Nitric Oxide, Angiotensin II, and Hypertension

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Although initially adaptive, the changes that accompany hypertension, namely, cell growth, endothelial dysfunction, and extracellular matrix deposition, eventually can become maladaptive and lead to end-organ disease such as heart failure, coronary artery disease, and renal failure. A functional imbalance between angiotensin II (Ang II) and nitric oxide (NO) plays an important pathogenetic role in hypertensive end-organ injury. NO, an endogenous vasodilator, inhibitor of vascular smooth muscle and mesangial cell growth, and natriuretic agent, is synthesized in the endothelium by a constitutive NO synthase. NO antagonizes the effects of Ang II on vascular tone, cell growth, and renal sodium excretion, and also down-regulates the synthesis of angiotensin-converting enzyme (ACE) and Ang II type 1 receptors. On the other hand, Ang II decreases NO bioavailability by promoting oxidative stress. A better understanding of the pathophysiologic mechanisms involved in hypertensive end-organ damage may aid in identifying markers of cardiovascular susceptibility to injury and in developing therapeutic interventions. We propose that those antihypertensive agents that lower blood pressure and concomitantly restore the homeostatic balance of vasoactive agents such as Ang II and NO within the vessel wall would be more effective in preventing or arresting end-organ disease.
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HYPERTENSION IS A MAJOR risk factor for coronary artery disease, congestive heart failure, cerebrovascular disease, peripheral vascular disease, and renal failure. Hypertension involves 3 major factors: abnormal vascular tone, abnormalities in volume and salt regulation, and vessel wall remodeling. Both nitric oxide (NO) and angiotensin II (Ang II) are important players in these pathogenetic mechanisms. The interactions of and balance between NO and Ang II are of key importance in hypertensive end-organ injury and are the focus of this review.

THE RENIN-ANGIOTENSIN AND NO SYSTEMS

The renin-angiotensin system (RAS) is an enzymatic cascade that starts with the cleavage of angiotensinogen by renin to form the inactive decapetide Ang I. Thereafter, Ang I is converted by angiotensin-converting enzyme (ACE) to form Ang II. ACE, or kininase, is a bivalent dipeptide carboxyl metallopeptidase present as a membrane-bound form in endothelial, epithelial, or neuroepithelial cells, including the heart, kidney, and brain, and as a soluble form in blood and numerous body fluids.[1] ACE cleaves the C-terminal dipeptide from Ang I and bradykinin. Thus, ACE strategically regulates the balance between the RAS and the kallikrein-kinin system.[2]

The main subtypes of Ang II receptors are Ang type 1 (AT_1) and Ang type 2 (AT_2). Both the AT_1 and AT_2 receptors belong to the superfamily of G-protein–coupled receptors that contain 7 transmembrane regions.[3] Their amino acid sequence seems to be highly conserved across species and across tissues within a species. AT_1 and AT_2 receptors have distinct signal transduction pathways. AT_1 receptor is distributed ubiquitously and abundantly in adult tissues, including blood vessels, heart, kidney, adrenal gland, liver, brain, and lung. The AT_1 receptor mediates all the classic and well-known effects of Ang II, such as an increase of blood pressure, vasoconstriction, cardiac contractility, aldosterone release from nerve endings, renal sodium and water reabsorption, and Ang II–induced growth in cardiovascular and renal tissues. NO can down-regulate AT_1 receptors in vascular tissue[4] and the adrenal gland[5] and mitigate the action of Ang II. Although AT_2 receptor actions have not been elucidated fully, AT_2 receptors have been associated with the synthesis and/or the release of both prostaglandins and NO and have been shown to exert antiproliferative effects.[6]

The circulating RAS participates in short-term regulation of the cardiovascular system, which becomes activated in acute conditions including hypotension, hypovolemia, and hemorrhage. In the case of chronic condition, including hypertension

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and chronic heart failure, activation of the RAS causes long-term regulation of cardiovascular homeostasis via sustained activation of local angiotensin and degradation of bradykinin, resulting in permanent structural changes.[2]

NO is synthesized in the endothelium by endothelial nitric oxide synthase (eNOS), which converts L-arginine to citrulline and NO. In endothelial cells, NOS is bound to cell membrane–associated caveolae.[7] Blood flow–induced shear stress on endothelial cells is the major physiologic stimulus for vascular NO production by eNOS.[8] This activation of eNOS has been shown to be directly dependent on phosphorylation by serine/threonine protein kinase Akt/PKB, and not primarily mediated by an increase in intracellular calcium levels.[9]

NO activates the enzyme soluble guanylate cyclase to generate the second messenger cyclic guanosine monophosphate (cGMP).[10] Activation of this enzyme is mediated by the binding of NO to the heme moiety of soluble guanylate cyclase to form the nitrosyl-heme adduct of soluble guanylate cyclase.[11] As a result, the heme iron is shifted out of the plane of the porphyrine ring configuration, which initiates the binding of guanosine triphosphate and the formation of cGMP. cGMP activates 2 specific cGMP-dependent protein kinases, protein kinase G (PKG) I and II. PKG I is the main kinase mediating vasodilatation and inhibition of platelet aggregation.[12]

Endothelial production of NO has become a major research area in vascular biology. Some of the most important effects that NO exerts in the vascular wall are potentially vasoprotective because these effects maintain important physiologic functions such as vasodilatation, anticoagulation, leukocyte adhesion, smooth muscle proliferation, and antioxidative capacity.[13]

INTERACTION OF ANG II AND NO IN THE REGULATION OF VASCULAR TONE

There is a countervailing interaction between endothelial NO and Ang II.[14-16] The balance between NO, Ang II, and vascular generation of reactive oxygen species appear crucial for maintaining the homeostasis of the cardiovascular and renal systems, particularly for regulation of vascular tone and modulation of growth-related pathologic changes.[14] During the pathogenesis of hypertension in both animal models and humans, this homeostatic balance becomes perturbed so that the actions of Ang II predominate over those of NO (Fig 1).[17]

ENDOTHELIAL FUNCTION

The endothelium is the site of the final step of synthesis of both Ang II and NO and a major site for their interaction. The vascular endothelium plays a key role in the control of vasomotor tone, local homeostasis, and vascular wall proliferation processes.[13] Endothelium-derived NO may contribute to the overall regulation of arterial blood pressure by virtue of its ability to relax vascular smooth muscle. Inhibition of NO production stimulates endothelial ACE activity and generation of Ang II and superoxide anion (O_2^-), induces vasoconstriction, and causes pronounced and sustained hypertension.[18,19] Mice lacking the eNOS gene have a slightly higher arterial blood pressure than wild-type animals,[20] whereas hypotension has been found in mice with overexpression of the NOS gene.[21]

Ang II has been shown to increase O_2^- production in rat vascular smooth muscle cells (VSMCs), mesangial cells, and human vascular endothelial cells through activation of the reduced forms of nicotinamide-adenine dinucleotide/nicotinamide-adenine dinucleotide phosphate oxidase.[22-24] Chemical antagonism between O_2^- and NO has been recognized as a potentially important modulator of vascular reactivity as well as being a source of peroxynitrite, a potent oxidant.[25] Increased production of O_2^- could be responsible for impairment of the balance between relaxing and contracting factors released from the endothelium, leading to expression of endothelium-dependent vasoconstriction and loss of endothelium-dependent vasorelaxation.[26,27] Furthermore, oxidative injury of endothelial cells induced by O_2^- converted to peroxynitrite, hydrogen peroxide, or hydroxyl radicals may inhibit production and release of relaxing factors. All of these properties support the concept that excessive production of O_2^- in endothelial cells may increase the tone of the underlying smooth muscle.[28]

This NO, Ang II, and O_2^- interaction in the regulation of vascular tone has been documented clearly in humans with increased Ang II owing to renovascular hypertension. Higashi et al[29] showed that the forearm blood flow in response to acetylcholine was lower in subjects with renovascular

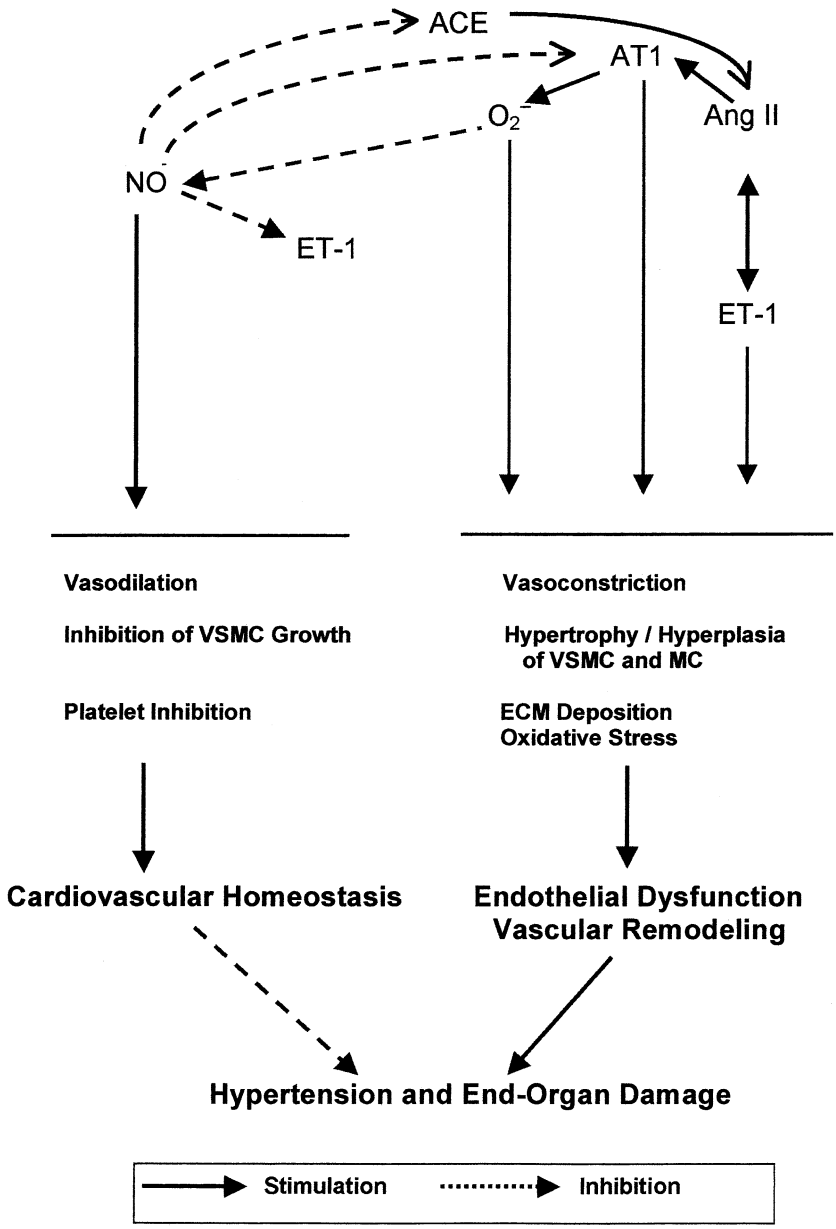

Fig 1. Imbalance among NO, Ang II, O_2^-, ET-1, and hypertensive end-organ injury. MC, mesangial cells.

hypertension than in controls. Angioplasty decreased systolic and diastolic blood pressures, forearm vascular resistance, and urinary excretion of 8-hydroxy-2′-deoxyguanosine and serum malondialdehyde-modified low-density lipoprotein, indexes of oxidative stress. Co-infusion of ascorbic acid (vitamin C) augmented the response of forearm blood flow to acetylcholine before angioplasty but not after angioplasty. These studies in patients with renovascular hypertension as well as ex vivo studies of human arteries have shown that the biological interaction between Ang II, O_2^-, and NO is fully operative in humans (Fig 2).

INTERACTION WITH ENDOTHELIN-1

The antagonistic effects between NO and Ang II appear also in interactions with other vasoactive substances. Endothelin-1 (ET-1) is a powerful vasoconstricting peptide released from the endothelium.[30] Ang II stimulates the endothelial cell to synthesize and release ET-1.[31] In Ang II–induced hypertension, ET-1 augments Ang II–induced va-

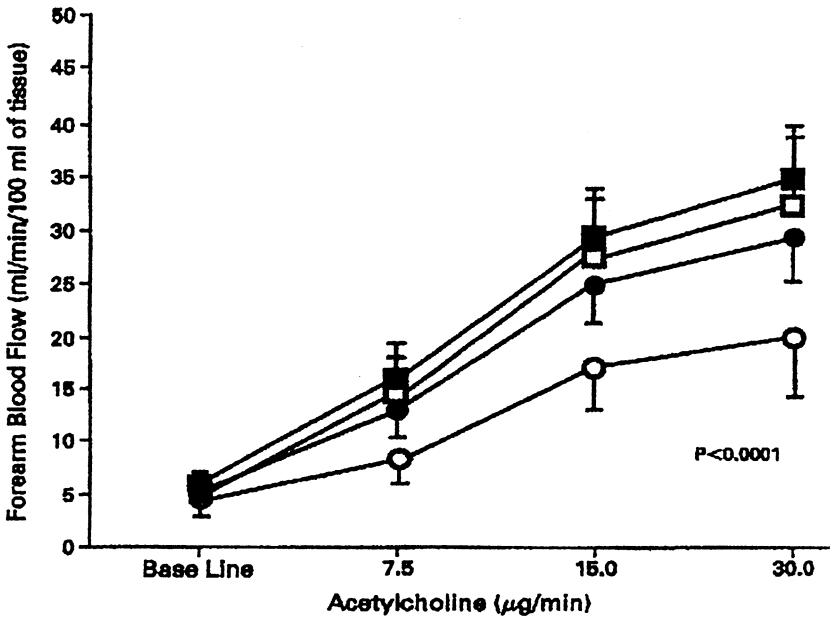

Fig 2. Effect of concomitant administration of the antioxidant ascorbic acid (24 mg/min, vitamin C) on the response of forearm blood flow to the administration of acetylcholine before and after angioplasty in subjects with renovascular hypertension. Data are expressed as mean ± SD. *P* < .0001, before angioplasty group compared with the other 3 groups. ○, Before angioplasty; ●, before angioplasty plus ascorbic acid; □, after angioplasty; ■, after angioplasty plus ascorbic acid. Reprinted with permission from Higashi Y, Sasaki S, Nakagawa K, et al: Endothelial function and oxidative stress in renovascular hypertension. N Engl J Med 346:1954-1962, 2002.[29] © 2002 Massachusetts Medical Society. All rights reserved.

soconstriction.[32] On the other hand, NO inhibits endothelial cell ET-1 production and action. In the porcine aorta, NOS inhibition augments the release of ET-1, whereas 8-bromo cGMP has an inhibitory effect on ET-1 release, suggesting that NO inhibits ET-1 production via a cGMP-dependent mechanism.[33] Selective endothelin A (ETA) or dual ETA/endothelin B (ETB) receptor antagonists blunt the acute pressor response caused by NOS inhibition,[34] whereas blockade of NO formation magnifies ET-1–induced vasoconstriction of various vascular territories.[35] NOS inhibition facilitates Ang II–related effects, which can be inhibited by both AT_1 receptor and endothelin receptor antagonists. Human and animal studies have suggested that there is a feedback mechanism between Ang II, endothelin, and NO synthesis that acts reciprocally to regulate vascular tone.[36,37]

REGULATION OF RENAL MICROCIRCULATION

In vivo studies have suggested that NO plays an important role in maintaining renal hemodynamics near the normal range in kidneys in response to increased Ang II levels.[38] In normotensive rat kidneys, intrarenal NO regulates afferent and efferent arteriolar tone and modulates both afferent and efferent arteriolar responsiveness to Ang II.[39] The relative degree to which NO affects the afferent and efferent arteriolar responsiveness to Ang II significantly affects renal function. In Ang II–infused hypertensive rats, inhibition of NOS with nitro-L-arginine (NLA) decreased afferent and efferent arteriolar diameters, and the decrease in diameter was significantly greater in afferent than in efferent arterioles. The addition of sodium nitroprusside, a NO donor, to increase local NO concentrations blunted the Ang II response in afferent arterioles but was not sufficient to alter the efferent arteriolar reactivity to Ang II. Thus, NO modulation of Ang II responsiveness is maintained in afferent but not efferent arterioles. The maintained NO-dependent tone and function in afferent arterioles may contribute to maintaining renal hemodynamics during the development of Ang II–dependent hypertension.[40]

INTERACTION OF ANG II AND NO IN CARDIOVASCULAR REMODELING

Vascular remodeling is an adaptive response of the vascular beds to alterations in blood flow or blood pressure.[41,42] Remodeling is dependent on a dynamic interaction between locally generated growth factors, vasoactive substances, and hemodynamic stimuli, and involves changes in cell growth, cell death, cell migration, and extracellular matrix (ECM) turnover. Although initially an adaptive process, remodeling contributes to hypertension and its complications, including left ven-

tricular hypertrophy, heart failure, myocardial ischemia, stroke, peripheral artery disease, and renal failure. The balance between NO and Ang II, which, as noted, plays a critical role in the modulation of vascular tone, also plays a predominant role in the regulation of cardiovascular remodeling.[43]

CARDIAC REMODELING

Left ventricular hypertrophy (LVH) is a well-established, strong, and independent risk factor for cardiovascular complications in hypertension, including heart failure and cardiac death.[44] LVH is characterized by an increase in myocyte size, myocyte gene reprogramming (enhanced expression of fetal phenotypes of genes such as β-myosin heavy chain), fibroblast proliferation, and an increased accumulation of ECM proteins, such as collagen types I and III and fibronectin, in the interstitium and around blood vessels within the heart.[45]

Accumulating in vitro and in vivo evidence show that Ang II, via the AT_1 receptor, is involved directly in all of these processes of cardiac remodeling independent of its effect of increasing blood pressure.[45] On the other hand, current data suggest that NO may inhibit the fibrotic and inflammatory cardiac changes thought to be an important pathologic process in several experimental models of hypertension.[46] Chronic inhibition of NO synthesis with oral administration of N^G-nitro-L-arginine methyl ester has been shown to increase cardiac tissue ACE activity, AT_1 receptor expression, gene expression of transforming growth factor-β 1 and ECM proteins, and activity of 70-kd S6 kinase, all thought to play a major role in cardiac remodeling.[18,19,46,47] Furthermore, treatment with ACE inhibitors or AT_1 receptor antagonists, but not hydralazine, prevented the N^G-nitro-L-arginine methyl ester–induced increases in the gene expression of transforming growth factor-β1 and ECM proteins, and also prevented the activation of 70-kd S6 kinase and the myocardial structural changes seen in this model.[18,47,48] The role of NO in mediating the antifibrotic and antihypertrophic effects of AT_2 receptor stimulation remains an active area of research.[49] A recent study showed in rat cardiomyocytes that stimulation of the AT_2 receptor by Ang II is accompanied by increased expression of eNOS, an effect mediated by the calcineurin pathway.[50] Moreover, in transgenic mice overexpressing the AT_2 receptor selectively in cardiomyocytes, the Ang II–induced perivascular fibrosis was attenuated significantly compared with wild-type mice, whereas there was no difference in the development of myocyte hypertrophy. Cotreatment with N^G-nitro-L-arginine methyl ester abolished the inhibition of perivascular fibrosis in the transgenic mice.[51] These findings suggest that an imbalance in the interplay of NO and Ang II may participate in the maladaptive cardiac remodeling that occurs in hypertension and restoration of this balance, in addition to blood pressure reduction, is necessary to prevent end-organ damage.

Studies in spontaneously hypertensive (SHR) and Dahl salt-sensitive (DS) rats, experimental models of salt-resistant and salt-sensitive hypertension, suggest that individual variability in susceptibility to hypertensive end-organ injury may be explained at least partially by genetic differences in vascular eNOS activity in response to hypertension. In SHR rats, left ventricular and aortic eNOS activity significantly increased compared with normotensive controls, whereas in hypertensive DS rats the left ventricular and aortic eNOS activity did not increase compared with normotensive controls.[14,52] At a similar blood pressure, hypertensive DS rats developed significantly greater LVH and aortic hypertrophy than SHR rats. Moreover, in DS rats, treatment with an ACE inhibitor and a diuretic normalized blood pressure, eNOS activity, and LVH, and reduced aortic hypertrophy. Thus, in susceptible individuals, decreased vascular NO bioavailability in response to a blood pressure increase may result in a relative increase in Ang II action that contributes to cardiac and vascular remodeling.

VASCULAR REMODELING

Increased peripheral vascular resistance is one of the hallmark features of hypertension. The resistance arteries, characterized by a lumen diameter of 100 to 350 μm, are the major determinants of peripheral resistance.[44,53] Structural changes in these resistance arteries are observed commonly in experimental animal models of hypertension and in human hypertension. The 2 alterations in vascular structure most commonly described are eutrophic and hypertrophic remodeling. Eutrophic remodeling describes the increased media to lumen ratio that results from a reduced outer diameter that narrows the lumen without net growth. In hypertrophic remodeling the growth of the media nar-

rows the lumen resulting in increased media cross-sectional area and media to lumen ratio.[54] There is increasing evidence that vascular remodeling in resistance arteries contributes to the development and complications of hypertension.[54] Furthermore, hypertension-induced remodeling in conduit arteries facilitates development of atherosclerosis and contributes to decreased vessel compliance, which results in increased systolic blood pressure.[53] The structural alterations exhibited in conduit arteries include increased lumen size and thickened media with increased ECM deposition. The functional balance between NO and Ang II plays a central role in the pathophysiology of vascular remodeling of resistance and conduit arteries through modulation of VSMC growth and ECM deposition.

The effect of Ang II on growth has been shown in vitro and in several in vivo models of hypertension and arterial injury.[42,45] Ang II regulates VSMC growth via direct and indirect pathways. On binding to the AT_1 receptor, Ang II stimulates the generation of inositol triphosphate and diacylglycerol, resulting in activation of calcium-dependent intracellular kinases and protein kinase C, respectively. Ang II also activates other growth-promoting kinases such as Raf-1, extracellular signal-regulated kinase (ERK)1/2, p38 mitogen-activated protein kinase, phosphatidylinositol 3-kinase (P13K), Akt, the Janus kinase (JAK)2, and tyrosine kinase (TYK)2. Additional growth mechanisms triggered by Ang II include production of growth factors such as ET-1, platelet-derived growth factor-A, platelet-derived growth factor-B, basic fibroblast growth factor (bFGF), insulin-like growth factor-1, transforming growth factor-β, vascular endothelial growth factor, and heparin-binding epidermal growth factor (HBEGF), and transactivation of growth factor receptors including the epidermal growth factor and platelet-derived growth factor receptors. Ang II also has been shown to stimulate production of several ECM components including fibronectin, collagen, laminin, and tenascin.[45,55] In vivo studies have shown that ACE inhibitors and AT_1-receptor blockers suppress these growth-promoting effects of Ang II in resistance and conduit arteries.[53,56-58] In addition, recent evidence suggests that the AT_2 receptor may counteract the growth-promoting effects of the AT_1 receptor. Studies in AT_1-receptor null mice and AT_2-receptor null mice have shown that Ang II stimulation of the AT_2 receptor exerts antiproliferative and pro-apoptotic effects in VSMCs and contributes to the decrease in neointimal formation in a cuff-induced vascular injury model.[59] Transfection of an AT_2-receptor expression vector into the rat carotid artery also was shown to attenuate neointimal formation in a balloon injury model.[42,60]

NO has been shown to inhibit the growth-stimulating effects of Ang II in conduit arteries, implying an antagonistic interaction between NO and Ang II in the modulation of vascular remodeling.[53,61,62] As opposed to Ang II, NO, generated by both eNOS or NO donors, exerts antiproliferative, antimitogenic, and antimigratory effects on cultured VSMCs.[13,63] In addition, NO has been shown to inhibit total protein and collagen synthesis in VSMCs[64] and to activate certain matrix metalloproteinases,[63] suggesting an antagonistic role to Ang II in the modulation of ECM turnover in the vessel wall. The inhibitory effects of NO on vascular wall growth have been verified in in vivo models of vascular injury by using NOS inhibitor–treated animals,[18,65,66] mice genetically deficient in eNOS,[63,67,68] transgenic mice that overexpress eNOS in the endothelium,[64] and animals that overexpress eNOS in VSMCs and/or the adventitia via eNOS gene transfer.[69,70] The mechanisms underlying these actions of NO have not been elucidated fully. Recent evidence suggests that activation of protein kinase A, at least partially mediated by cGMP-induced inhibition of phosphodiesterase III, contributes to the antiproliferative activity of NO by regulating the expression of cell cycle proteins and by inhibiting Raf-1.[13] The inhibition of arginase and ornithine decarboxylase by NO, independently of cGMP, is another mechanism shown to mediate the antiproliferative effects of NO on VSMCs.[13] The phosphorylation of ERK 1/2 is an important mediator of Ang II–induced VSMC growth. Blockade of the AT_1 receptor and the ERK 1/2 pathway both have been shown to attenuate this growth response to Ang II.[45,55] It has been reported recently that NO is an endogenous inhibitor of ERK 1/2 phosphorylation, thus counteracting the growth-promoting effects of Ang II in conduit arteries.[61]

In human hypertension, reactive oxygen species such as superoxide anion and hydrogen peroxide contribute to the pathogenesis of vascular remodeling and exacerbate cardiovascular damage. Ang II has been shown to activate the reduced form of nicotinamide-adenine dinucleotide phosphate oxidase in endothelial cells, VSMCs, adventitial fibro-

blasts, and glomerular mesangial cells.[14,24,71,72] The reduced form of nicotinamide-adenine dinucleotide phosphate oxidases produce superoxide anions that avidly interact with NO extracellularly and reduce its bioactivity, thereby counteracting the tonic inhibition on VSMC growth. Loss of NO bioactivity also alters vascular reactivity and contributes to the development and maintenance of hypertension. Moreover, O_2^- and hydrogen peroxide have been shown to be second messengers in the cascade of Ang II–initiated growth responses that participate in the pathologic vascular remodeling that occurs in hypertension.[42,71-73] Thus, vascular remodeling likely is dependent on the net balance between VSMC-generated reactive oxygen species, diffusible reactive oxygen species produced in the endothelium and adventitia, and diffusible endothelium-derived NO.

INTERACTION OF ANG II AND NO IN THE REGULATION OF SODIUM BALANCE

The capability of the kidneys to excrete sodium is considered to be an important determinant of arterial blood pressure. Studies performed in different animal models of hypertension suggest that a functional imbalance between the counteracting effects of Ang II and NO on tubular sodium reabsorption, renal hemodynamics, tubuloglomerular feedback, and pressure natriuresis plays an important pathogenetic role in the development of hypertension.[74]

MODULATION OF TUBULAR SODIUM REABSORPTIVE FUNCTION BY ANG II AND NO

In addition to its vasoconstricting and growth-promoting effects, Ang II indirectly and directly enhances both proximal and distal tubule reabsorptive function, thereby exerting its antinatriuretic effect. By modifying the production and release of aldosterone in the adrenal gland, Ang II indirectly promotes sodium retention.[75] The direct stimulatory action of Ang II on proximal tubule reabsorption is mediated by activation of the AT_1 receptor present on both the basolateral and luminal membranes.[76] Ang II increases sodium-potassium-adenosine triphosphate (Na^+/K^+ ATPase) activity and sodium/bicarbonate ($Na+/HCO_3^-$) cotransport on the basolateral membrane, and stimulates the sodium/hydrogen (Na^+/H^+) antiporter on the luminal membrane, thus increasing sodium, water, and bicarbonate reabsorption.[77]

Although the role of Ang II in the regulation of distal tubule reabsorptive function has not been elucidated fully, recent evidence suggests that luminal AT_1 receptors mediate Ang II–induced net bicarbonate and fluid reabsorption in these segments. In vivo micropuncture experiments showed that Ang II increases sodium/potassium/chloride transport in the medullary thick ascending loop of Henle.[77] Moreover, a recent study using isolated, perfused, cortical collecting duct segments also showed that Ang II directly stimulates epithelial sodium channel activity via AT_1 receptor binding.[78]

The AT_2 receptor also is present in the kidney and may contribute to the stimulatory action of Ang II on proximal tubule reabsorptive function because both AT_1 and AT_2 receptor antagonists have been shown to independently and synergistically inhibit the proximal tubule reabsorption rate.[76] On the other hand, the AT_2 receptor may play a role in counteracting the antinatriuretic effect of Ang II. In vivo renal microdialysis experiments suggested that sodium depletion or Ang II administration augmented renal NO production through activation by Ang II of the AT_2 receptor.[79] These findings imply a central role of the AT_2 receptor in maintaining a functional balance between the opposing effects of Ang II and NO on tubular reabsorptive function.

Studies evaluating the effect of intrarenal NOS inhibition indicated that NO plays a key role in the preservation of normal renal excretory function.[80] Furthermore, NO administered intrarenally has been shown to serve as a diuretic and natriuretic agent.[80] Experimental evidence from proximal tubule and cortical collecting duct cells and isolated, perfused, proximal tubule and collecting duct segments showed that this effect of NO is mediated by direct inhibition of epithelial transport mechanisms.[81] NO inhibits the Na^+/H^+ antiporter on the luminal membrane of the proximal tubule and attenuates the Na+/K+ ATPase activity on the basolateral membrane of the proximal tubule and collecting duct segments.[82] However, accumulating experimental evidence suggests that the effects of intrarenal NOS inhibitors or NO donors on tubular reabsorptive function also are mediated indirectly by the associated changes in peritubular hemodynamics or interstitial pressure.[80,83]

MODULATION OF RENAL HEMODYNAMICS BY ANG II AND NO

The counterbalancing effects of Ang II and NO also are evident in the renal microcirculation. It has been well established that Ang II causes a dose-dependent decrease in renal blood flow, a smaller reduction in glomerular filtration rate, and an increase in filtration fraction.[76] These effects are mediated by AT_1-receptor–induced vasoconstriction of the afferent and efferent arterioles, thereby increasing both pre- and postglomerular resistances. This renal hemodynamic response to Ang II is similar to the renal hemodynamic response to acute systemic NOS inhibition, implying that NO may counteract the vascular actions of Ang II.[82]

The functional balance between Ang II and NO influences medullary hemodynamics to a greater extent than cortical hemodynamics.[80,84] In vitro and in vivo studies have revealed a higher density of Ang II receptors and a greater content of Ang II in the medulla than in the cortex.[84] In normotensive and hypertensive rats, Ang II stimulates medullary NO generation, which in turn prevents its vasoconstricting effects on the medullary circulation.[82,85,86] Moreover, recent reports have suggested that an increased susceptibility to the hypertensive actions of Ang II may result from an impaired NO counterregulatory system in the medulla.[86,87]

MODULATION OF THE TUBULOGLOMERULAR FEEDBACK (TGF) MECHANISM BY ANG II AND NO

TGF describes the mechanism whereby increases or decrease in sodium chloride delivery to the macula densa transmit a signal to the afferent arterioles to constrict or dilate, respectively, to maintain stability of the filtered load. Systemic or peritubular capillary infusion of Ang II has been shown to enhance the sensitivity of the TGF mechanism, whereas inhibitors of the RAS have been shown to exert the opposite effect.[76] Current data indicate that Ang II, via the AT_1 receptor, amplifies TGF responsiveness by stimulating macula densa transport of sodium chloride and not by directly constricting the afferent arteriole.[77] The role of Ang II as a modulator of TGF sensitivity has been confirmed in studies of AT_1-receptor knockout and ACE-deficient mice, which have been shown to have a markedly attenuated TGF response to increases in distal nephron sodium chloride delivery.[88,89] Micropuncture experiments also have shown that in the presence of a NOS inhibitor, the effect of Ang II on TGF responsiveness is enhanced significantly. In these studies, AT_1-receptor blockade suppressed the Ang II–induced TGF activation during NO inhibition.[90] An increase in sodium chloride delivery to the macula densa has been shown to stimulate apical sodium-hydrogen exchange, which in turn promotes NO generation by neuronal NO synthase.[80,91] NO is considered to be an important modulator of TGF responsiveness, at least partially, by counteracting the vasoconstrictor stimuli mediating TGF responses.[80] Collectively, these findings imply that the functional balance between Ang II and NO plays a central role in maintaining the sensitivity of the TGF mechanism, which is a key factor underlying the effects of Ang II and NO on renal sodium excretion.[77]

SALT-SENSITIVE HYPERTENSION AND THE MODULATION OF THE PRESSURE-NATRIURESIS RELATIONSHIP BY ANG II AND NO

In hypertension, salt sensitivity is a marker for a disproportionate susceptibility to cardiovascular and renovascular injury. Three large studies of patients with essential hypertension showed that patients who were salt sensitive more often had LVH, cardiovascular events, and/or microalbuminuria than non–salt-sensitive hypertensive patients.[92-94] These findings highlight the important link between salt-sensitive hypertension and cardiovascular injury. Salt-sensitive hypertension has been linked to a decrease in renal NO production, inappropriate activation of the RAS, or both.[14]

One of the defects characterized in this form of hypertension is a blunted pressure-natriuresis relationship, so that a higher blood pressure is needed to achieve the same level of sodium excretion. Ang II and NO are key modulators of pressure-natriuretic responses.[77,80] Based on in vivo and in vitro experimental data, it has been proposed that increases in intrarenal NO generation in response to acute increases in renal arterial pressure may directly inhibit distal tubular sodium transport, leading to an increase in sodium excretion.[80] AT_2-receptor knockout mice have been shown to exhibit a higher blood pressure and an antinatriuretic shift in the pressure-natriuresis relationship.[95,96] A recent study in AT_2-receptor knockout

mice treated with a NOS inhibitor for 1 week showed a greater blood pressure increase and rightward shift of the pressure-natriuresis curve compared with NOS inhibitor–treated wild-type mice.[96] AT_1-receptor blockade normalized blood pressure in both strains, suggesting that the AT_1 receptor mediates the impaired renal sodium excretion and blood pressure increase induced by NO synthesis blockade. However, in experiments conducted in rats treated for 8 weeks with a NOS inhibitor, RAS blockade normalized blood pressure but had no effect on the pressure-natriuresis responses, implying that correction of NOS inhibitor–induced hypertension is not enough to overcome the renal alterations associated with the chronic deficiency of NO.[97] These findings highlight the complex interactions between Ang II and NO and indicate that they play an important pathogenetic role in the development of hypertension.

PHARMACOLOGIC RESTORATION OF ANG II AND NO BALANCE: CLINICAL IMPLICATIONS

Dysfunction of blood vessels and of neuroendocrine systems plays a major role in the development of hypertension. Modern therapeutic strategies in human hypertension focus on preserving endothelial integrity and on preventing the development of end-organ damage. To the extent that vascular disease is characterized by an imbalance between a relative increase in Ang II and/or superoxide production and a relative deficit of NO bioactivity, modulating the activity of vasoactive substances generated by the endothelium has important implications for the treatment of hypertension and the prevention of end-organ damage.[52,98-101] ACE inhibitors, dual ACE/neutral endopeptidase (NEP) inhibitors, AT_1 receptor blockers, and the 3-hydroxy-3-methylgutaryl-CoA reductase inhibitors (statins) have been shown to improve endothelial vasodilator function and to exert beneficial effects on vascular remodeling by restoring the balance between NO, Ang II, and O_2^-.

ACE INHIBITORS

ACE inhibitors can reduce blood pressure effectively in hypertensive patients and are associated with improvements in endothelial function. Beyond inhibiting the renin-angiotensin system, ACE inhibitors potentiate bradykinin stimulation of NO release.[102] In addition, ACE inhibitors stabilize the B_2-receptor and reduce oxidative stress and tissue ET-1 levels.[103] ACE inhibitors have been shown to improve endothelial function in subcutaneous arteries, epicardial arteries, and the renal circulation. The Trial on Reversing Endothelial Dysfunction showed that 6 months of treatment with quinapril was associated with significantly improved, angiographically documented, vasodilative responses to acetylcholine in coronary artery segments.[104] Higashi et al[105] studied 296 patients with arterial hypertension. They found that although ACE inhibitors and other frequently prescribed classes of antihypertensive agents (calcium antagonists, β-blockers, and diuretics) were equally effective in decreasing blood pressure, only ACE inhibitors augmented the response of forearm blood flow to reactive hyperemia, an index of endothelium-dependent vasorelaxation.

ACE/NEP INHIBITORS

NEP is an endothelial cell surface zinc metallopeptidase with a similar structure and catalytic site as ACE.[100] NEP is the major enzymatic pathway for degradation of natriuretic peptide and a secondary enzymatic pathway for degradation of kinin and adrenomedullin.[106] Dual ACE/NEP inhibitors decrease vascular resistance and blood pressure, and improve sodium and water balance by simultaneously inhibiting the RAS and potentiating the natriuretic peptide and kinin systems. Within the blood vessel wall, omapatrilat, a potent dual NEP/ACE inhibitor, has been shown to reduce Ang II–induced vasoconstriction and vessel wall growth and enhance bradykinin-induced NO release/formation.[100] Studies on both DOCA-salt hypertensive and SHR rats showed that omapatrilat was more effective in decreasing blood pressure, preventing vascular remodeling, and improving endothelial function of resistance arteries than ACE inhibition alone. In patients with hypertension, omapatrilat produced greater decreases in both systolic and diastolic blood pressure than ACE inhibition alone.[107-109] These data suggest that combined inhibition of ACE and NEP may produce greater benefits in hypertension and end-organ injury than ACE inhibition alone. Thus, the ACE/NEP inhibitors may be a new and promising approach to treat hypertension and other cardiovascular diseases.[110]

ANGIOTENSIN RECEPTOR BLOCKERS

With mechanisms similar to those of ACE inhibitors, AT_1-receptor blockers possess a number

of unique properties that may add to the benefits observed clinically. For instance, low-dose candesartan, when given to hypertensive rats, normalized vascular NO production and improved vascular morphology.[111] AT_1 receptor blockers enhance endothelial prostaglandin release and NO formation by shifting the balance to AT_2 and B_2-receptor stimulation.[112] In the experimental model of Ang II–induced hypertension, losartan enhanced endothelium-dependent relaxation to acetylcholine and prevented the increase in tissue ET-1 content. In SHR rats, blockade of AT_1 receptors decreased blood pressure, improved endothelial function, and inhibited vascular superoxide production.[113,114] In patients with hypertension, AT_1 receptor blockers have been shown to be as effective as ACE inhibitors, calcium channel antagonists, β-blockers, and diuretics in decreasing blood pressure.[115] However, the effects of Ang II antagonists on endothelial dysfunction in human hypertension have not been defined.

STATINS

Statins are potent inhibitors of cholesterol biosynthesis. However, evidence has mounted steadily that the pleiotropic effects of statins, including up-regulation of eNOS activity, inhibition of ET-1 and AT_1 receptor expression, improvement of endothelial dysfunction, and anti-oxidant and anti-inflammatory effects, are also important mediators of the beneficial role of these drugs on cardiovascular disease.[116,117] In SHR rats, atorvastatin treatment decreased arterial blood pressure, improved endothelial dysfunction, down-regulated aortic AT_1 receptor expression, up-regulated eNOS activity, and inhibited superoxide production.[118] In hypertensive DS rats, pravastatin treatment decreased blood pressure and proteinuria, and prevented renal injury.[119] Therefore, these effects of statins may be a new additional treatment for hypertension.

CALCIUM CHANNEL BLOCKERS

The calcium channel blocker amlodipine is an effective antihypertensive agent.[120] In addition to its direct vasodilating effect, amlodipine influences the functional balance between Ang II and NO within the vessel wall. Experimental studies in small and large coronary arteries and in the aorta indicate that amlodipine stimulates NO generation to a similar extent as ACE inhibitors.[121] This effect of amlodipine may not be exerted by other calcium channel blockers and potentially may provide beneficial effects on end-organ disease.[121]

CONCLUSION

In summary, it has been well established that by decreasing the hemodynamic stress of high blood pressure, antihypertensive agents reduce endothelial injury. Some agents, particularly inhibitors of the RAS and perhaps some of the calcium channel blockers, decrease blood pressure and concomitantly block Ang II actions, enhance NO bioactivity, and/or decrease reactive oxygen species production, thereby restoring the homeostatic balance of these vasoactive factors in the vessel wall. These drug-specific actions beyond decreasing blood pressure may exert cardioprotective, vasculoprotective, and renoprotective effects by preventing the maladaptive changes that accompany hypertension, namely, cell growth and migration, endothelial dysfunction, and increased extracellular matrix deposition.

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