From bedside to bench to bedside: role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension

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RESISTANCE ARTERIES are vessels of ~100–300 μm in lumen diameter that are an important site of resistance to blood flow. Small artery-dependent increased peripheral resistance may participate in development and complications of hypertension. The degree of remodeling of small arteries has prognostic significance over a 10-yr period, with worse prognosis for hypertensive subjects with greater remodeling. In almost all hypertensive subjects, a reduction in lumen and an increase in the media-to-lumen ratio are found, without increase in its media cross section, as a result of rearrangement of smooth muscle cells and increased collagen and fibronectin. Approximately 60% of hypertensive patients exhibit endothelial dysfunction already in stage 1 hypertension. Study of human vascular smooth muscle cells and of vessels from experimental animals has demonstrated that ANG II, aldosterone, and endothelin exert remodeling effects in large measure by activation of NADPH oxidase, and to lesser degree by stimulating xanthine oxidase and mitochondrial reactive oxygen species generation. Stimulation of angiotensin type 1 (AT1) receptors (AT1R) leads to increased reactive oxygen species in part via activation of nonreceptor tyrosine kinases such as c-src, and of PKC and phospholipase D, and thereby contributes to endothelial dysfunction by inactivating nitric oxide (NO). Treatment of hypertensive patients with angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers, but not β-blockers, corrects small artery structure and endothelial dysfunction, which may favorably affect outcome in the long term, beyond the 3–5 yr of randomized clinical trials during which most antihypertensives affect outcome similarly as long as blood pressure is well controlled.

RESISTANCE ARTERIES IN HYPERTENSION: FROM CLINIC TO LABORATORY

A major objective of translational research from bench to bedside is to test in humans novel therapeutic modalities developed through experimentation. However, because our understanding of many human diseases, such as hypertension, is still very limited, there is a critical need for bedside to bench research. We and other researchers have demonstrated that in human hypertension, resistance arteries undergo remodeling and altered reactivity. What remains uncertain, however, is how these processes occur and whether they are primary causative events or secondary adaptive phenomena. To answer some of these questions, it is imperative to extend bedside observations and findings to the bench, where molecular and cellular processes underlying vascular changes in hypertension can be studied in isolated vessels and cells in vitro conditions. Ideally, one would like to examine cells directly involved in the pathological process under investigation. In hypertension, this includes vascular smooth muscle cells in resistance arteries. However, because of ethical and practical constraints, this is not always possible, and most studies investigating subcellular processes in hypertension are conducted, in large part, in cells from animal models, where hypertension is spontaneous or induced experimentally. When human cells are examined, they are usually derived from large arteries and veins obtained during surgery, from postmortem specimens, or from immortalized cell lines. Because these conditions do not control for blood pressure status and other variables of subjects from whom the cells are derived, it is inordinately difficult to extrapolate findings from such cell models to what may be happening in vascular cells during the development of hypertension. Because circulating blood cells are easily accessible from humans, many investigators have studied cellular events and signaling cascades in platelets, erythrocytes, and leukocytes as markers of events in vascular cells. However, this model is suboptimal because circulating cells are devoid of a supporting extracellular matrix and adventitia and have very different morphological and functional phenotypes compared with vascular smooth muscle cells.

Over the past 10 yr, we successfully developed a system for the study of isolated vessels and vascular smooth muscle cells derived from human small arteries obtained from gluteal biopsies of subcutaneous tissue. This unique approach provides multiple benefits. First, small arteries and vascular cells derived from these are obtained from healthy individuals and well-characterized hypertensive patients, with or without treatment. Second, the vascular smooth muscle cells are derived from resistance arteries, which contribute to blood pressure regulation, elevation of peripheral resistance and development of hypertension. Third, low-passaged cells that maintain their morphological and functional properties are studied. With the use of this approach, implementing “bedside to bench” research is realized, gaining a fuller understanding of cellular processes and signaling pathways contributing to vascular remodeling in hypertension. Our long-term goal is to identify putative genes and/or proteins fundamentally involved in hypertensive vascular pathology that could be used as targets for manipulation in the prevention and management of human hypertension. Ultimately and ideally, our findings will echo back from “bench to bedside” to test novel therapeutic strategies developed through experimentation. We will recapitulate our own scientific itinerary from the bedside and studies of
human small artery remodeling to the bench and the use of cells derived from these human small arteries as well as cells from vessels from experimental animals, and with the insights gained in the latter, back to the bedside, with studies of the action of agents that block these mechanisms, in particular the inhibitors of the renin-angiotensin system, and their effect on vascular remodeling in hypertensive humans.

STRUCTURAL CHANGES IN RESISTANCE ARTERIES IN HUMAN ESSENTIAL HYPERTENSION

Increased peripheral resistance is the hallmark of essential hypertension (94) (Fig. 1). Increased peripheral resistance results primarily from the increased energy dissipation that occurs when blood flows through small resistance arteries with reduced lumen. Resistance arteries have a lumen of 100–300 μm (11) and play an important role in the development of hypertension (136). Small artery remodeling may also lead to complications of hypertension (137), including myocardial ischemia (13, 61, 78), stroke (22), and renal failure (79).

Recently it was demonstrated that increased media-to-lumen ratio is associated with increased cardiovascular events over a 10-yr period of follow-up of 150 subjects, which included normotensive controls, essential hypertensive individuals, and subjects with hyperaldosteronism, pheochromocytoma, renovascular hypertension, and diabetes (124).

Resistance arteries from normotensive and hypertensive patients, and from persons with diabetes and dyslipidemia, have been mainly investigated by obtaining biopsies of gluteal subcutaneous tissue, followed by dissection of small arteries. This technique, which was first introduced by Heagerty and Mulvany (1), is invasive but well tolerated and allows studies of well-characterized hypertensive subjects, individuals with dyslipidemia, and persons with diabetes, etc. The first finding with this technique has been that in essential hypertension small arteries do not exhibit hypertrophy, but rather “eutrophic remodeling” (62, 107, 140–144, 146), in which the outer diameter and lumen of these vessels are smaller, the media-to-lumen ratio is greater, but the cross-sectional area of the media is not different from that of age and sex-matched normotensive subjects (62, 107, 142). The increased media-to-lumen ratio (105, 143, 144) is the most reproducible parameter in longitudinal and cross-sectional studies (135). In small arteries from patients with essential hypertension there does not appear to be any smooth muscle cell hypertrophy or hyperplasia in the media (81) whereas there may be in secondary hypertension (125–127). Smooth muscle cells are rearranged around a smaller lumen, and this is accompanied by increased extracellular matrix deposition (71, 72, 74). The restructuring of smooth muscle cells may be the result of increased vasoconstriction (6) or of increased apoptosis in the periphery of the vessel with enhanced growth toward the lumen (73) and could also entail motility changes in smooth muscle cells contributing to this cellular rearrangement. Recently, we found that whereas structural remodeling of small arteries is found in all hypertensive patients, impairment of endothelial function is found in ~60% and left ventricular hypertrophy in 45% of hypertensive patients (115) with stage I hypertension (according to the recent Joint National Committee VII classification) (21). We have concluded that altered structure of small arteries may be the first manifestation of target organ damage in hypertensive humans before the appearance of microalbuminuria, thickening of the intima media of the carotid arteries, or development of cardiac hypertrophy. It still remains to be established whether remodeling of resistance arteries precedes the development of hypertension or is a consequence of elevated blood pressure. In patients with severe hypertension, secondary hypertension, and in acromegalic patients (103, 122, 125–127), small arteries undergo hypertrophic remodeling, where media growth encroaches on the lumen to increase the media-to-lumen ratio and media cross-sectional area.

Role of ANG II in Vascular Structural Changes in Hypertension

Vascular smooth muscle cells are dynamic, multifunctional cells that contribute to arterial remodeling through numerous processes, including cell growth (hyperplasia and hypertrophy), apoptosis, elongation of cells, reorganization of cells and/or altered extracellular matrix composition (8, 52, 72, 81, 125, 127) (Fig. 2). Hyperplasia refers to an increase in vascular smooth muscle cell number associated with DNA synthesis and is stimulated by ANG II (55, 127, 130). Hypertrophy, a reversible process, refers to increased cell size due to increased protein synthesis and/or increased intracellular cell water volume (68). ANG II stimulates hypertrophy by stimulating protein synthesis and by inducing activation of transmembrane transport systems, which influence transmembrane movement of ions and water. In experimental models of hypertension, hyperplasia, and hypertrophy have been demonstrated to contribute, to varying degrees, to vascular remodeling (3, 32, 106, 153). We demonstrated that ANG II stimulates both hyperplasia...
sia and hypertrophy and that in human vascular smooth muscle cells from resistance arteries of essential hypertensive patients, these growth responses are enhanced (175, 176).

In eutrophic remodeling, characteristic of vessels in mild essential hypertension, apoptosis and vascular fibrosis may also be important (72–74). Apoptosis, gene-regulated cell death, is involved in the fine tuning of media growth and is increased in some vascular beds (36, 151) and decreased in others (31) in hypertensive rats. In addition, ACE inhibitors and AT1 R blockers could contribute to regression of vascular wall growth through activation of proapoptotic pathways. Apoptosis may also play a role in microvascular rarefaction, which has been implicated in the development of hypertension (56). Furthermore, there is evidence that detachment of vascular smooth muscle cells and endothelial cells (anoikis) may also contribute to vascular dysfunction and remodeling in hypertension (98, 159).

Vascular fibrosis involves the accumulation of extracellular matrix proteins, such as collagen, elastin, fibrillin, fibronectin, and proteoglycans, in the vascular media. Increased collagen deposition in the vascular media has been demonstrated in experimental hypertension and in subcutaneous resistance arteries from essential hypertensive patients (74, 120, 130, 164). Increased collagen I and III mRNA and enhanced collagen protein synthesis by fibroblasts have also been demonstrated in patients with essential hypertension (28). Experimental studies indicate that collagen accumulation may be related, in part, to increased ANG II-stimulated synthesis (43, 130, 177). In addition to inducing production, ANG II regulates collagen degradation by attenuating interstitial matrix metalloproteinase (MMP) activity and by enhancing tissue inhibitor of metalloproteinase-1 production (16, 189). A similar role may be played by endothelin-1 in blood vessels and the heart (4). In patients with untreated hypertension, serum levels of MMP-1 (88) and MMP-9 (91) have been reduced with normalization after 1 yr of lisinopril treatment (88). These data further support a role for ANG II in profibrotic processes in human hypertension.

Fig. 2. Molecular and cellular mechanisms whereby ANG II influences vascular structure in hypertension. ANG II binds to the ANG type 1 (AT1) receptor (AT1R), leading to activation of receptor tyrosine kinases, such as epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR) and insulin-like growth factor-1 receptor (IGF-1R), and nonreceptor tyrosine kinases, such as c-Src. In addition, ANG II:AT1R binding induces activation of NAD(P)H oxidase resulting in intracellular generation of reactive oxygen species (ROS), which influence redox-sensitive signaling molecules, such as mitogen-activated protein (MAP) kinases (p38MAP kinase, JNK, ERK5, and ERK2), transcription factors [NF-κB, AP-1, and hypoxia-inducible factor (HIF-1)] and matrix metalloproteinases (MMP). ANG II may downregulate (indicated by minus sign) peroxisome proliferator-activated receptors (PPARs), which have anti-inflammatory effects, thus enhancing vascular inflammation. These signaling events regulate vascular smooth muscle cell growth, extracellular matrix (ECM) protein production and inflammatory responses. Under pathological conditions, altered ANG II signaling leads to altered growth, fibrosis, and inflammation, which contribute to structural remodeling in hypertension. PAI, plasminogen activator inhibitor; RXR, retinoid X receptor.
Increasingly it has been appreciated that evidence of an inflammatory reaction may be recorded in association with the hypertensive process (7, 17, 73, 91, 165). It is unclear whether angiotensin II, endothelin-1, or aldosterone, agents that participate in the pathophysiology of hypertension and that induce inflammation in the heart, the vasculature, and the kidney (12, 82, 129, 171, 193) or blood pressure elevation by itself, through effects on adhesion molecules, chemokines, or endothelin-1 induced by cyclic mechanical strain (18, 195, 201) are associated with the inflammatory response that is found in hypertension. As well, the intriguing possibility that inflammation may activate the renin-angiotensin system and contribute to vascular remodeling and hypertension has been raised (194). Recently, it has been demonstrated that the activators of nuclear receptors, such as the peroxisome proliferator-activated receptors (PPARs), that are well-known hypolipidemic agents (the fibrates, PPAR α-agonists), or insulin sensitizers (glitazones, PPAR-γ agonists), downregulate the cardiac (37) and vascular inflammatory response in experimental animals (34, 35, 69) and serum markers of inflammation in humans (60). Furthermore, these inflammatory markers have been found to predict the risk of developing hypertension (42, 150). Chronic subclinical inflammation has been associated with the insulin resistance syndrome (45) that may precede the development of hypertension (57). Thus PPARs and vasoactive substances may be endogenous modulators of the inflammatory process that plays a role in structural changes that occur in the vasculature in hypertension, and inflammation may contribute to accelerate vascular damage in cardiovascular disease including hypertension. In fact, ANG II has been shown to down-regulate PPARs, an effect mediated through activation of NF-κB (167). Blockade of the action of these agents or activation of PPARs may contribute to slow down cardiovascular damage in hypertension.

Vascular inflammation is 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. 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$_2^-$) and H$_2$O$_2$, cytokines, adhesion molecules, and activation of redox-sensitive inflammatory genes (156). Vascular O$_2^-$ and H$_2$O$_2$ function intracellularly as second messengers, influencing redox-sensitive signaling molecules that regulate vascular smooth muscle cell contraction/dilatation, cell growth (hypertrophy and hyperplasia), apoptosis, and extracellular matrix protein content (172, 191, 203). ANG II also modulates expression of proinflammatory molecules in the vessel wall, such as VCAM-1, ICAM, and E-selectin expression through redox-dependent pathways (23, 82, 119). In vascular smooth muscle cells, ANG II stimulates VCAM-1 production, chemokine monocyte chemotactic protein-1, and the cytokine IL-6 (116), which stimulate the recruitment of mononuclear leukocytes into the vessel media. Many of these factors are increased in plasma from hypertensive patients and it has been suggested that elevated circulating levels of cytokines and chemokines may reflect vascular inflammation and target organ damage in hypertensive patients (66, 96).

To support the role of endogenous ANG II in vascular inflammation, AT$_1$/R blockers have been shown to reduce serum levels of VCAM-1, TNF-α, and O$_2^-$ in patients with early atherosclerosis (110).

**FUNCTIONAL ABNORMALITIES OF RESISTANCE ARTERIES IN HYPERTENSION**

Enhanced myogenic tone has been reported in experimental hypertension (76), and it has been suggested that this is a primary event in hypertension that may lead to the chronic vascular changes described above. Most vasoconstrictor agents such as endothelin-1 and vasopressin as well as norepinephrine appear to elicit normal or diminished constrictor responses in clinical hypertension (1, 62, 141, 142). Whether there is amplification of vasoconstrictor responses by the structural or mechanical reduction of lumen diameter according to the law of Laplace (47, 48, 80, 199, 200) is controversial (75). However, ANG II appears to frequently elicit enhanced vasoconstrictor responses in essential hypertension (142). This effect may be direct or indirect through increased sympathetic activity (29, 117). Enhanced ANG II-induced vascular contraction is attributed to increased cytoplasmic free Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) and is probably due to postreceptor signaling changes (179, 180) (Fig. 3). Recent studies (20) demonstrate that increased sensitivity of the contractile machinery through RhoA/Rho kinase-dependent pathways may also contribute to increased vascular contractility in hypertension.

The endothelium is critically involved in modulating vascular relaxation through release of endothelial-derived NO, stimulation of vascular smooth muscle cell soluble guanylate cyclase, 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 (44, 85, 104). Endothelial dysfunction has been demonstrated in vessels from hypertensive humans (30, 113, 114) and in many experimental models of hypertension (30, 33, 92, 92, 95) but is also found with aging (84, 104), diabetes, dyslipidemia, obesity, smoking, hyperhomocysteinemia, and other conditions in the absence of elevated blood pressure (139). Impaired endothelial function may result from reduced generation of NO due to reduced expression/abundance of endothelial NO synthase (eNOS) or decreased activation of eNOS, and increases in oxidative stress that reduce the bioavailability of NO through conversion to peroxynitrite (14, 100, 185). Increased concentrations of asymmetric dimethylarginine that inhibit NOS may be a mechanism for reduced production of NO in hypertension (10, 26). Oxidant stress may also lead to reduction in tetrahydrobiopterin, which induces the uncoupling of NOS, which then produces O$_2^-$ rather than NO (86). Enhanced production of the vasoconstrictor peptide endothelin-1 is another mechanism whereby abnormal endothelial function may contribute to vaso spasms, blood pressure elevation and complications of hypertension (reviewed in Ref. 138). Increased generation of arachidonic acid-derived products with vasoconstrictor properties (endothelium-derived contracting factors) is another manifestation of endothelial dysfunction (30, 33). Alterations in vasodilator mechanisms have been identified also in endothelial-independent systems. Although the endothelium was considered the major regulator of vascular relaxation through the NO/cGMP pathway, recent experimental evidence...
indicates that endothelium-independent processes also play a role in vascular tone modulation (93). However, there is little convincing data to support these findings in human hypertension. 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 (113, 140). 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 awaits clarification.

**ANG II Signaling in Human Vascular Smooth Muscle Cells and in Experimental Animals**

Angiotensin receptors. The many actions of ANG II are mediated via specific, highly complex intracellular signaling pathways that are stimulated after initial binding of the peptide to its cell-surface receptors. Two major receptor subtypes have been cloned and characterized, AT1R and AT2R (70, 108, 155, 180). In humans, AT1R is widely expressed and is located in numerous tissues, including the blood vessels, heart, kidney, adrenal glands, and liver. In contrast, AT2R is present primarily in fetal tissue, decreasing rapidly after birth, with relatively low amounts normally expressed in adult tissue (67). In pathological conditions associated with cardiac and vascular remodeling or inflammation, AT2R expression is upregulated (15). Both receptors play a role in the regulation of vascular smooth muscle, although they differ in their action. Whereas the AT1R is associated with growth, inflammation and vasoconstriction, the AT2R is associated, in general, with apoptosis and vasodilation (64, 158, 169). Recent data (2, 89) suggest that
AT1-R may also exert hypertrophic and proinflammatory effects, but this awaits further clarification.

**Growth signaling by ANG II in vascular smooth muscle cells.** Although ANG II is classically described as a vasoconstrictor agent, it is now clear that this hormone has potent growth, proinflammatory and profibrotic actions. Like growth factors, ANG II induces cell hyperplasia and hypertrophy by stimulating phosphorylation of tyrosine kinases, activation of mitogen-activated protein (MAP) kinases, mobilization of intracellular Ca$^{2+}$ and production of reactive oxygen species (134, 183). ANG II binding to AT1-R induces phosphorylation of multiple tyrosine kinases, including c-Src, JAK, focal adhesion kinase (FAK), Pyk2, p130Cas and phosphatidylinositol 3-kinase (40, 183). Findings from our laboratory in human vascular smooth muscle cells identified c-Src as one of the kinases immediately phosphorylated in response to ANG II, and involved not only in trophic effects but also in smooth muscle cell contraction (182). Furthermore, we showed that ANG II-induced activation of c-Src is augmented and that this is associated with increased growth in cells from hypertensive patients (178). c-Src is a major regulator of many distal targets, including PLC-γ, Pyk2, FAK, JAK, Shc, MAP kinases, phosphatidylinositol 3-kinase, and NAD(P)H oxidase (50, 59, 180). These proteins influence cell survival, metabolism, cytoskeletal reorganization and membrane trafficking and have been identified as having important growth promoting and anti-apoptotic actions (49, 132).

Of the many growth-signaling pathways, the MAP kinase family is best characterized (163). In vascular smooth muscle cells ANG II activates all four of the major MAP kinases, including ERK1/2, p38 MAP kinase, c-Jun NH2-terminal kinases (JNK) and ERK5 (83, 99, 202). 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 and vascular smooth muscle cells from hypertensive patients ANG II-stimulated activation of tyrosine kinases, ERK1/2, JNK, and p38MAP kinase is augmented (51, 197). These processes have been associated with enhanced vascular smooth muscle cell growth, inflammation, fibrosis, as well as increased vascular contractility.

ANG II can also activate receptor tyrosine kinases, even though it may not bind directly to these receptors. This process of transactivation has been demonstrated for EGF receptor, PDGF receptor, subtype β, and IGF-1 receptor (131, 174). Processes underlying ANG II-induced receptor tyrosine kinase transactivation include activation of tyrosine kinases (Pyk2 and Src), redox-sensitive processes and possibly modulation of MMPs that release heparin-binding EGF (131, 174, 187). ANG II also increases production of vasoactive hormones and growth factors in hypertension, such as ET-1, PDGF, TGFβ, basic FGF, and IGF-1, which could promote cell proliferation, protein synthesis, and fibrosis, further contributing to growth processes in arterial remodeling.

**ANG II signaling through reactive oxygen species in vascular smooth muscle cells.** Growing evidence indicates that ANG II mediates many of its cellular actions by stimulating formation of intracellular reactive oxygen species, which are highly reactive, bioactive, short-lived molecules that derive from a reduction of molecular oxygen (162). Reactive oxygen species participate in numerous biological processes by acting as second messengers to modulate the activation of various signaling molecules and pathways (46, 87). ANG II increases production of reactive oxygen species in all types of vascular cells, including endothelial cells, smooth muscle cells, and adventitial fibroblasts (87, 90, 112).

A major source of oxygen intermediates in the vascular wall is ANG II-modulated nonphagocytic NAD(P)H oxidase, which is upregulated in hypertension (188, 196). Neutrophil NAD(P)H oxidase is the prototype oxidase and comprises five subunits, gp91phox (Nox2), p22phox, p47phox, p67phox, and p40phox, all of which have been detected, to varying degrees, in vascular cells (25). Studies in rat vascular smooth muscle cells indicate that gp91phox is replaced by the homologues Nox1 and Nox4 (157, 160). However, in vascular smooth muscle cells from human resistance arteries we demonstrated that gp91phox is the major Nox isofrom present (173). These differences may be important functionally because Nox1 has been implicated to be involved in cell growth, Nox4 in delayed cell growth and gp91phox may act as a proxn pump in addition to being part of the NAD(P)H oxidase complex (65, 97, 157, 160, 173). Whereas ANG II upregulates Nox1 and gp91phox, it downregulates Nox4 (160, 173). Moreover whereas gp91phox is primarily cell membrane-associated, Nox1 localizes in caveolae and Nox4 in focal adhesion. Thus these findings reflect important differences between human and rat cells and highlight the fact that caution should be applied when extrapolating results from animal studies to humans.

O$_2^-$ and H$_2$O$_2$ activate multiple signaling molecules, including MAP kinases (p38 MAP kinase, JNK, ERK-5, and ERK1/2), nonreceptor tyrosine kinases (Src, JAK2, STAT, p21Ras, Pyk2, and Akt), receptor tyrosine kinases (EGFR, IGFR, and PDGFR), protein tyrosine phosphatases and redox-sensitive transcription factors (NF-κB, AP-1, and HIF-1) (109, 170). Activation of these molecules participates in cell growth, migration, expression of proinflammatory genes, production of extracellular matrix proteins and contraction (39). All of these processes play important roles in vascular remodeling in hypertension. Exact mechanisms whereby reactive oxygen species modify signaling molecules remain unclear, but oxidative modification of proteins is important (19, 77).

**ANG II-mediated activation of NAD(P)H oxidase involves c-Src, PKC, PLA$_2$, and PLD as well as increased synthesis of gp91phox, p22phox, p47phox and p67phox (53, 175, 184).** In hypertension, these processes are augmented, contributing to increased activation of the oxidase and consequent oxidative stress (181). Furthermore, various polymorphisms in the promoter of the p22(phox) gene have been identified in hypertensive patients, which may also play a role in increased NAD(P)H-driven generation of O$_2^-$ in hypertension (101).

Inhibition of NAD(P)H oxidase activity is now being considered, at least experimentally, as a possible therapeutic target in the treatment of hypertension (41). In fact, it has been suggested that some of the beneficial actions of ACE inhibitors and AT1 receptor antagonists may be mediated, in part, by decreasing vascular oxidative stress. These effects have been attributed to direct inhibition of NAD(P)H oxidase activity, as shown for AT1 receptor blockers, and to intrinsic antioxidant properties of the agents. Recent clinical studies support a direct
antioxidant effect of AT1 receptor blockers, since treatment of hypertensive patients with candesartan reduced oxidative stress and inflammation independently of blood pressure-lowering actions (38).

EFFECT OF RENIN-ANGIOTENSIN BLOCKADE ON SMALL ARTERIES IN HUMAN HYPERTENSION

As indicated earlier, we have attempted to go from the bedside to the bench to elucidate some of the mechanisms involved in the regulation of the vasculature and its abnormalities in essential hypertension, eventually to come back to the bedside to determine whether indeed interfering with those mechanisms could improve the vascular status of patients and eventually their outcome. Accordingly, we have taken the agents that interfere with these mechanisms and used them on patients, and questioned whether the use of these drugs would correct the remodeling of the vasculature of hypertensive patients. We treated hypertensive patients in double-blind randomized studies for 1 (143) or 2 yr (144, 145) with the ACE inhibitor cilazapril compared with the β-blocker atenolol. The ACE inhibitor corrected the structure and improved the endothelial function of small gluteal subcutaneous arteries whereas the β-blocker had no effect despite equal blood pressure control. Thybo et al. (168) obtained similar results with the ACE inhibitor perindopril compared with atenolol for 1 yr. Rizzoni et al. (121) examined hypertensive subjects treated with lisinopril for 3 yr, and found that the media-to-lumen ratio was slightly reduced, and endothelial function slightly improved. We also investigated treatment with the angiotensin receptor blocker (ARB) losartan, again compared with atenolol (146). The ARB normalized the structure of small arteries and the endothelial dysfunction, whereas again the atenolol-treated group had no change (146). More recently, we evaluated the effect of switching patients who had been randomized to treatment with atenolol to the ARB irbesartan (147). Under atenolol use for 1 yr, the structure and function of small arteries had remained abnormal; however, 1 yr after the patient was switched to the ARB and achieved equal blood pressure control, structure and endothelial function were normalized.

Blockade of ANG II-induced oxidative stress and its growth-promoting, profibrotic and proinflammatory action may also play a role in correction of remodeling and endothelial dysfunction. ARBs increase circulating ANG II, which may stimulate unblocked AT2Rs, which could contribute to the improved structure and function (15). Vasodilatation may also play a role, as vasoconstriction has been shown to induce inward eutrophic remodeling (6). Blood pressure reduction does not seem to contribute because atenolol-treated patients had their blood pressure was equally well controlled but exhibited no improvement. A recent study (111) has suggested that β-adrenergic receptors in smooth muscle cells inhibit cell growth and collagen production. β-Blockade could exacerbate the latter and antagonize any beneficial effect of lowering of blood pressure.

It should, however, be clarified that whereas β-blockers, or at least atenolol, do not seem to improve vascular remodeling or endothelial dysfunction, they have other beneficial properties in hypertension, particularly in relation to cardioprotection through their anti-ischemic effects, as they reduce oxygen consumption by the heart. For this reason, although atenolol was not shown to be vasculoprotective, β-blockers are indeed indicated as the first choice of therapy in patients with hypertension, and are a compelling indication in those with ischemic heart disease unless there are specific contraindications such as asthma, severe peripheral vascular disease, bradycardia, or atrioventricular block (21). Even though in the Atherosclerosis Risk in Community study (58) β-blockers were found to have induced de novo diabetes mellitus to greater degree than other antihypertensive agents, in the UK Prospective Diabetes Study (186), atenolol was shown to be equally efficacious as the angiotensin-converting enzyme (ACE) inhibitor captopril, in preventing cardiovascular events. However, this aspect of UK Prospective Diabetes Study has been criticized as being underpowered to find differences between these individual agents. Most trials have been unable to detect differences between antihypertensive agents to prevent cardiovascular events, as discussed below.

Relevance of Regression of Small Artery Remodeling in Hypertensive Humans

The changes that are demonstrated in subcutaneous resistance arteries are similar to those found in experimental animals in the heart, kidney, and brain. We have shown that endothelial function of small arteries from gluteal subcutaneous biopsies correlates closely with endothelium-dependent brachial artery flow-mediated dilatation (114), which in turn correlates with coronary vasomotion (5). Coronary vasomotion has been shown to relate to cardiovascular outcome (133). The structure of small arteries dissected from gluteal subcutaneous tissue correlates with coronary flow (123) and with the type of cardiac remodeling present (103, 122). The media-to-lumen ratio of small arteries was greater in patients with dilated cardiac hypertrophy, which has poor prognosis. The same group demonstrated that patients with a greater media-to-lumen ratio of small arteries suffer more cardiovascular events (124). Treatment with the ACE inhibitors enalapril (102) and perindopril (149) has been shown to improve coronary reserve, and perindopril also diminished pericoronary fibrosis in small vessels in hypertensive subjects (149). The calcium channel blockers nifedipine gastrointestinal transport system (192) or lacidipine (161) improved forearm blood flow responses to acetylcholine, agreeing with findings in small arteries from gluteal subcutaneous biopsies (140, 148, 152). These different results demonstrate the pathophysiological relevance of gluteal subcutaneous small arteries, which appear to be good surrogates for more critical vascular beds.

Numerous meta-analyses of randomized clinical trials (9, 154), and the recent Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (54) and Second Australian National Blood Pressure studies (198) demonstrate that most agents reduce cardiovascular events largely to a similar degree as long as blood pressure is adequately controlled. This could suggest that whether agents do or do not have vasculoprotective effects, the results of treatment are similar. However, it should be recalled that most randomized clinical trials last 3–5 yr. The study of Rizzoni et al. (124) suggests that divergence of survival without events between individuals with greater or lesser degree of remodeling of small arteries occurs after 3–4 yr. Furthermore, the Heart Outcomes Prevention Evaluation study (63) supported the idea that ACE inhibitors protect from cardiovascular events beyond blood pressure lowering, although in the
Perindopril Protection Against Recurrent Stroke Study (118) blood pressure lowering seemed to play a very important role in secondary stroke prevention, and even in the Heart Outcomes Prevention Evaluation, blood pressure lowering may have had a critical role. The recent Losartan Intervention for Endpoint study (24) has suggested that ARBs are superior to β-blockers, and this trial is the one that most favors the hypothesis of beneficial effects of vascular protection beyond blood pressure lowering. Thus it is possible that benefits of vascular protection are found after long-term treatment and may not be demonstrable within the period of time (3–5 yr) that most randomized clinical trials take place.

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