Angiotensin and Cytoskeletal Proteins: Role in Vascular Remodeling

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Vascular remodeling occurs during normal development and is involved in various physiologic events. However, the adaptive structural changes of the vasculature can also be pathologic, leading to vascular disease such as hypertension, atherosclerosis, and vein graft disease. Preeclampsia may develop as a consequence of inappropriate vascular remodeling during pregnancy. Angiotensin II contributes to vascular remodeling by activating signal transduction cascades that promote vasoconstriction, growth, and inflammation. The cytoskeleton also participates in structural adaptation responses of the vasculature; cytoskeletal filaments may mediate vasoactive responses, transduce mechanical stimuli, and are involved in pharmacologic signal transduction. It has become clear that many of the cytoskeletal changes during vascular remodeling can be induced by angiotensin II. Recently, the small G-protein Rho has attracted much attention. The Rho/Rho-kinase system is activated by angiotensin II, is a prominent regulator of the cytoskeleton, and is involved in pathologic vascular remodeling.

Introduction

Blood vessels are able to sense and respond to a great diversity of stimuli. Acute responses include modulation of smooth muscle tone leading to constriction or dilation. However, persistent stimulation of blood vessels induces structural changes, *ie*, remodeling. The kind of remodeling response depends on the stimulus and the vessel type, and may include changes in lumen diameter (inward or outward remodeling [1]); medial cross-sectional area (hypotrophic, eutrophic, or hypertrophic remodeling [1]); and media-to-lumen ratio. Vascular remodeling is involved in normal development and in many physiologic events, such as the expansive remodeling of the uterine circulation during pregnancy [2]. On the other hand, pathologic vascular remodeling has been demonstrated as well, for example during hypertension, atherosclerosis, and vein graft disease [3–6]. The process of vascular remodeling is complex, with many different subprocesses such as growth, apoptosis, dedifferentiation, rearrangement of vessel components, extracellular matrix deposition, and formation of focal adhesions, with similar or different temporal patterns. In this review, we focus on the roles of angiotensin II (Ang II) and the cytoskeleton in vascular remodeling.

Angiotensin II

Angiotensin II is the principal effector of the renin-angiotensin-system (RAS). Renin, mainly produced by the juxtaglomerular cells in the kidneys, is a circulating enzyme that converts angiotensinogen into Ang I. Ang I is further transformed to Ang II by angiotensin converting enzyme (ACE). ACE exists in several forms, but is primarily bound to the vascular endothelium throughout the body. Over the years it has become clear that besides the systemic RAS, a local RAS is present in the vasculature, which may play an important role in vascular remodeling [3,7–9]. In addition, other enzymatic systems have been described by which Ang II can be produced, including cathepsin G, elastase, tonin, tissue plasminogen activator, and chymase. However, their relative contribution to total Ang II production is not clear [8,10–12]. Ang II acts on two receptor types, angiotensin type 1 and 2 (AT_1 and AT_2) receptors. Activation of the AT_1 receptor is responsible for the powerful vasoconstrictive and growth promoting actions of Ang II, whereas AT_2 activation often elicits opposite actions.

Angiotensin Receptor Signaling

AT_1 receptors activate many signal transduction cascades with complex interactions leading to cellular growth, migration, and proliferation. Activation of the G-protein–coupled AT_1 receptor induces rapid protein tyrosine phosphorylation of src family kinases and substrates of src, such as focal adhesion kinase (FAK); proline-rich tyrosine kinase 2; paxillin, tensin, Janus kinase 2 (JAK2); and signal transduction and activator of transcription 1 (STAT1). This leads to activation of regulatory proteins (Shc, Grb2, Sos); small G-proteins (Ras, Rho); mitogen-activated protein (MAP) kinases (extracellular signal-regulated kinase [ERK1/2], p38, *c-jun* N-terminal kinases [JNK]); expression of protooncogenes (*c-fos*, *c-jun*, *c-*

myc); activation of transcription factors (AP-1, nuclear transcription factor κB [NFκB]); and gene expression (Fig. 1) [8,13,14,15•,16–21,22••].

AT_1 activation leads to rapid activation of phospholipase (PL) C , PLD, and PLA_2 via src [23]. PLC activation induces formation of diacylglycerol and inositol triphosphate, leading to activation of protein kinase C and Ca^{2+} release from intracellular stores, respectively [24]. AT_1 stimulation also increases vascular smooth muscle cell (VSMC) $[Ca^{2+}]_i$ by activation of sarcolemmal Ca^{2+} channels, which leads to smooth muscle contraction [8]. The AngII-induced activation of Ca^{2+} channels was shown to be regulated by src and FAK [25]. In addition, Ang II has been demonstrated to induce rat aorta contraction by activation of the p38 MAP kinase pathway (Fig. 1) [26].

Cascades induced by PLD and PLA_2 lead to activation of a NADH/NADPH oxidase, probably through formation of phosphatic acid or arachidonic acid, and induce production of reactive oxygen species (ROS). The superoxide anion ($•O_2^-$) has a short half-life (seconds), and is rapidly converted by superoxide dismutase to the more stable ROS hydrogen peroxide (H_2O_2). Catalase and glutathione peroxidase finally convert H_2O_2 to H_2O [27,28]. The molecular identity of the oxidase that is responsible for ROS generation in VSMC is not clear yet. Recent evidence points towards several essential components of the oxidase enzyme: the cytosolic $p47^{phox}$ (phagocyte oxidase), $p67^{phox}$, and the membrane-bound $p22^{phox}$, $p91^{phox}$, and nox-1 (nonphagocytic oxidase), which is a $p91^{phox}$ homologue [28–32]. ROS are mediators of Ang II-induced VSMC proliferation. Many of the growth-associated signaling molecules that are activated by Ang II, such as src, JAK2, STAT1, PLD, Ras, and p38 and JNK MAP kinases are redox sensitive. Thus, ROS may mediate Ang II-induced VSMC proliferation by manipulation of these signaling molecules [28]. Besides promoting VSMC proliferation, ROS are also involved in expression of interleukin-6, monocyte chemoattractant molecule-1, and vascular cell adhesion molecule through activation of NFκB [6,13,18], suggesting a role for ROS in the inflammatory response to Ang II. Inflammation leads to enhanced production of ACE and Ang II, and this creates a positive feedback mechanism that amplifies the actions of Ang II [3,6]. Furthermore, ROS can modulate activity of matrix metalloproteinases, which modify the deposition of extracellular matrix molecules and thereby may affect the vessel structure [6,33]. Finally, ROS inactivate the vasodilator molecule nitric oxide, leading to impairment of endothelium-dependent relaxation [3,28].

Part of the growth promotory action of AT_1 activation may be mediated by other growth factors or activation of their receptors. AT_1 activation has been shown to induce rapid (within minutes) transactivation (tyrosine phosphorylation) of receptors for epidermal growth factor [19,34,35], insulin-like growth factor-1, and platelet derived growth factor-β [16,34,36,37]. The mechanisms of receptor transactivation are not clear, but ROS and src are candidate transducers (Fig. 1) [16,34]. In addition to the rapid receptor transactivation, Ang II has been reported to stimulate expression of various growth factors, including platelet derived growth factor-β, basic fibroblast growth factor, vascular endothelial growth factor, insulin-like growth factor-1 (proproliferative), and transforming growth factor-β (antiproliferative) [3,8,16,17,34]. AT_1 activity also interacts with the endothelin metabolism. Hypertension induced by Ang II infusion can be suppressed by ET_A-receptor antagonism, which suggests that endothelin partly mediates Ang II-induced hypertension [38]. Furthermore, AT_1 stimulation has been shown to promote endothelin synthesis and release [39]. Thus, Ang II may mediate growth and remodeling responses by autocrine or paracrine production of growth factors.

The physiologic role and signal transduction mechanisms of the AT_2 receptor are not yet clearly defined. Activation of the AT_2 receptor generates vasodilation, and is involved in growth inhibition, apoptosis, and differentiation. The G-protein–coupled AT_2 receptor is abundantly expressed in neonatal and embryonic blood vessels, and has lower (but detectable) levels of expression in adult vessels. However, the AT_2 receptor is re-expressed after vascular injury and in the development of proliferative vascular lesions. It is hypothesized that during vascular remodeling, the AT_2 receptor modulates the growth responses elicited by the AT_1 receptor or other growth stimuli [3].

Besides these direct effects, Ang II may also promote vascular remodeling indirectly through effects on blood pressure and sympathetic nerve activity [40,41], but this is beyond the scope of this review.

Role of Angiotensin in Pathologic Vascular Remodeling

Evidence is accumulating that Ang II, through AT_1 activation, is involved in many types of pathologic vascular remodeling. Hypertension is associated with arterial remodeling; large arteries show hypertrophic remodeling, arterioles (diameter < 100 μm) undergo rarefaction, and small arteries (diameter 100–500 μm) display inward remodeling, leading to the characteristic increased peripheral resistance [5]. The involvement of Ang II in hypertension-related arterial remodeling, and in the severity of the arterial hypertrophy, is evident from many clinical and experimental observations. Schiffrin *et al*. [42••] showed that in patients with essential hypertension, resistance arteries show inward eutrophic remodeling. In these patients, the AT_1 antagonist losartan and β-blocker atenolol induced similar blood pressure reduction. However, losartan, and not atenolol, corrected resistance artery structure [42••]. In spontaneously hypertensive rats (SHR), both AT_1 blockade and ACE inhibition normalized resistance artery structure [43]. Patients with renovascular hypertension, which is

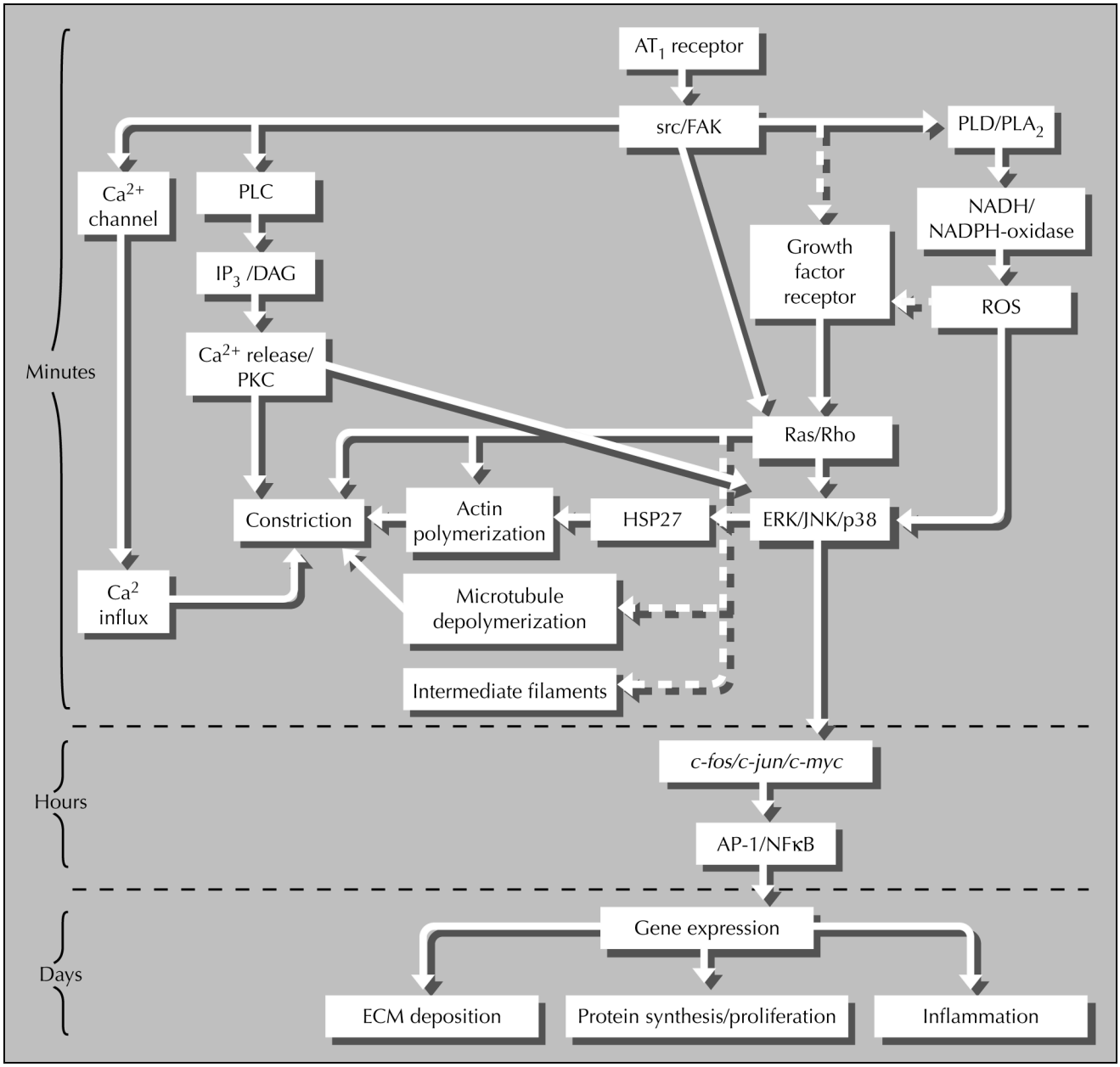

Figure 1. Angiotensin II signaling through the AT_1 receptor. Stimulation of the G-protein–coupled AT_1 receptor leads to activation of several signal transduction cascades that promote vascular constriction and gene expression, and finally cause changes in the architecture of blood vessels (see text for details). All *solid arrows* depict established routes of activation, the *dashed arrows* represent hypothesized activation pathways. DAG—diaglycerol; ECM—extracellular matrix; ERK—extracellular signal-regulated kinase; FAK—focal adhesion kinase; HSP—heat shock protein; IP_3—inositol triphosphate 3; NFκB—nuclear transcription factor κB; PKC—protein kinase C; PL—phospholipase; ROS—reactive oxygen species.

characterized by high circulating renin levels, show disproportional arterial hypertrophy compared with essential hypertension [44•]. In mRen transgenic rats with enhanced renin expression in the vasculature, similar observations were made [45]. Several recent studies showed enhanced Ang II-induced signal transduction in VSMC from hypertensive patients or SHR. In VSMC of essential hypertensive patients compared with normotensive patients, AT_1 receptor expression was unaltered, but Ang II induced an enhanced phosphorylation of src and ERK, enhanced *c-fos* expression, AP-1 DNA binding, and protein and DNA synthesis [22••]. Similarly, in VSMC of SHR compared with Wistar-Kyoto (WKY) rats, Ang II induced enhanced PLD activity [46], phosphorylation of p38 and ERK, and enhanced DNA and collagen synthesis [20,21]. Moreover, the Ang II-induced $[Ca^{2+}]_i$ response is differently modulated by extracellular matrix molecules in VSMC of SHR and WKY, leading to enhanced Ca^{2+} release from intracellular stores and an increased Ca^{2+} influx in SHR. This may contribute to increased arterial smooth muscle tone and increased peripheral resistance in SHR [47].

Pregnancy is associated with outward remodeling of uterine spiral arteries. If this remodeling does not sufficiently occur, pre-eclampsia may develop [2]. Defective RAS has been implicated in the development of pre-eclampsia. It was shown that all components of the RAS are expressed in and around remodeling spiral arteries, and that especially altered expression levels of angiotensinogen may be a critical factor in the development of pre-eclampsia [9]. Furthermore, our group recently showed that in tissue-ACE–deficient mice, uterine arteries have a larger lumen diameter compared with wild-type mice. Unexpectedly, pregnancy induced a more hypertrophic outward remodeling in the tissue-ACE–deficient mice [48]. These results show that RAS is involved in pregnancy-related vascular remodeling, but the exact contribution of the RAS components needs further investigation.

Angiotensin II is also implicated in several other forms of pathologic vascular remodeling, such as atherosclerosis, vein graft disease after coronary bypass surgery, and vascular injury after percutaneous transluminal coronary angioplasty. These pathologies are characterized by narrowing of the lumen, neointima formation, VSMC migration and proliferation, and endothelial dysfunction. The involvement of Ang II is clear because both ACE inhibitors and AT_1 blockers have been shown to reverse the above-mentioned pathologic changes of vessel structure [3,4,17]. They have been shown to act by inhibition of Ang II-induced oxidative stress [49] and inflammation [13,14]. In atherosclerosis, an increased accumulation of tissue-ACE has been reported [3,50], as well as an AT_1 receptor upregulation [49]. For both vein graft disease and balloon-injured arteries, there is evidence for the involvement of chymase, besides ACE, as an Ang II-generating system [10,12].

Cytoskeleton

The cytoskeleton is a dynamic three-dimensional network of filamentous polymers that has many functions in the cell, such as cell shape, deformability, and transduction of mechanical stimuli [51]. The cytoskeleton consists of three components: actin filaments, microtubules, and intermediate filaments (IFs). It is becoming increasingly clear that the cytoskeleton has a role in acute (up to several hours) vasoactive responses.

Cytoskeleton in acute vasoactive responses

The involvement of the three cytoskeletal components in acute vasoactive responses has been demonstrated. Mice lacking the IF vimentin (vimentin-/-) showed impaired flow-induced, nitric oxide-mediated, dilation of mesenteric resistance arteries, although the myogenic reactivity was not modified [52]. Disruption of actin filaments prolonged nitric oxide synthase mRNA half-life in endothelial cells [53], and inhibited Ca^{2+} channels in VSMC [54], which would both promote vasodilation. On the other hand, polymerization of actin causes constriction. Ang II has been shown to induce contraction of rat aorta through phosphorylation of heat shock protein (HSP)27, an important regulator of actin polymerization. This response was dependent on activation of p38 MAP kinase and generation of ROS [26]. Depolymerization of microtubules blocked flow-dependent dilation of arterioles [55], and is associated with endothelium-independent, slow (steady-state after 1 hour), force production mediated by myosin light chain phosphorylation [56,57]. Recently, Bakker *et al.* [58••] showed that in an isolated resistance artery in organ culture, a maintained (days) vasoconstriction caused structural narrowing of the artery. This suggests that if the cytoskeleton is involved in vasoactive responses that persist, it may be able to mediate or modify vascular remodeling.

Cytoskeleton in vascular remodeling

For all three components of the cytoskeleton there is evidence for a role in vascular remodeling. It is known from experiments in cultured endothelial cells that shear stress modification induces a coordinated displacement of vimentin filaments [59]. This cytoskeletal reorganization probably participates in the shape changes and alignment of the endothelial cells, and thus may contribute to the remodeling of whole blood vessels after altered flow. Indeed, Schiffers *et al.* [60•] recently reported that vimentin-/- mice show an altered carotid artery remodeling in response to a 4-week period of modified flow. Thus, vimentin and possibly other IFs participate in the mechanotransduction of altered shear stress, but their role in the remodeling process is not clear yet.

Depolymerization of microtubules has been shown to potentiate the phenylephrine-induced constriction in mesenteric arteries of normotensive rats, but not in the mesenteric bed of deoxycorticosterone acetate (DOCA) hypertensive rats. This experimental finding supports the hypothesis that the microtubular network in vessels from the hypertensive rats is somewhat dissembled, which would contribute to the increased reactivity in this model of hypertension [35].

Actin disorganization decreased Ang II-induced Ca^{2+} release from intracellular stores and Ca^{2+} influx in cultured rat aortic VSMC from SHR, but not from WKY rats. This suggests a more prominent role of the actin cytoskeleton in Ca^{2+} handling mechanisms in SHR [61]. Furthermore, the inward remodeling of rabbit carotid artery, induced by a ligation that reduced shear stress, was associated with reorganization of the actin cytoskeleton in the endothelium, with fewer central stress fibers and more peripheral actin bundles. This was accompanied by enhanced leukocyte adhesion and changes of the endothelial cells to a much less elongated "cobblestone" phenotype, indicative for the initiation of atherosclerosis [62]. Likewise, loss of stress fibers was shown to be associated with accumulation of macrophages and atherosclerotic plaque formation in rabbits with hypercholesterolemia [63].

These findings show that the cytoskeleton participates in both acute vasoactive responses and the remodeling process, but the responsible mechanisms are not fully elucidated. Focal adhesion sites deserve special attention. Here, actin-containing stress fibers and the extracellular matrix interact with integrins in the plasma membrane. Focal adhesion sites already have been implicated in transduction of mechanical stimuli, such as pressure and flow [25]. However, experimental findings showing association of second messenger molecules, such as src and FAK, to the cytoskeleton [15•,64,65], and the regulation of the cytoskeleton by Rho (see below), suggests participation of the cytoskeleton in agonist-induced signal transduction as well.

Rho and Vascular Remodeling

The Rho protein family belongs to the Ras superfamily of small G-proteins that has four main representatives: Rho, Cdc42, Rnd, and Rac. They are key regulators of the actin cytoskeleton, and as such they are involved in many fundamental vascular processes, including smooth muscle contraction, cell adhesion, cell motility, and cell shape. Recently, Rho G-proteins have also been reported to regulate the organization of other cytoskeletal proteins, such as mircotubules [66] or IF vimentin [67], but the functional consequences of these Rho actions for the vasculature are not known. In vivo, the activation of Rho is regulated by guanine nucleotide exchange factors, guanine nucleotide dissociation inhibitors, and GTPase-activating proteins. The members of the Rho subfamily, RhoA, RhoB, and RhoC, are very similar, and many of the used inhibitors, or mutant guanine nucleotide exchange factors, cannot specifically target one of them. Therefore, we use the term Rho, although RhoA, which is the best characterized Rho subfamily member, probably regulates most pathways discussed in this review. Rho exerts most of its effects through Rho-kinase (p160ROCK) [68,69]. Because of its newly discovered roles in Ca^{2+} sensitization of smooth muscle contraction (by inactivation of myosin light chain phosphatase), focal adhesion assembly, stress fiber formation, and G-protein–coupled receptor signaling, many recent studies investigating the mechanisms of pathologic vascular remodeling focused on Rho.

In 1997, Uehata *et al*. [70] reported that Y27632, a specific inhibitor of p160ROCK, inhibited smooth muscle contraction by reducing Ca^{2+} sensititvity, reduced stress fiber formation in cultured cells, and corrected hypertension in several hypertensive rat models [70]. Recent studies showed that Rho-kinase inhibition normalized the enhanced Ca^{2+} sensitization in SHR to WKY levels, preferentially reduced the augmented arterial tone in SHR compared with WKY rats, and also prevented hypertrophic remodeling of coronary arterioles in SHR [71•,72••]. Moreover, it was found that the expression and activity of Rho-kinase were augmented in SHR [72••]. In VSMC, stretch-induced Rho translocation and stretch-induced ERK activation were suppressed by both Rho-kinase inhibition and actin disruption [73]. In endothelial cells, gene transfer of dominant-negative mutants of Rho and Rho-kinase inhibited shear stress–induced cell alignment and stress fiber formation [74]. In VSMC, Rho-kinase inhibition reduced Ang II-induced expression of monocyte chemoattractant molecule-1 and plasminogen activator inhibitor-1 [75,76]. Rho-kinase inhibition or transfer of dominant-negative Rho-kinase reduced constrictive remodeling and vasospastic serotonin responses after interleukin-1β infusion in porcine coronary arterioles, a model of atherosclerosis [77,78•]. The same treatment also suppressed neointima formation in porcine femoral artery after balloon injury [79•]. Collectively, these recent findings show that inhibition of Rho-kinase reduced mechanotransduction, atherosclerosis-related Ang II-signaling and pathologic vascular remodeling in different animal models for hypertension, atherosclerosis, and response to injury. Thus, Rho-kinase can be regarded as a novel therapeutic target in the treatment of these forms of vascular disease.

Conclusions

Angiotensin II and the cytoskeleton participate in pathologic vascular remodeling. We suggest two ways by which Ang II is involved: 1) increased Ang II production, and 2) sensitization of the AT_1 response. The Ang II production may increase through elevated plasma renin levels, enhanced expression of angiotensinogen or ACE in the vasculature, or by upregulation of other Ang II-generating systems, such as chymase [3,9–11,44•,50]. Mechanisms of AT_1 sensitization are unknown, but the finding that Ang II-induced src phosphorylation, one of the first events in Ang II signaling, was enhanced in hypertensive patients [22••] suggests modifications in the link between the AT_1 receptor and src tyrosine kinase. It can be speculated that this link is altered by specific subunits of the G-proteins that are coupled to the AT_1 receptor, but this hypothesis has to be tested in future experiments.

The cytoskeleton appears to participate in vascular remodeling by changes in polymerization, assembly of focal adhesions, and formation of stress fibers. At least part of these cytoskeletal changes can be induced by Ang II, which indicates interactions between cytoskeletal dynamics and Ang II-induced signaling (Fig. 1). Ang II stimulates formation of focal adhesions and stress fibers [16,64,65]. Ang II, through generation of ROS and activation of p38 and HSP27 can also induce actin polymerization and contraction [26]. On the other hand, disruption of the actin cytoskeleton suppressed Ang II-dependent signaling and protein synthesis [15•]. Moreover, Ang II leads to activation of Rho, a key regulator of the actin cytoskeleton [68].

Many studies suggest that pathologic vascular remodeling is due to augmented Rho or Rho-kinase activity. It is not known what causes this upregulation, and it is also

not clear by what signal transduction mechanisms Rho activation leads to pathologic vascular remodeling. Candidate effector mechanisms involve 1) enhanced Ca^{2+} sensitization, generating more smooth muscle tone that may contribute to hypertension, enhanced agonist-induced contractions, and spasms [35,68,69,77,78•]; 2) rearrangement of actin cytoskeleton, stress fiber formation, and focal adhesion assembly, which may potentiate smooth muscle contraction and possibly sensitize the smooth muscle cells to mechanical stimulation [25,68]; and 3) inflammation, through expression of proinflammatory molecules [75,76].

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