The Role of Angiotensin II in Regulating Vascular Structural and Functional Changes in Hypertension

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A major hemodynamic abnormality in hypertension is increased peripheral resistance due to changes in vascular structure and function. Structural changes include reduced lumen diameter and arterial wall thickening. Functional changes include increased vasoconstriction and/or decreased vasodilation. These processes are influenced by many humoral factors, of which angiotensin II (Ang II) seems to be critical. At the cellular level, Ang II stimulates vascular smooth muscle cell growth, increases collagen deposition, induces inflammation, increases contractility, and decreases dilation. Molecular mechanisms associated with these changes in hypertension include upregulation of many signaling pathways, including tyrosine kinases, mitogen-activated protein kinases, RhoA/Rho kinase, and increased generation of reactive oxygen species. This review focuses on the role of Ang II in vascular functional and structural changes of small arteries in hypertension. In addition, cellular processes whereby Ang II influences vessels in hypertension are discussed. Finally, novel concepts related to signaling pathways by which Ang II regulates vascular smooth muscle cells in hypertension are introduced.

Introduction

Hypertension develops as a result of multiple complex interactions between susceptibility genes and environmental factors that influence cardiac, renal, and vascular function to increase arterial pressure. A key factor underlying hypertension is increased peripheral vascular resistance, due in large part to functional, structural, and mechanical alterations of resistance arteries. Functional changes, which are usually acute responses, include increased vascular reactivity to vasoconstrictor agents and decreased vasodilation and reflect abnormal excitation-contraction coupling, altered electrical properties of vascular smooth muscle cells, and impaired endothelial cell function [1,2]. Structural alterations due to persistent, chronic stimulation of vessels include reduced lumen diameter and media thickening (vascular remodeling) [3,4] and involve changes in vascular smooth muscle cell growth, cell migration, dedifferentiation, rearrangement of vascular components, increased abundance of extracellular matrix components, and formation of focal adhesions [5••,6] (Table 1). Mechanical changes include altered stiffness and distensibility and may be due to changes in collagen:elastin content [7]. Another recently identified factor contributing to vascular dysfunction in hypertension is inflammation of the vascular wall associated with migration of proinflammatory cells, increased expression of redox-sensitive proinflammatory genes, and proteins and fibrosis [8••,9]. Vascular smooth muscle cells are central to these processes. Consequently, much research has focused on elucidating physiologic mechanisms and pathophysiologic events that regulate vascular smooth muscle cell function in health and cardiovascular disease.

Among the many humoral factors involved in vascular alterations in hypertension, angiotensin II (Ang II) appears to be one of the most important. Ang II is a multifunctional peptide that has numerous actions on vascular smooth muscle: it modulates vasomotor tone through its potent vasoconstrictor effects, it regulates cell growth and apoptosis, it influences cell migration and extracellular matrix deposition, it is proinflammatory, and it stimulates production of other growth factors and vasoactive agents [10–12].

The multiple actions of Ang II are mediated via specific, highly complex intracellular signaling pathways that are stimulated following an initial binding of the peptide to its cell-surface receptors. Two major receptor subtypes have been cloned and characterized, AT_1R and AT_2R [10–13]. Both receptors play a role in the regulation of vascular smooth muscle, although they differ in their action. Whereas the AT_1R is associated with growth, inflammation, and vasoconstriction, the AT_2R is associated with apoptosis and vasodilation [10–13]. The significance of Ang II in vascular pathology associated with hypertension is supported by experimental and clinical studies demonstrating that angiotensin-converting enzyme (ACE)

Table 1. Angiotensin II-regulated cellular processes that influence vascular functional and structural changes in hypertension*

Functional changes	Structural changes
Increased myocyte contraction	Vascular smooth muscle cell hyperplasia
Decreased vasodilation	Vascular smooth muscle cell hypertrophy
Endothelial damage	Decreased/increased apoptosis
Increased endothelial permeability	Increased collagen production
Myocyte migration	Decreased collagen degradation
	Recruitment of inflammatory cells
	Expression of adhesion molecules
	Cytoskeletal rearrangement
	Formation of focal adhesions

*These changes contribute to increased peripheral resistance.

inhibitors and AT_1R blockers not only lower blood pressure, but also regress arterial remodeling, improve endothelial function, reduce vasomotor tone, decrease inflammation, and normalize aberrant signaling events in vascular smooth muscle cells [14–16]. This review focuses on the role of Ang II in vascular functional and structural changes associated with hypertension and discusses some of the molecular mechanisms underlying these processes. Other agents such as endothelin-1 (ET-1); vasopressin (AVP); aldosterone and norepinephrine; growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and insulin-like growth factor-1 (IGF-1); and mechanical factors such as stretch/strain, pressure, and shear stress may also be important in vascular pathologic processes and complications of hypertension. However, these will not be discussed in this paper, and the reader is referred to recent reviews [5••,6,17,18].

Vascular Structural Changes in Hypertension: Role of Angiotensin II

Increased peripheral resistance in hypertension results from a general narrowing of resistance arteries (arteries with diameter < 300 μm), due in large part to changes in vascular structure, also known as remodeling [3,4]. Vascular remodeling has been classified according to the nature of changes in lumen diameter (inward or outward) and by changes in media mass (increased = hypertrophic, decreased = atrophic, no change = eutrophic). Major structural changes in established hypertension include reduced diameter and increased media thickness. In human mild essential hypertension, spontaneously hypertensive rats (SHR), and 2-kidney, 1-clip Goldblatt rats, vessels exhibit inward eutrophic remodeling where lumen diameter is reduced, media:lumen ratio is increased, and media cross-sectional area is unaltered [3,4,5••,19]. In patients with severe hypertension or renovascular hypertension, and in deoxycorticosterone acetate-salt rats, 1-kidney, 1-clip Goldblatt rats, and Dahl salt-sensitive rats, small arteries undergo hypertrophic remodeling, where media growth encroaches on the lumen to increase the media:lumen ratio and media cross-sectional area [3,4,5••,20,21].

Vascular smooth muscle cell growth

Cellular processes associated with arterial remodeling include vascular smooth muscle cell growth (hyperplasia and hypertrophy), apoptosis, elongation of vascular smooth muscle cells, reorganization of cells around the lumen, and/or altered extracellular matrix composition [3,22,23]. Hyperplasia refers to an increase in vascular smooth muscle cell number associated with DNA synthesis and is stimulated by Ang II. Hyperplasia may be an important component of hypertension as evidenced by an increase in smooth muscle cell proliferation rate and the number of cell layers in the media of arteries from hypertensive animals [23,24]. These processes appear to be genetically determined and independent of hypertension, as recently demonstrated by Hu *et al.* [25•]. In that study, vascular smooth muscle cells from young SHR exhibited exaggerated growth; increased expression of genes associated with metabolic enzymes, adhesion molecules, and cytokines as assessed by GeneChip (Affymetrix, Santa Clara, CA) technology; and enhanced production of Ang II [25•]. Hypertrophy, a reversible process, refers to increased cell size due to increased protein synthesis and/or increased intracellular cell water volume. Ang II stimulates hypertrophy by stimulating protein synthesis and by inducing activation of transmembrane transport systems, such as Na^+/K^+ATPase, Na^+/H^+ exchanger, Na^+-dependent Mg^{2+} transporter, and $Na^+/K^+/2Cl$ cotransporter, which influence transmembrane movement of ions and water [5••,26,27]. In experimental models of hypertension, hyperplasia and hypertrophy have been demonstrated to contribute, to varying degrees, to vascular remodeling. In intramyocardial arteries in SHR, the volume and number of arterial smooth muscle cells is increased [28], and in Ang II-induced hypertensive rats, arterial smooth muscle cell thickness is increased without a change in the number of cell layers [29]. In prehypertensive SHR, structural changes of small arteries are associated with an

increase in the media volume, increased number of smooth muscle cell layers, and elongation of vascular smooth muscle cells [30]. Mesenteric resistance arteries from SHR have a greater number of cell layers than normotensive controls, and this is normalized when rats are treated chronically with ACE inhibitors or AT_1R blockers [31], further confirming the importance of Ang II in growth processes associated with vascular remodeling in hypertension.

Apoptosis

In eutrophic remodeling, characteristic of small arteries in mild essential hypertension, increased vascular smooth muscle cell growth does not seem to be a major factor contributing to media thickening. In this condition, apoptosis and vascular fibrosis may be more important. The exact role of apoptosis in arterial remodeling remains unclear, and it is unknown whether apoptosis is a growth-associated compensatory process or a primary event. However, an imbalance between growth and apoptosis could be important. In resistance arteries of young SHR, apoptosis is reduced and growth is enhanced [32]. On the other hand, in Ang II-infused normotensive rats, aortic apoptosis is increased [33]. Treatment of SHR with an AT_1R blocker increased the vascular smooth muscle cell apoptotic rate, whereas AT_2R blockade attenuated these effects, suggesting an antiapoptotic (cell survival) role for AT_1R and a proapoptotic role for AT_2R in SHR [34]. To further support the proapoptotic, antigrowth effect of AT_2R in vivo, Wu *et al*. [35] recently demonstrated that valsartan improved cardiovascular remodeling in aortic-banded wild-type mice, whereas valsartan effects were blunted in AT_2R null mice. Numerous studies have suggested that ACE inhibitors and AT_1R blockers may contribute to regression of vascular wall growth through activation of proapoptotic pathways [36,37].

Vascular fibrosis

Vascular fibrosis involves the accumulation of extracellular matrix proteins, particularly collagen, in the vascular media. Changes in extracellular matrix may precede the vascular dysfunction associated with hypertension. Risler *et al*. [38] demonstrated that synthesis of secreted and membrane-bound sulfated proteoglycans by cultured vascular smooth muscle cells from young SHR was greater than that from age-matched controls. Increased collagen deposition in the vascular media has been demonstrated in experimental hypertension and in subcutaneous resistance arteries from essential hypertensive patients [3,39]. Increased collagen I and III mRNA and enhanced collagen protein synthesis have also been demonstrated in fibroblasts from patients with essential hypertension [40]. Collagen accumulation may be due to increased Ang II-induced synthesis. In Ang II-dependent hypertension in TGRen2 transgenic rats, vascular hypertrophy is attributed to increased collagen deposition and dedifferentiation of vascular smooth muscle cells to a fetal-type smooth muscle cell phenotype [41]. In isolated vascular smooth muscle cells from SHR, Ang II directly stimulates collagen production via a p38 mitogen-activated protein (MAP) kinase–dependent pathway [42]. In addition to stimulating production, Ang II regulates collagen degradation by attenuating interstitial matrix metalloproteinase (MMP) activity and by enhancing tissue inhibitor of metalloproteinase-1 (TIMP-1) production. In young SHR, activity of MMP1 and MMP3 is reduced, whereas in adult SHR, MMP2 activity is decreased [43]. These effects promote accumulation of fibronectin, proteoglycans, and collagen, which contribute to remodeling in hypertension. In SHR treated with AT_1R blockers, TIMP-1 expression and collagenase activity were normalized [44], supporting the role for Ang II in these processes.

Inflammation

Vascular inflammation, characterized by recruitment of monocytes and lymphocytes into the subendothelial space, production of chemotactic cytokines, increased expression of adhesion molecules, reactive smooth muscle cell proliferation, and altered extracellular matrix production and degradation, may also contribute to vascular structural changes in hypertension [8••,45]. These processes, together with lipid oxidation, are proatherogenic, particularly in damaged arteries in hypertension. Ang II has significant proinflammatory actions in the vascular wall, inducing the production of reactive oxygen species, such as superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), cytokines, adhesion molecules, and activation of redox-sensitive inflammatory genes [8••,46]. Vascular $O_2^{\bullet-}$ and H_2O_2 function extracellulary to modulate endothelium-dependent changes in vasomotor tone and intracellularly as a second messenger to produce long-term phenotypic alterations of cells [46,47••]. Reactive oxygen species regulate activity of pro-MMP2 and pro-MMP9, which further influence extracellular matrix protein content [48•].

Angiotensin II also modulates expression of proinflammatory molecules in the vessel wall that influence monocyte recruitment into the damaged hypertensive vessel. In endothelial cells, Ang II upregulates vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule, and E-selectin expression through a redox-dependent pathway [8••,49]. In vascular smooth muscle cells, Ang II stimulates VCAM-1 production, chemokine monocyte chemotactic protein-1 (MCP-1), and the cytokine interleukin (IL)-6 [8••,49], which stimulate the recruitment of mononuclear leukocytes into the vessel media. To support the role of endogenous Ang II in vascular inflammation, AT_1R blockers have been shown to reduce serum levels of VCAM-1, tumor necrosis factor-α, and superoxide in patients with early atherosclerosis [50].

Vascular Functional Changes in Hypertension: Role of Angiotensin II

In addition to structural changes, abnormal regulation of vascular caliber due to increased vasoconstriction and/or decreased vasodilation contributes to increased peripheral resistance and consequently to blood pressure elevation. These are usually acute events in response to multiple stimuli.

Increased vasoconstriction

Of the many vasoconstrictor agonists implicated in vascular hyper-responsiveness in hypertension, Ang II appears to be one of the most important. Whereas contractile responses to ET-1, AVP, and norepinephrine are reported to be decreased, unchanged, or rarely increased, vascular reactivity to Ang II has, for the most part, been found to be enhanced in experimental and human hypertension [51–54]. These effects may be direct or indirect through increased sympathetic activity [55]. Regulation of Ang II-induced vascular contraction is generally attributed to a G protein-mediated increase in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$), which is the signal activating the contractile machinery of vascular smooth muscle cells. Ca^{2+} activates the Ca^{2+}/calmodulin-dependent myosin light chain (MLC) of myosin, inducing a myosin:actin interaction [10]. Relaxation results from dephosphorylation of MLC by MLC phosphatase. Accordingly, the contractile state of vascular smooth muscle is dependent upon the relative activities of these enzymes. In addition to changes in $[Ca^{2+}]_i$, Ca^{2+} sensitivity of MLC phosphorylation contributes to regulation of the contractile state. Both MLC kinase and phosphatase are downstream targets of multiple signaling molecules including Ca^{2+}/calmodulin kinase II, protein kinase C, arachidonic acid, extracellular signal-regulated kinase (ERK1/2), and cGMP-dependent protein kinase [10–13]. Recent studies demonstrate that RhoA/Rho kinase–dependent pathways also influence MLC kinase and phosphatase at a constant $[Ca^{2+}]_i$, and probably constitute a major mechanism underlying increased vascular contractility in hypertension [56]. Other systems implicated in enhanced Ang II-mediated contractility in hypertension include 5-lipoxygenase-derived products, particularly the cysteinyl leukotrienes [57] and epoxide hydrolase [58]. Studies in intact arteries and isolated vascular smooth muscle cells from experimental hypertensive rats and hypertensive patients have demonstrated that many of the above Ang II-mediated events are upregulated and contribute, at least in part, to increased reactivity, exaggerated vasoconstriction, and enhanced vascular tone in hypertension (Fig. 1).

Decreased vasodilation

Alterations in vasodilator mechanisms have been identified both in endothelial-dependent and endothelial-independent systems. The endothelium plays a critical role in modulating vascular relaxation by release of endothelial-derived nitric oxide (NO), stimulation of vascular smooth muscle cell soluble guanylate cyclase (sGC), and the subsequent increase in intracellular cGMP. Altered vascular tone in hypertension is associated with impaired endothelium-dependent vasodilation due, in large part, to reduced NO signaling [59]. Among the mechanisms implicated in perturbed endothelial-derived bioavailability of NO in hypertension are 1) reduced expression/abundance of endothelial nitric oxide synthase (eNOS), the major endothelial NO-generating enzyme system; 2) decreased activation of eNOS, possibly due to tetrahydrobiopterin (eNOS cofactor) deficiency; and 3) increased quenching of NO by increased Ang II-mediated generation of $O_2^{\bullet-}$ [60]. Impaired endothelium-mediated vasodilation has been demonstrated in many experimental models of hypertension, as well as in essential hypertensive patients [15,19,59,61,62•]. These endothelium-dependent aspects have been reviewed recently elsewhere and will not be discussed further [63,64].

The endothelium was classically considered to be the major regulator of vascular relaxation through the NO/cGMP pathway. However, growing evidence indicates that endothelium-independent processes also play a role in modulation of vascular tone, and that abnormalities in these events contribute to aberrations in vasorelaxation in hypertension. This is supported by studies demonstrating that endothelium-independent vasodilators release NO, activate GC, and generate cGMP in the vascular wall [65]. In addition, vascular smooth muscle relaxes in response to classical endothelium-independent agents such as adenosine, prostacyclin, forskolin, and β-receptor agonists [66]. In vascular smooth muscle cells, activation of receptors linked to adenyl cyclase and the consequent increase in intracellular cAMP levels is an important endothelium-independent mechanism mediating vasodilation. Impaired receptor-mediated vasodilation has been demonstrated in genetic and experimental models of hypertension and has been attributed to alterations in the transmembrane signaling processes linking β-adrenoceptor receptor activation with the stimulation of adenyl cyclase. Other studies suggest that dysregulation of sGC is important in impaired endothelium-independent vasodilation in hypertension [60,67]. Despite data implicating endothelium-independent processes in impaired vasodilation in experimental hypertension, there is little convincing data to support these findings in human hypertension. In fact, clinical studies demonstrate, for the most part, that vasodilatory responses to acetylcholine (endothelium dependent) but not to sodium nitroprusside (endothelium independent) are attenuated in human hypertension [64]. Furthermore only endothelium-dependent relaxation seems to be positively influenced by antihypertensive therapy. Hence, the clinical significance of experimental hypertensive models exhibiting impaired endothelium-independent dilation await clarification.

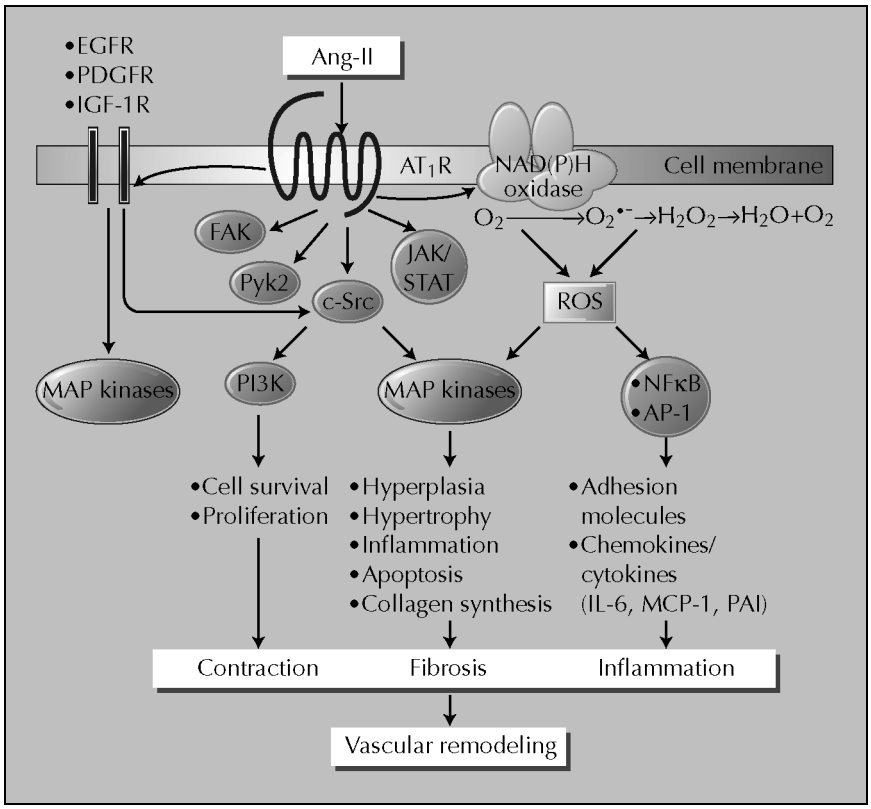

Figure 1. Angiotensin II (Ang II)-mediated cellular events regulating vascular structure. Ang II binds to the AT_1R leading to activation of tyrosine kinases, mitogen-activated protein (MAP) kinases, and NAD(P)H oxidase. These signaling events regulate vascular smooth muscle cell function. Under pathologic conditions, increased signaling leads to altered growth, fibrosis, and inflammatory processes, which contribute to structural remodeling in hypertension. EGFR— epidermal growth factor receptor; FAK—focal adhesion kinase; IGF-1R—insulin-like growth factor 1 receptor; IL—interleukin; JAK—janus family kinase; MCP-1— monocyte chemotactic protein 1; NF-κB—nuclear factor-κB; PAI—platelet activator inhibitor; PDGFR—platelet-derived growth factor receptor; PI3K—phosphatidylinositol 3-kinase; ROS—reactive oxygen species.

Molecular Mechanisms Whereby Angiotensin II Influences Vascular Changes in Hypertension

Angiotensin II induces its vascular effects by acting directly through Ang II receptors, indirectly through the release of other factors, by transactivating receptor tyrosine kinases, and via cross-talk with intracellular signaling pathways of other vasoactive agents and growth factors (Fig. 2). Although Ang II is classically described as a vasoconstrictor agent, it is now clear that this peptide has potent growth and proinflammatory actions. Some novel concepts related to signaling pathways whereby Ang II modulates vascular changes in hypertension are discussed below.

Regulation of vascular smooth muscle cell growth by angiotensin II

Similar to growth factors, Ang II induces cell hyperplasia and hypertrophy by stimulating phosphorylation of tyrosine kinases, activation of MAP kinases, and production of reactive oxygen species [10–13]. These signaling molecules are required for the growth-promoting actions of Ang II. Ligand binding to AT_1R induces phosphorylation of multiple tyrosine kinases, including c-Src, janus family kinases (JAK), focal adhesion kinase (FAK), Pyk2, p130Cas, and phosphatidylinositol 3-kinase (PI3K). One of the earliest kinases to be phosphorylated in response to Ang II is c-Src [68]. c-Src is a major regulator of many downstream proteins involved in growth signaling, including phospholipase C-γ, Pyk2, FAK, JAK, Shc, MAP kinases, PI3K, and NAD(P)H oxidase, and its activation is increased in hypertension [68]. JAK proteins phosphorylate STAT proteins and are key mediators of mRNA expression and are characterized as early response genes [69]. Ang II-induced activation of FAK causes its translocation to sites of focal adhesion with the extracellular matrix and phosphorylation of paxillin and talin. These processes regulate vascular smooth muscle cell morphology and migration. p130Cas is an adapter protein that plays a role in cytoskeletal rearrangement and is critical in arterial development. PI3K stimulates Akt, a threonine-serine kinase. These proteins influence cell survival, metabolism, cytoskeletal reorganization, and membrane trafficking, and have been identified as having growth-promoting, antiapoptotic effects [70].

Angiotensin II also activates major members of the MAP kinase family, ERK1/2, p38 MAP kinase, c-Jun N-terminal kinases (JNK), and ERK5 [10–13]. ERK1/2, phosphorylated by MEK1/2 (MAP/ERK kinase), is a key growth signaling kinase, whereas JNK and p38 MAP kinase, phosphorylated by MEK4/7 and MEK3/6 respectively, influence cell survival, apoptosis, differentiation, and inflammation. ERK5, a redox-sensitive MAP kinase, is involved in protein synthesis, cell cycle progression, and cell growth. In cardiac, renal, and vascular tissue from hypertensive rats, basal and Ang II-stimulated activation of tyrosine kinases and ERK1/2 is increased [10–13]. These processes have been associated with enhanced vascular smooth muscle cell growth, inflammation, fibrosis, as well as increased vascular contractility. We recently demonstrated in in vivo studies that blockade

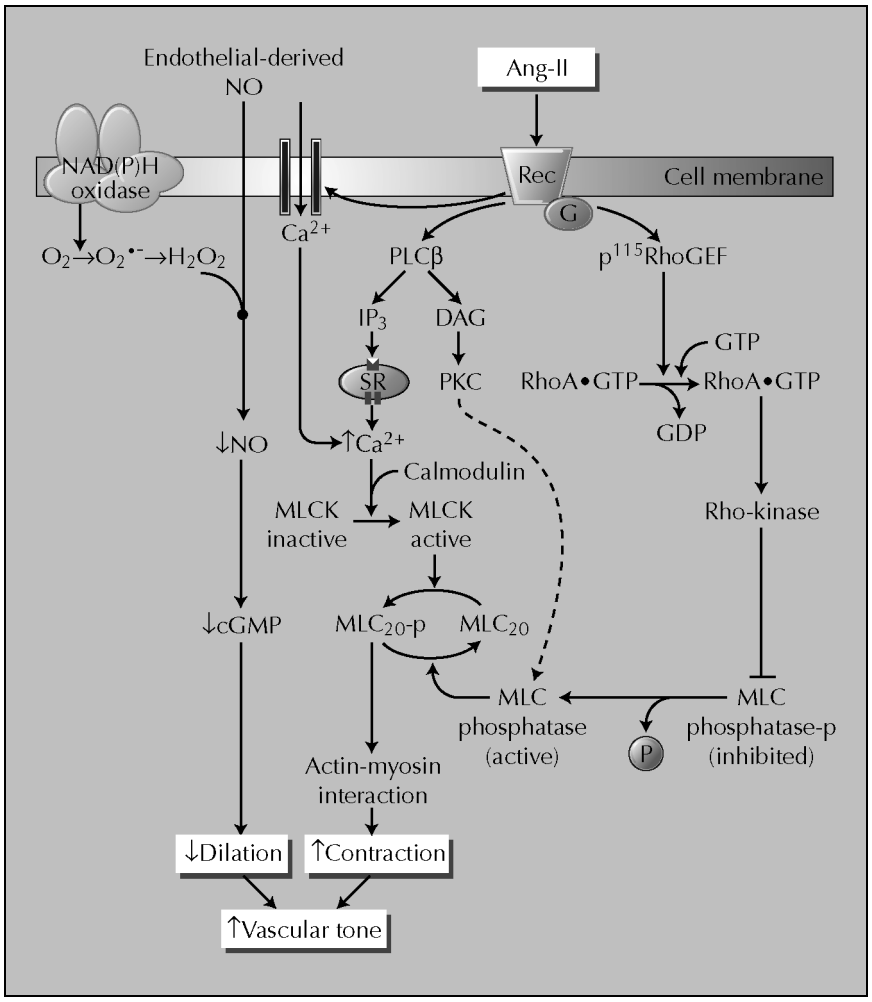

Figure 2. Signal transduction mechanisms involved in angiotensin II (Ang II)-induced changes in vascular contractility in hypertension. Ang II increases intracellular free Ca^{2+} concentration $[CA^{2+}]_i$ by stimulating Ca^{2+} influx and through mobilization from sacroplasmic reticulum (SR) stores. Increased activity of the RhoA/Rho kinase Ca^{2+}-sensitizing pathway and protein kinase C (PKC)-dependent pathway (*dashed line*) also contribute to contractility. Increased Ang II-stimulated NAD(P)H oxidase–mediated generation of $O_2^{\bullet-}$ quenches endothelial-derived nitric oxide (NO), resulting in decreased cGMP-induced vasodilation. DAG—diacylglycerol; GAP—GTPase-activating protein; GEF—guanine nucleotide exchange factor; IP_3—inositol triphosphate; MLC(K)—myosin light chain (kinase); PLC—phospholipase C.

of the ERK1/2 pathway by PD98059 improves vascular dysfunction in hypertensive rats, independent of blood pressure–lowering actions [71].

Recent studies suggest that Ang II activates growth-signaling pathways primarily by transactivating receptor tyrosine kinases. Ang II can activate receptor tyrosine kinases, even though it does not directly bind to these receptors. This process of transactivation has been demonstrated for EGF receptor, PDGF receptor, subtype β PDGF receptor, and IGF-1 receptor [72]. Mechanisms underlying Ang II-induced transactivation of RTKs include activation of tyrosine kinases (Pyk2 and Src), redox-sensitive processes, and possibly stimulation of MMPs that release heparin-binding EGF [72]. Ang II also increases production of various vasoactive agents and growth factors in hypertension, such as ET-1, PDGF, transforming growth factor-β, basic fibroblast growth factor, and IGF-1, which could promote cell proliferation, protein synthesis, and fibrosis, further contributing to growth processes underlying vascular remodeling.

Regulation of redox-sensitive pathways by angiotensin II in vascular smooth muscle cells

In the vasculature, reactive oxygen species modulate vascular tone and structure. $O_2^{\bullet-}$ and H_2O_2 have been shown to induce vascular contraction and vascular smooth muscle cell growth, whereas $NO^{\bullet}$ plays a pivotal role in endothelium-dependent relaxation. Furthermore, oxygen free radicals are proinflammatory and stimulate monocyte migration and formation of oxidized low-density lipoprotein, which is toxic to vascular cells and impairs vascular endothelial function [8••]. Consequently, increased bioavailability of reactive oxygen species (oxidative stress) may underlie pathologic processes associated with vascular dysfunction and structural remodeling in hypertension [47••].

Angiotensin II increases production of reactive oxygen species in all cell types of the vasculature, including smooth muscle cells, endothelial cells, and adventitial fibroblasts. The major source of oxygen intermediates in the vascular wall is Ang II-modulated nonphagocytic NAD(P)H oxidase, which is upregulated in hypertension [73•,74]. Activation of this oxidase by Ang II involves c-Src, PLA_2, and PLD, as well as increased synthesis of the NAD(P)H oxidase subunits gp91phox, p22phox, p47phox and p67phox [73,74]. In Ang II-dependent models of hypertension, vascular production of superoxide anions is increased [75,76]. This effect is mediated via Ang II-stimulated activation of vascular NAD(P)H oxidase.

In Ang II-dependent hypertensive rats, treatment with liposome-encapsulated superoxide dismutase (SOD), SOD mimetics, or antioxidant vitamins reduced production of vascular reactive oxygen species, decreased blood pressure, regressed vascular remodeling, and improved endothelial function [75,76]. NAD(P)H oxidase-generated $O_2^{\bullet-}$ contributes to increased Ang II-mediated vascular smooth muscle cell growth in hypertension [73•,74]. Both $O_2^{\bullet-}$ and H_2O_2 are potent mitogens that elicit effects via MAP kinases (p38 MAP kinase, JNK, ERK-5) and tyrosine kinases (Src, JAK2, STAT, p21Ras, Pyk2, and Akt), through transactivation of receptor tyrosine kinases, and by increasing expression of growth-inducing genes [47••]. Blockade of NAD(P)H oxidase activity inhibits Ang II-induced vascular smooth muscle cell hypertrophy and regresses vascular remodeling [73•,76], supporting a role for reactive oxygen species as inducers of enhanced vascular growth in hypertension.

Oxidative stress also plays an important role in vascular inflammation. Ang II activates the redox-sensitive transcription factor, nuclear factor (NF)-κB, which is associated with upregulation of adhesion molecules, stimulation of chemokine and cytokine production, and recruitment of monocytes to the arterial wall [8••,47••]. Inhibition of NF-κB activity abrogates Ang II-mediated expression of IL-6, VCAM-1, and MCP-1 [49]. These Ang II-regulated processes, which play a role in the progression of atherosclerosis, are also involved in vascular remodeling and dysfunction in hypertension. In Ang II-infused rats, which exhibit vascular oxidative stress, endothelial dysfunction and media thickening, expression of VCAM-1 and PECAM was increased and activity of NF-κB was augmented [77•]. Accordingly, growing evidence indicates that vascular damage in hypertension is associated with inflammatory responses. Oxidative stress and inflammation, mediated in large part by Ang II, may reflect the continuum from hypertensive vascular damage to atherosclerosis.

Increased $O_2^{\bullet-}$ in hypertension also impairs endothelium-dependent vascular relaxation and increases vascular contractile reactivity. These effects may be mediated directly by increasing cytosolic Ca^{2+} concentration through increased Ca^{2+} influx and increased intracellular mobilization, or indirectly by reducing concentrations of the vasodilator $NO^{\bullet}$ through quenching by $O_2^{\bullet-}$ [78]. Oxygen radicals also induce endothelial permeability, with extravasation of plasma proteins and other macromolecules, which could further impair endothelial function and aggravate vascular damage.

Role of RhoA/Rho kinase in angiotensin II-mediated vascular changes in hypertension

It is becoming increasingly evident that the RhoA/Rho kinase pathway may play an important role in hypertension. Inhibition of this pathway lowers blood pressure in hypertensive rats [79] and improves forearm blood flow in hypertensive patients [80]. RhoA is a low-molecular-weight guanosine triphosphatase that is regulated by Ang II. RhoA activation leads to stimulation of Rho kinase, which promotes contraction of cells via the phosphorylation of the myosin-binding subunit of MLC phosphatase (thereby inhibiting phosphatase activity) [56]. Inhibition of Rho kinase by Y-27632, or fasudil, results in relaxation of isolated vessels due to inhibition of Ca^{2+} sensitization of vascular smooth muscle contraction. Recent findings suggest that increased vascular reactivity and increased tone in hypertension may be due to increased Ca^{2+} sensitization due to upregulation of the RhoA/Rho kinase cascade [56]. RhoA/Rho kinase may also play a role in vascular remodeling and inflammation in hypertension. Ang II-induced hypertrophy and MCP-1 expression in vascular smooth muscle cells involves a Rho kinase–dependent mechanism [81]. In Ang II-induced hypertensive rats, increased plasminogen activator inhibitor-1 gene expression and cardiovascular remodeling were normalized by Rho kinase inhibitors [82]. Furthermore, increased activation of RhoA/Rho kinase in L-NAME-induced hypertension was reduced by AT_1R blockade [83]. Taken together, these data suggest that RhoA/Rho kinase regulates vascular smooth muscle cell processes that influence both vascular contractility and remodeling in Ang II-dependent hypertension. Although exact pathways whereby Ang II signals through RhoA await clarification, this system may provide a novel therapeutic target to reduce peripheral vascular resistance in hypertension.

Conclusions

Abnormal vascular function (increased constrictor and decreased dilator responses) and altered structure (vascular remodeling) are major factors underlying vascular pathology in hypertension. These processes are influenced by Ang II, which stimulates vascular smooth muscle cell contraction, inhibits NO-mediated vasodilation, augments cell growth, increases content of extracellular matrix proteins, inhibits apoptosis, induces migration, and promotes inflammation. Mechanisms whereby Ang II mediates these cellular events in hypertension seem to occur at the postreceptor level and appear to be associated with upregulation of Ang II-stimulated G protein-coupled phospholipases, tyrosine kinase– and MAP kinase–dependent pathways, oxidative stress, and RhoA/Rho kinase cascades. Interaction between these pathways is highly complex and dysregulation at any level could manifest as pathologic functional and structural vascular changes in hypertension. Although there has been major progress in the elucidation of Ang II-mediated signaling in vascular smooth muscle cells, we still know little about the processes that underlie aberrant signaling in hypertension and at what level some pathways become more important than others. With molecular and pharmacologic tools that target specific molecules, identification of distinct signaling abnormalities in hypertension should be possible. This would facilitate improved management of vascular

changes, thereby ameliorating development of hypertension and preventing target organ damage. In the mean time, we should continue to use agents that interrupt the renin-angiotensin system such as ACE inhibitors or AT_1R blockers, which not only lower blood pressure, but also improve vascular structural and functional changes in hypertension. These effects could contribute to improved cardiovascular outcomes as recently demonstrated in the Heart Outcomes Prevention Evaluation (HOPE) study [84••] and the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) study [85••].

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