stimulatory and growth-inhibitory factors appears to regulate vascular remodeling and intimal lesion formation. A growing body of evidence indicates that vasoconstrictor substances (e.g., ang II) promote increased growth of vascular smooth muscle cells, whereas vasodilators (e.g., endothelium-derived NO) inhibit the growth of vascular smooth muscle cells.[1, 2]

Ang II serves as a useful archetype of a vasoactive substance that can modulate the cellular processes of vascular remodeling. It is now well documented that, in addition to the circulating renin-angiotensin system, the vessel wall expresses a paracrine vascular angiotensin system that may generate ang II locally within the vasculature.[18] Experimental studies have shown that blockade of ang II effectively reverses the changes in vascular structure associated with hypertension.[19, 20] In accordance with these in vivo studies, in vitro studies suggest that ang II can induce either hypertrophy or

hyperplasia of cultured vascular smooth muscle cells, an effect that is potentiated by mechanical forces imposed by hemodynamic stimuli.[21, 22] The angiotensin type I receptor is coupled to cellular signaling pathways such as tyrosine kinases (src- and mitogen-activated protein kinase) that mediate the induction of cell growth.[23] Ang II is a bifunctional growth factor that induces increased expression of proliferative autocrine factors such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), and of antiproliferative autocrine factors such as transforming growth factor-β1 (TGFβ1) in cultured vascular smooth muscle cells.[21] Thus, the growth response of vascular smooth muscle cells to ang II–hypertrophy versus hyperplasia—depends on the relative balance of proliferative (PDGF, bFGF) versus antiproliferative (TGFβ1) autocrine growth factors. In addition to these well-defined mediators, recent reports suggest that the induction of endothelin-1, insulin-like growth factor I, and heparin-binding epidermal growth factor may also contribute to the growth-stimulatory effects of ang II.[2] These in vitro models have provided important mechanistic insights and suggest that the net growth response to ang II is dependent on the balance of mediators within the cellular milieu.

Our knowledge of the mediators involved in vascular remodeling has been based primarily on the indirect evidence provided by pharmacologic studies that are confounded by changes in systemic hemodynamics or on in vitro studies that are limited by the artificial nature of the cell culture system. In order to define the role of these mediators in vivo, we have employed a novel experimental approach that utilizes the technology of in vivo genetic engineering to modify the expression of autocrine-paracrine factors within the vessel wall in the intact animal. We have demonstrated that transfecting the ACE gene into an intact rat carotid artery effectively increased the local expression of ACE within the vessel and thereby simulated the increase in local ACE activity observed in hypertensive vessels.[24] This increase in vascular ACE activity stimulated an increase in DNA synthesis that could be inhibited by an angiotensin type I receptor antagonist. Furthermore, the growth response stimulated by local generation of ang II induced the characteristic medial layer hypertrophy and increase in wall:lumen ratio observed in hypertensive vessels. It is noteworthy that the vascular remodeling response induced by a local increase in ACE expression occurred without effects on systemic hemodynamics or influences on the circulating renin-angiotensin system. Thus, this novel experimental approach provides the first direct evidence that the paracrine vascular angiotensin system has the capacity to induce the vascular remodeling characteristic of hypertensive vessels in vivo independent of an influence on systemic hemodynamics.

The current paradigm of the pathogenesis of vascular disease has often focused on the regulation of cell growth and matrix modifications as the critical pathobiologic processes involved in determining vessel structure. Although these processes are important, an exciting new area of research indicates that the paradigm of vascular remodeling and lesion formation must include the process of programmed cell death, or apoptosis. Apoptosis is a form of "cell suicide" in which a carefully regulated genetic program is activated that deletes a cell from a tissue without inducing an inflammatory response; it is therefore quite distinct from necrotic cell death. This powerful biologic process appears to play a crucial role in mediating changes in tissue architecture that occur during ontogeny as well as pathobiologic processes such as glomerulonephritis, acquired immunodeficiency syndrome (AIDS), and cancer. Indeed, recent studies of human vascular lesions have documented apoptosis in human atherosclerotic plaques and restenotic lesions after angioplasty.[25, 26]

Although the precise role of apoptosis as a determinant of vascular structure remains to be further defined, evidence indicates that cell-growth mediators such as PDGF are also important modulators of vascular cell programmed cell death.[27] Indeed, recent in vitro studies in our laboratory have shown that ang II is an effective inhibitor of vascular smooth muscle cell programmed cell death.[28] These in vitro observations have been confirmed by in vivo studies that have documented that the capacity of ACE inhibitors to induce the regression of vascular lesions is associated with increased apoptosis of vascular cells as well as the inhibition of cell growth.[29] Overall, these data suggest that ang II may promote vascular lesion formation by increasing the vascular cell population through two mechanisms: increased cell growth and decreased programmed cell death. One may speculate that the targeted induction of apoptosis may represent an exciting new therapeutic strategy for modifying cardiovascular tissue function and structure.

In addition to its effects on vascular cellularity, ang II may also mediate remodeling and lesion formation by altering extracellular matrix composition via its effect on thrombospondin, fibronectin, tenascin, glycosaminoglycans expression, and plasminogen activator activity.[30–32] Moreover, the migration of vascular smooth muscle cells and endothelial cells during structural modifications can be modulated by ang II.[33] Thus, ang II is a pleiotropic local mediator capable of modulating cell growth, apoptosis, migration, and matrix modulation—all the biologic mechanisms of vascular remodeling and intima formation. Similar pleiotropic effects on vascular smooth muscle cell behavior have been described for other vasoconstrictors, including norepinephrine, endothelin-1,and thromboxane.[34–36] Hence, vasoconstrictors may play an important role in determining vascular structure by influencing the various biologic mechanisms of vascular remodeling.

Mechanisms of Vascular Remodeling and Intimal Lesion Formation: Role of Nitric Oxide

Endogenous vasodilators such as NO and natriuretic peptides appear to have a countervailing influence to ang II as determinants of vascular architecture. Vasodilators generally inhibit vascular smooth muscle cell growth in in vitro models.[37,38] Recent studies suggest that vasodilators may also promote a decrease in vascular smooth muscle cellularity by inducing apoptosis.[29] Similarly, experiments performed with

intact animals have documented that the local generation of NO inhibits vascular lesion formation after vessel injury.[39] Moreover, under certain circumstances NO may alter matrix composition by modulating the activity of the metalloproteinases that degrade matrix proteins.[40] Thus, NO appears to inhibit increases in vascular smooth muscle cellularity and expansion of the extracellular matrix associated with hypertensive vascular remodeling and atherosclerotic lesion formation.

The process of vascular remodeling is particularly important as a determinant of lumen size. One of the best examples of the plasticity of the vasculature is evident from the flow-stimulated remodeling response induced by an arteriovenous shunt. The factors that induce the enlargement of lumen dimensions under these circumstances have not been characterized. However, recent experimental studies have shown that if the well-described flow-stimulated increase in NO generation[41] is prevented by pharmacologic inhibitors, the vessel chronically exposed to increased flow fails to undergo appropriate enlargement remodeling.[42] It has also been observed that chronic pharmacologic blockade of NO generation results in a hypertensive state characterized by fibrosis and shrinkage remodeling within the coronary microvasculature.[8] Taken together, these observations suggest that decreased NO generation is associated with shrinkage remodeling, whereas increased NO generation is associated with enlargement remodeling. Thus, several lines of evidence indicate that NO is an endogenous inhibitory factor that attenuates the process of occlusive vascular lesion formation characteristic of hypertensive and atherosclerotic disease.[43]

Endothelial Dysfunction: An Imbalance in Reactive Nitrogen and Oxygen Species

The endothelium is a multifactorial determinant of tissue function via its regulation of vessel tone, thrombosis, inflammation, and structure. The normal endothelium appears to have an intrinsic capacity to prevent vascular disease. However, an impairment of endothelial function manifested as abnormal endothelium-dependent vasorelaxation has been documented in a variety of vascular diseases, including hypertension and atherosclerosis in both animal models and humans.[44–46] In fact, this perturbation has been described in normotensive subjects who merely have a positive family history of risk factors such as hypertension.[47] Thus, in many cases the onset of endothelial dysfunction may precede the development of clinically evident vascular disease. Unfortunately, the molecular basis of endothelial dysfunction in vascular disease remains to be further defined.

Although there are several potential etiologies of decreased NO bioactivity, several lines of evidence suggest that increased catabolism of NO may be a principal factor in promoting endothelial dysfunction. It is important to emphasize that NO is itself a free radical—a highly reactive nitrogen species. Consequently, the biologic function of this vasoactive factor is determined in large part by the redox state of the tissue.[48] An increase in oxidative stress will mitigate the vasodilatory bioactivity of NO. A potential role for the redox state as a determinant of vascular homeostasis is demonstrated by animal model studies in which administration of antioxidants such as superoxide dismutase induced a lowering of blood pressure.[49] This antihypertensive effect is mediated in part by enhancing the bioactivity of NO. In support of this hypothesis, an increase in superoxide anion generation has been documented in the vasculature of genetically hypertensive animals compared with normotensive controls.[50] Moreover, recent in vivo studies have shown that the hypertensive state induced by infusion of ang II is due in part to increased generation of the free radical superoxide anion.[51] This ang II-stimulated increase in oxidative stress potentiates the direct vasoconstrictor effects of ang II by promoting increased catabolism of NO and endothelial dysfunction. Thus, in addition to its direct vasoconstrictive effects, ang II appears to promote chronic hypertension by modulating the vascular redox state and promoting the catabolism of the vasodilator NO.

The balance between NO and reactive oxygen species may also be an important role determinant of vessel structure. In vitro studies have documented that reactive oxygen species may function as signaling molecules that regulate vascular cell growth and programmed cell death.[52, 53] In fact, the growth-stimulatory effects of ang II on vascular smooth muscle cells appear to be mediated in part by the induction of reactive oxygen species that function as signaling molecules.[52] Similarly, the generation of reactive oxygen species may promote atherosclerosis by several mechanisms, including oxidation of low-density lipoprotein cholesterol and upregulation of leukocyte adhesion molecules and chemokines.[54] Thus, the development of endothelial dysfunction characterized by an imbalance between NO and reactive oxygen species may be an important pathogenic event in hypertension that determines the level of the blood pressure, promotes alterations in vessel structure, and contributes to clinical complications such as coronary artery disease.

One may speculate that the endothelium may be a new target for therapeutic interventions that will alter the course of vascular disease. Indeed, one of the salutary effects of antihypertensive treatment is the reversal of endothelial dysfunction.[45, 55] Future studies will further clarify the role of endothelial dysfunction in the natural history of hypertensive vascular disease and the clinical implications of reversing this abnormality.

Altering the Path of Vascular Disease: Potential Role of Angiotensin-Converting Enzyme Inhibition

As noted above, the generation of ang II is governed by both a circulating renin-angiotensin system and a tissue angiotensin system.[18] The tissue angiotensin system appears to be upregulated in the context of cardiovascular disease. Animal and human studies have documented increased expression of tissue ACE in the heart in the context of ventricular remodeling and heart failure postmyocardial infarction,[56–58] and expression of tissue ACE is increased in the vasculature in

the context of hypertension in various models.[2, 59] Moreover, we have recently documented that atherosclerotic human coronary vessels express high levels of ACE immunoreactivity and ang II within the plaque,[60] most prominently in the monocyte-macrophages that are major constituents of the plaque cellular population. Studies of human peripheral monocytes have also documented high levels of ACE expression and ang II within inflammatory cells.[61] Thus, the changes in ventricular and vascular structure observed under pathologic conditions are characterized by increased activity of a tissue angiotensin system. Given the capacity of ang II to modulate cell growth as well as programmed cell death, migration, and matrix modification, the blockade of ang II generation may have profound effects on ventricular and vascular structure and function.

Angiotensin-converting enzyme also functions as a kininase responsible for the degradation of bradykinin. Some of the most compelling evidence of the physiologic role of the kallikrein-kinin system in cardiovascular homeostasis has been provided by results in genetically engineered animal models. In these models, augmentation of kallikrein-bradykinin activity is associated with significant decreases in blood pressure.[62] Conversely, animals that lack the bradykinin type 2 receptor exhibit a hypertensive phenotype.[63] Thus, bradykinin appears to be an important modulator of vascular tone. This effect may be due in part to the fact that bradykinin is a potent inducer of NO generation. In vitro and in vivo studies have shown blocking bradykinin degradation by inhibiting ACE is an effective means of augmenting endothelial generation of NO.[64, 65]

Angiotensin-converting enzyme is strategically positioned to influence the activity of at least three local vasoactive systems—ang II, bradykinin, and NO. Accordingly, the various effects of blocking ACE on cardiovascular function and structure may be mediated in part by each or many of these factors. To the degree that vascular disease is characterized by an imbalance between a relative increase in ang II generation and a relative deficit of NO bioactivity, it is postulated that ACE inhibition may effectively restore the appropriate homeostatic balance between these vasoactive systems. This hypothesis, generated on the basis of animal model studies, has recently been tested in clinical trials. Compelling evidence indicates that long-term administration of ACE inhibitors reverses endothelial dysfunction in patients with either hypertension or atherosclerotic vascular disease.[44, 45 ,65] Thus, the beneficial effects of ACE inhibition may relate in part to changes in endothelial function that involve coordinate changes in the relative balance between ang II, bradykinin, and NO.

Angiotensin–converting enzyme inhibitors appear to have particular efficacy in reversing vascular remodeling and preventing the eventual development of hypertension in genetically predisposed animals[19, 66] and in clinical studies of patients with essential hypertension.[20, 67–69] This efficacy exists even compared with other antihypertensive agents. Such observations support the hypothesis that antihypertensive agents that reduce blood pressure and reverse the remodeling process may change the natural history of the disease.

Experimental studies suggest that alterations in microvascular structure within the kidney are important in the development of renal dysfunction and eventual organ failure in hypertensive patients, and ang II may have an important pathogenic role in the progression of this form of renovascular disease. Clinical studies have confirmed that ACE inhibitors have particular efficacy in modifying the natural history of renovascular diseases such as insulin-dependent diabetes,[70] noninsulin-dependent diabetes,[71] and various etiologies of glomerular damage.[72]

Angiotensin-converting enzyme inhibitors are the vasodilators of choice in altering the natural history of congestive heart failure due to their influence on ventricular remodeling.[73] In several clinical trials in patients with left ventricular dysfunction, ACE inhibition reduced the incidence of recurrent myocardial infarctions, indicating that ACE inhibitors may alter the natural history of coronary artery disease,[74] possibly via direct effects on coronary vascular function and structure. While the mechanisms are not well understood, animal model studies indicate that ACE inhibition within the heart enhances NO generation from coronary microvessels, an effect that is mediated via the accumulation of bradykinin.[64, 65] The clinical significance of this observation has been substantiated by the recent observation that ACE inhibition reverses endothelial dysfunction in patients with coronary atherosclerosis.[44] These findings suggest that ACE inhibition has a salutary effect on coronary blood flow and reactivity in patients susceptible to myocardial ischemia.

Other experimental studies have recently documented that ACE inhibition reduces myocardial oxygen consumption in association with an increase in NO generation.[64] Such findings are consistent with previous studies demonstrating that NO has a direct effect on muscle oxidative metabolism. These observations raise the possibility that ACE inhibition may prevent myocardial ischemia by optimizing the balance between myocardial oxygen supply and demand. Two mechanisms may be involved: enhancing blood flow and reducing myocardial oxygen demands. This response may be useful in reducing the sequelae of chronic ischemic heart disease.

Finally, the ultimate goal in altering the path of coronary heart disease is to prevent acute ischemic syndromes such as unstable angina and myocardial infarction. Pathologic studies indicate that these episodes are related to two phenomena—plaque rupture and plaque erosion.[75, 76] Plaque rupture is the most prevalent etiology of acute coronary thrombosis, accounting for 60% of cases in an autopsy series. Plaque rupture involves an inflammatory process in which leukocytes infiltrating the plaque promote increased expression and activity of metalloproteinases which may weaken the integrity of the thin fibrous cap and predispose it to rupture.[77] There are several reasons why ang II may contribute to this pathogenic process. Ang II stimulates the redox-sensitive, proinflammatory transcription factor NFkB, which, in turn, induces the coordinate up-regulation of cytokines, chemoattractants, and leukocyte adhesion molecules that promote the local inflammatory response within the vascular lesion.[78–80] Furthermore, the oxidative stress induced by factors such as ang II may pos-

sibly activate the metalloproteinases expressed within the plaque and potentially induce plaque rupture.[40, 51, 52] This proinflammatory effect of angiotensin that may predispose to plaque rupture can be counteracted by the actions of NO, which inhibits transcription factor NFkB and downregulates the expression of inflammatory cytokines, chemoattractants, and leukocyte adhesion molecules.[81, 82] Thus, the ang II-NO balance may be critical to modulating the propensity of lesions to rupture and cause acute ischemic syndromes.

In contrast to plaque rupture, the pathogenesis of plaque erosion is characterized by endothelial cell loss, exposure of the procoagulant subendothelial space, and in situ thrombosis. The cause of endothelial cell loss is unknown, but it is intriguing to speculate that this denudation of the endothelium may result from endothelial cell death by apoptosis. In this regard it is noteworthy that ang II reduces the capacity of the endothelium to regenerate by inhibiting cell replication via the angiotensin type 2 receptor.[83] Moreover, the angiotensin type 2 receptor can mediate cell loss by inducing apoptosis.[84] Conversely, recent studies indicate that NO preserves the integrity of the endothelium by enhancing regeneration[85] and preventing endothelial cell apoptosis in response to cytotoxic cytokines.[86] These findings support the concept that the maintenance of the appropriate ang II-NO balance may play an important role in vascular homeostasis and the prevention of acute ischemic events.

Clinical studies are under way that will directly test the hypothesis that chronic administration of ACE inhibitors in normotensive subjects with coronary disease will prevent ischemic events. It is hoped that these studies will move us closer to developing pharmacotherapies that modify the molecular events that eventually cause end-stage heart disease

Conclusion

The current challenge facing clinicians is to develop pharmacotherapies that move beyond the treatment of symptoms toward an agenda in cardiovascular therapeutics in which interventions actually prevent the development of end-stage coronary heart disease. The development of new strategies to alter the natural history of cardiovascular disease will be fostered by insights into the fundamental pathobiologic mechanisms that promote the morbidity and mortality of these disorders. An emerging body of evidence indicates that locally generated vasoactive substances such as ang II and NO are important determinants of the natural history of vascular disease. It is anticipated that ongoing clinical trials will extend the concept that modulating the activity of vasoactive substances generated by the endothelium has important implications for altering the course of coronary heart disease.

References

1. Gibbons GH, Dzau VJ: The emerging concept of vascular remodeling. *N Engl J Med* 1994;330:1431–1438
2. Gibbons GH: Mechanisms of vascular remodeling in hypertension: role of autocrine–paracrine vasoactive factors. *Curr Opin Nephrol Hypertens* 1995;4:189–196
3. Folkow B: Physiological aspects of primary hypertension. *Physiol Rev* 1982;62:347–504
4. Heagerty AM, Aalkjaer C, Bund SJ, Korsgaard N, Mulvany MJ: Small artery structure in hypertension: Dual processes of remodeling and growth. *Hypertension* 1993;21:391–397
5. Sivertsson R: The hemodynamic importance of structural vascular changes in essential hypertension. *Acta Physiol Scand* 1970; 343(suppl):1–56
6. Cook TA, Yates PO: A histometric study of cerebral and renal arteries in normotensives and chronic hypertensives. *J Pathol* 1972;108:129–135
7. Fazekas F, Kleinart R, Offenbacher H, Payer F, Schmidt, Kleinart G, Radner H, Lechner H: The morphologic correlate of incidental punctate white matter hyperintensities on MR images. *Am J Nephrol Res* 1991;12:915–921
8. Ito A, Egashira K, Kadokami T, Fukumoto Y, Takayanagi T, Nakaike R, Kuga T, Sueishi K, Shimokawa H, Takeshita A: Chronic inhibition of endothelium–derived nitric oxide synthesis causes coronary microvascular structural changes and hyperreactivity to serotonin in pigs. *Circulation* 1995;92(9):2636–2644
9. Liao Y, Cooper RS, McGee DL, Mensah GA, Ghali JK: The relative effects of left ventricular hypertrophy, coronary artery disease, and ventricular dysfunction on survival among black adults. *J Am Med Assoc* 1995;273(20):1592–1597
10. Hampton JA, Bernardo DA, Khan NA, Lacher DA, Rapp JP, Gohara AF, Goldblatt PJ: Morphometric evaluation of the renal arterial system of Dahl salt–sensitive and salt–resistant rats on a high salt diet. II: Interlobular arteries and intralobular arterioles. *Lab Invest* 1989;60(6):839–846
11. Klag MJ, Whelton PK, Randall BL, Neaton JD, Brancati FL, Ford CE, Shulman NB, Stamler J: Blood pressure and end-stage renal disease in men. *N Engl J Med* 1996;334(1):13–18
12. Boutouyrie P, Laurent S, Girerd X, Benetos A, Lacolley P, Abergel E, Safar M: Common carotid artery stiffness and patterns of left ventricular hypertrophy in hypertensive patients. *Hypertension* 1995;25(4, pt 1):651–659
13. Mintz GS, Popma JJ, Pichard AD, Kent KM, Satler LF, Wong C, Hong MK, Kovach JA, Leon MB: Arterial remodeling after coronary angioplasty: A serial intravascular ultrasound study. *Circulation* 1996;94:35–43
14. Lim TT, Liang DH, Botas J, Schroeder JS, Oesterle SN, Yeung AC: Role of compensatory enlargement and shrinkage in transplant coronary artery disease: Serial intravascular ultrasound study. *Circulation* 1997;95:855–859
15. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ: Compensatory enlargement of human atherosclerotic coronary arteries. *N Engl J Med* 1987;316:1371–1375
16. Hermiller JB, Tenaglia AN, Kisslo KB, Phillips HR, Bashore TM, Stack RS, Davidson CJ: In vivo validation of compensatory enlargement of atherosclerotic coronary arteries. *Am J Cardiol* 1993; 71:665–668
17. Chobanian AV, Alexander RW: Exacerbation of atherosclerosis by hypertension: Potential mechanisms and clinical implications. *Arch Intern Med* 1996;156(17):1952–1956
18. Dzau VJ, Re R: Tissue angiotensin system in cardiovascular medicine: A paradigm shift? *Circulation* 1994;89:493–498
19. Thybo NK, Korsgaard N, Eriksen S, Christensen KL, Mulvany MJ: Dose–dependent effects of perindopril on blood pressure and small artery structure. *Hypertension* 1994;23:659–666
20. Thybo NK, Stephens N, Cooper A, Aalkjaer C, Heagerty AM, Mulvany MJ: Effect of antihypertensive treatment on small arteries of patients with previously untreated essential hypertension. *Hypertension* 1995;25(4, pt 1):474–481
21. Itoh H, Mukoyama M, Pratt RE, Gibbons GH, Dzau VJ: Multiple autocrine growth factors modulate vascular smooth muscle cell

growth response to angiotensin II. *J Clin Invest* 1993;91: 2268–2274
22. Sudhir K, Wilson E, Chatterjee K, Ives HE: Mechanical strain and collagen potentiate mitogenic activity of angiotensin II in rat vascular smooth muscle cells. *J Clin Invest* 1993;92:3003–3007
23. Griendling KK, Ushio–Fukai M, Lassegue B, Alexander RW: Angiotensin II signaling in vascular smooth muscle: New concepts. *Hypertension* 1997;29(part 2):366–373
24. Morishita R, Gibbons GH, Ellison KE, Lee W, Zhang L, Yu H, Kaneda Y, Ogihara T, Dzau VJ: Evidence for direct local effect of angiotensin in vascular hypertrophy: In vivo gene transfer of angiotensin converting enzyme. *J Clin Invest* 1994;94:978–984
25. Isner JM, Kearney M, Bortman S, Passeri J: Apoptosis in human atherosclerosis and restenosis. *Circulation* 1995;91(11):2703–2711
26. Geng YJ, Libby P: Evidence for apoptosis in advanced human atheroma: Colocalization with interleukin–1 beta–converting enzyme. *Am J Pathol* 1995;147(2):251–266
27. Bennett MR, Evan GI, Schwartz SM: Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. *J Clin Invest* 1995;95(5):2266–2274
28. Pollman MJ, Yamada T, Horiuhi M, Gibbons GH: Vasoactive substances regulate vascular smooth muscle cell apoptosis: Countervailing influences of nitric oxide and angiotensin II. *Circ Res* 1996;79:748–756
29. de Blois D, Tea BS, Dam TV, Tremblay J, Hamet P: Smooth muscle apoptosis during vascular regression in spontaneously hypertensive rats. *Hypertension* 1997;29:340–349
30. Levy BI, Benessiano J, Henrion D, Caputo L, Heymes C, Duriez M, Poitevin P, Samuel JL: Chronic blockade of AT2–subtype receptors prevents the effect of angiotensin II on the rat vascular structure. *J Clin Invest* 1996;98(2):418–425
31. van Leeuwen RTJ, Kol A, Andreotti F, Kluft C, Maseri A, Sperti G: Angiotensin II increases plasminogen activator inhibitor type 1 and tissue-type plasminogen activator messenger RNA in cultured rat aortic smooth muscle cells. *Circulation* 1994;90:362–368
32. de Blois D, Lambardi DM, Su EJ, Clowes AW, Schwartz SM, Giachelli CM: Angiotensin II induction of osteopontin expression and DNA replication in rat arteries. *Hypertension* 1996;28: 1055–1063
33. Dubey RK, Jackson EK, Lüscher TF: Nitric oxide inhibits angiotensin II-induced migration of rat aortic smooth muscle cell: Role of cyclic-nucleotides and angiotensin1 receptors. *J Clin Invest* 1995;96(1):141–149
34. Majesky MW, Daemen MJAP, Schwartz SM: Alpha 1-adrenergic stimulation of platelet-derived growth factor A-chain gene expression in rat aorta. *J Biol Chem* 1990;265:1082–1088
35. Bevan RD: Trophic effects of peripheral adrenergic nerves on vascular structure. *Hypertension* 1984;6(suppl III):III–19–III–26
36. Li JS, Larivière R, Schiffrin EL: Effect of a nonselective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate-salt hypertensive rats: Evidence for a role of endothelin in vascular hypertrophy. *Hypertension* 1994;24:183–188
37. Garg UC, Hassid A: Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 1989;83:1774–1777
38. Morishita R, Gibbons GH, Pratt RE, Tomita N, Kaneda Y, Ogihara T, Dzau VJ: Autocrine and paracrine effects of atrial natriuretic peptide gene transfer on vascular smooth muscle and endothelial cellular growth. *J Clin Invest* 1994;94:824–829
39. von der Leyen H, Gibbons GH, Morishita R, Lewis NP, Zhang L, Kaneda Y, Cooke JP, Dzau VJ: Gene therapy inhibiting neointimal vascular lesion: In vivo transfer of endothelial cell nitric oxide synthase gene. *Proc Natl Acad Sci USA* 1995;92:1137–1141
40. Rajagopalan S, Meng SXP, Ramasamy S, Harrison DG, Galis Z: Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro: Implications for atherosclerotic plaque stability. *J Clin Invest* 1996;98:2572–2579
41. Ohno M, Gibbons GH, Dzau VJ, Cooke JP: Shear stress elevates endothelial cGMP: Role of a potassium channel and G protein coupling. *Circulation* 1993;88(1):193–197
42. Tronc F, Wassef M, Esposito B, Henrion D, Glagov S, Tedgui A: Role of NO in flow-induced remodeling of the rabbit common carotid artery. *Arterioscler Thromb Vasc Biol* 1996;16(10): 1256–1262
43. Moroi M, Gold HK, Yasuda T, Fishman MC, Huang PL: Mice mutant in endothelial nitric oxide synthase: Vessel growth and response to injury (abstr). *Circulation* 1996;94:I–154
44. Mancini GB, Henry GC, Macaya C, O'Neill BJ, Pucillo AL, Carere RG, Wargovich TJ, Mudra H, Lüscher TF, Klibaner MI, Haber HE, Uprichard ACG, Pepine CJ, Pitt B: 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(3):258–265
45. Iwatsubo H, Nagano M, Sakai T, Kumamoto K, Morita R, Higaki J, Ogihara T, Kata T: Converting enzyme inhibitor improves forearm reactive hyperemia in essential hypertension. *Hypertension* 1997; 29(part 2):286–290
46. Panza JA, Garcia CE, Kilcoyne CM, Quyyumi AA, Cannon RO III: Impaired endothelium–dependent vasodilation in patients with essential hypertension: Evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. *Circulation* 1995;91(6):1732–1738
47. Taddei S, Virdis A, Mattei P, Arzilli F, Salvetti A: Endothelium-dependent forearm vasodilation is reduced in normotensive subjects with familial history of hypertension. *J Cardiovasc Pharmacol* 1992;20(suppl 12):S193–S195
48. Stamler JS: Redox signaling: Nitrosylation and related target interactions of nitric oxide. *Cell* 1994;78(6):931–936
49. Nakazono K, Watanabe N, Matsuno K, Sasaki J, Sato T, Inoue M: Does superoxide underlie the pathogenesis of hypertension? *Proc Natl Acad Sci USA* 1991;88(22):10045–10048
50. Tschudi MR, Mesaros S, Lüscher TF, Malinski T: Direct in situ measurement of nitric oxide in mesenteric resistance arteries: Increased decomposition by superoxide in hypertension. *Hypertension* 1996;27(1):32–35
51. 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(8):1916–1923
52. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, Griendling KK: p22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. *J Biol Chem* 1996;271:23317–23321
53. Tsai JC, Jain M, Hsieh CM, Lee WS, Yoshizumi M, Patterson C, Perrella MA, Cooke C, Wang H, Haber E, Schlegel R, Lee ME: Induction of apoptosis by pyrrolidinedithiocarbamate and N-acetylcysteine in vascular smooth muscle cells. *J Biol Chem* 1996;271(7):3667–3670
54. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ: Atherosclerosis: Basic mechanisms. Oxidation, inflammation, and genetics. *Circulation* 1995; 91(9):2488–2496
55. Lüscher TF, Vanhoutte PM, Raij L: Antihypertensive treatment normalizes decreased endothelium-dependent relaxations in rats with salt-induced hypertension. *Hypertension* 1987;9(suppl III): III-193–III-197
56. Sun Y, Weber KT: Angiotensin converting enzyme and myofibroblasts during tissue repair in the rat heart. *J Mol Cell Cardiol* 1996;28(5):851–858
57. Zisman LS, Abraham WT, Meixell GE, Vamvakias BN, Quaife RA, Lowes BD, Roden RL, Pecock 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(3):1490–1498
58. 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(7):1513–1518
59. 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(2):278–287
60. 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(11):2756–2767
61. Kitazono T, Padgett RC, Armstrong ML, Tompkins PK, Heistad DD: Evidence that angiotensin II is present in human monocytes. *Circulation* 1995;91(4):1129–1134
62. Xiong W, Chao J, Chao L: Muscle delivery of human kallikrein gene reduces blood pressure in hypertensive rats. *Hypertension* 1995;25(4, pt 2):715–719
63. Alfie ME, Yang X, Hess F, Carretero OA: Salt-sensitive hypertension in bradykinin B-2 receptor knockout mice. *Biochem Biophys Res Commun* 1996;224:625–630
64. Zhang X, Xie YW, Nasjletti A, Xu X, Wolin MS, Hintze TH: ACE inhibitors promote nitric oxide accumulation to modulate myocardial oxygen consumption. *Circulation* 1997;95(1):176–182
65. 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
66. Harrap SB, Van der Merwe WM, Griffin SA, Macpherson F, Lever AF: Brief angiotensin converting enzyme inhibitor treatment in young spontaneously hypertensive rats reduces blood pressure long-term. *Hypertension* 1990;16:603–614
67. Dahlof B, Hansson L: The influence of antihypertensive therapy on the structural arteriolar changes in essential hypertension: Different effects of enalapril and hydrochlorothiazide. *J Intern Med* 1993; 234:271–279
68. Thybo NK, Stephens N, Cooper A, Aalkjaer C, Heagerty AM, Mulvany MJ: Effect of antihypertensive treatment on small arteries of patients with previously untreated essential hypertension. *Hypertension* 1995;25(4, pt 1):474–481
69. Skov K, Fenger-Gron J, Mulvany MJ: Effects of an angiotensin-converting enzyme inhibitor, a calcium antagonist, and an endothelin receptor antagonist on renal afferent arteriolar structure. *Hypertension* 1996;28(3):464–471
70. 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(20): 1456–1462
71. Ravid M, Lang R, Rachmani R, Lishner M: Long-term renoprotective effect of angiotensin-converting enzyme inhibition in non-insulin-dependent diabetes mellitus: A 7-year follow-up study. *Arch Intern Med* 1996;156(3):286–289
72. Maschio G, Alberti D, Janin G, Locatelli F, Mann JF, Motolese M, Ponticelli C, Ritz E, Zucchelli P: Effect of the angiotensin-converting-enzyme inhibitor benazepril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group. *N Engl J Med* 1996;334(15):939–945
73. Rutherford JD, Pfeffer MA, Moyé LA, Davis BR, Flaker GC, Kowey PR, Lamas GA, Miller HS, Packer M, Rouleau JL, : Effects of captopril on ischemic events after myocardial infarction: Results of the Survival and Ventricular Enlargement trial. SAVE Investigators. *Circulation* 1994;90(4):1731–1738
74. Pepine CJ: Ongoing clinical trials of angiotensin-converting enzyme inhibitors for treatment of coronary artery disease in patients with preserved left ventricular function. *J Am Coll Cardiol* 1996; 27(5):1048–1052
75. Farb A, Burke AP, Tang AL, Liang TY, Mannan P, Smialek J, Virmani R: Coronary plaque erosion without rupture into a lipid core: A frequent cause of coronary thrombosis in sudden coronary death. *Circulation* 1996;93(7):1354–1363
76. Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R: Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med* 1997;336(18): 1276–1282
77. Fuster V: Elucidation of the role of plaque instability and rupture in acute coronary events. *Am J Cardiol* 1995;76(9):24C–33C
78. Hernandez-Presa M, Bustos C, Ortego M, Tunon J, Renedo G, Ruiz-Ortega M, Egido J: Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-kappa B activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. *Circulation* 1997;95(6):1532–1541
79. Li J, Brasier AR: Angiotensinogen gene activation by angiotensin II is mediated by the rel A (nuclear factor-kappaB p65) transcription factor: One mechanism for the renin angiotensin system positive feedback loop in hepatocytes. *Mol Endocrinol* 1996;10(3): 252–264
80. Collins T, Read MA, Neish AS, Whitley MZ, Thanos D, Maniatis T: Transcriptional regulation of endothelial cell adhesion molecules: NF-kappa B and cytokine-inducible enhancers. *FASEB J* 1995;9(10):899–909
81. Khan BV, Harrison DG, Olbrych MT, Alexander RW, Medford RM: Nitric oxide regulates vascular cell adhesion molecule 1 gene expression and redox-sensitive transcriptional events in human vascular endothelial cells. *Proc Natl Acad Sci USA* 1996; 93(17):9114–9119
82. De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA Jr, Shin WS, Liao JK: Nitric oxide decreases cytokine-induced endothelial activation: Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. *J Clin Invest* 1995;96(1):60–68
83. Stoll M, Steckelings UM, Paul M, Bottari SP, Metzger R, Unger T: The angiotensin AT2-receptor mediates inhibition of cell proliferation in coronary endothelial cells. *J Clin Invest* 1995;95(2): 651–657
84. Yamada T, Horiuchi M, Dzau VJ: Angiotensin II type 2 receptor mediates programmed cell death. *Proc Natl Acad Sci USA* 1996; 93(1):156–160
85. Ziche M, Morbidelli L, Masini E, Amerini S, Granger HJ, Maggi CA, Geppetti P, Ledda F: Nitric oxide mediates angiogenesis in vivo and endothelial cell growth and migration in vitro promoted by substance P. *J Clin Invest* 1994;94(5):2036–2044
86. Dimmeler S, Haendeler J, Nehls M, Zeiher AM: Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. *J Exp Med* 1997;185(4):601–607