Signal Transduction of Mechanical Stresses in the Vascular Wall

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Abstract—The vascular wall is constantly subjected to a variety of mechanical forces in the form of stretch (tensile stress), due to blood pressure, and shear stress, due to blood flow. Alterations in either of these stresses are known to result in vascular remodeling, an adaptation characterized by modified morphology and function of the blood vessels, allowing the vessels to cope with physiological or pathological conditions. The processes involved in vascular remodeling include cellular hypertrophy and hyperplasia, as well as enhanced protein synthesis or extracellular matrix protein reorganization. In vitro studies using vascular cells have attempted to identify the mechanisms behind structural alterations. Possible pathways include ion channels, integrin interaction between cells and the extracellular matrix, activation of various tyrosine kinases (such as c-Src, focal adhesion kinase, and mitogen-activated protein kinases), and autocrine production and release of growth factors. These pathways lie upstream of de novo synthesis of immediate response genes and total protein synthesis, both of which are likely to be involved in the process of vascular remodeling. **(*Hypertension*. 1998;32:338-345.)**

Key Words: shear stress ■ stretch ■ muscle, smooth ■ endothelium ■ MAP kinases

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Blood vessels are permanently subjected to mechanical forces in the form of stretch, which because of the pulsatile nature of blood flow exposes vessels to cyclic mechanical strain, and shear stress. Blood pressure is the major determinant of vessel stretch. It creates radial and tangential forces that counteract the effects of intraluminal pressure and that affect all cell types in the vessel. In comparison, fluid shear stress results from the friction of blood against the vessel wall, and it acts in parallel to the vessel surface. Accordingly, shear is sensed principally by endothelial cells, strategically located at the interface between the blood and the vessel wall. Alterations in stretch or shear stress invariably produce transformations in the vessel wall that will aim to accommodate the new conditions and ultimately restore basal levels of tensile stress and shear stress.[1–3] Hence, while acute changes in stretch or shear stress correlate with transient adjustments in vessel diameter, mediated through release of vasoactive agonists or change in myogenic tone, chronically altered mechanical forces usually instigate important adaptive alterations of vessel wall shape and composition. The concept of vascular remodeling has therefore been used to describe the transformations that occur in vessels undergoing mechanical stresses. For example, experimental hypertension is accompanied by increased wall thickness, resulting in resistance arteries and arterioles from VSMC hyperplasia and in conductance arteries from hypertrophy.[4,5] Likewise, reduced mechanical strain translates into vessel atrophy.

Several reports describe the effects of mechanical stretch on hypertrophy of the heart, and the pathways leading to these events have been studied extensively in cardiac cells (reviewed in Reference 6). More recently, investigators have identified how mechanical forces are sensed and transduced into biochemical signals by multiple pathways within the vascular cells, resulting in various biological responses. Located at the cell surface, integrins are likely to be key mechanosensors. In parallel, ion channels and other unknown stretch receptors presumably transduce the mechanical signal. As a result, several intracellular signaling pathways are activated, including the FAK pathway, the MAP kinase cascade, and the renin-angiotensin system (Figure 1).

Mechanical Forces and Vascular Cell Phenotype

Effects of Stretch

Mechanical stretching of VSMCs produces a variety of responses that account for vessel morphology. On one hand, stretch may be at the very core of the VSMC differentiated state. Indeed, in a model of cultured rabbit aorta, we determined that a certain level of stretch is crucial for the maintenance of the differentiated phenotype of the VSMC. Vessels placed in conditions of abnormally low intraluminal pressure (10 mm Hg) showed decreased content, over 3 to 6 days, of smooth muscle marker proteins h-caldesmon and filamin despite the presence of fetal calf serum, a known

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Selected Abbreviations and Acronyms

Ang II = angiotensin II
ECM = extracellular matrix
ERK = extracellular signal–related kinase
FAK = focal adhesion kinase
MAP = mitogen-activated protein
MEK = MAP kinase kinase
MEKK = MAP kinase kinase kinase
NFκB = nuclear factor-κB
PDGF = platelet-derived growth factor
PKC = protein kinase C
VSMC = vascular smooth muscle cell

mitogen, in the culture medium. In comparison, loss of these proteins was prevented in aortic segments kept at physiological intraluminal pressure (80 mm Hg).[7] The presence of endothelium was not essential for maintenance of VSMC marker proteins. These results corroborate earlier experiments, in which cyclic stretching of cultured VSMCs was shown to increase (or rather prevent the decrease of) the expression of smooth muscle myosin heavy chains and myosin light chain kinase.[8] Furthermore, cyclic stretching (12 to 72 hours) of VSMCs augmented smooth muscle myosin heavy chain SM-1 and SM-2 protein content and decreased nonmuscle myosin-A compared with static VSMC cultures.[9] Because only SM-1 showed enhanced mRNA expression under cyclic strain,[9] the relative increase in SM-2 was presumably due to reduced degradation of this marker protein. Therefore, constant mechanical stimulation appears to be required for maintenance of normal contractile phenotype of VSMCs in the arterial wall. Loss of stretch, together with loss of ECM contacts, is probably a major cause of differentiation of VSMCs in culture. However, while a certain level of stretch is required to maintain VSMCs in a quiescent state,

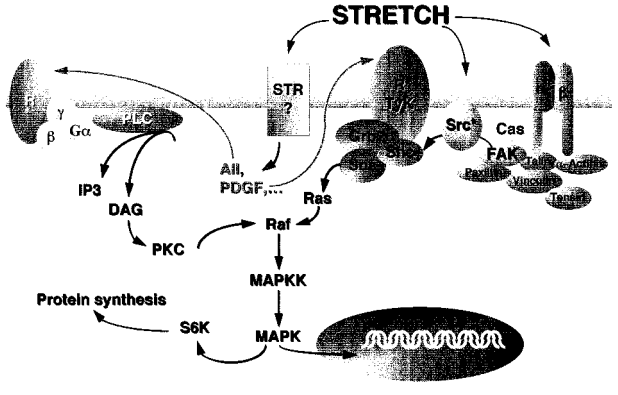

Figure 1. Multiple pathways of transduction of mechanical stretch in vascular cells. Mechanical forces may act on α/β heterodimer integrins and could activate nonreceptor membrane tyrosine kinases including c-Src*. Activated c-Src* may stimulate FAK autophosphorylation, allowing association of the Shc-Grb2-Sos complex and downstream activation of MAP kinase. MAP kinase may simulate growth or protein synthesis via activation of S6 kinase (S6K). Alternatively, stretch acts on unknown stretch receptors (STR) that stimulate the renin-angiotensin system, leading to the production of Ang II (AII), which in turn acts on G-protein coupled receptors. Autocrine production of growth factors including PDGF may activate tyrosine kinase receptors (R TyK).

overstretching triggers adaptive processes that result in increased protein synthesis and hypertrophy.

In fact, organ culture of aortas maintained during 3 to 6 days at high intraluminal pressure (150 mm Hg) revealed that both total protein synthesis and fibronectin content are enhanced by stretch.[10] Interestingly, DNA synthesis remains unchanged in the same preparations, in keeping with observations of pressure-induced hypertrophy but not hyperploidy in large vessels in vivo[4] and contrary to DNA synthesis induction by stretch in VSMC culture.[11,12] In the latter case, absence of interaction with the ECM environment is certainly responsible for the hyperplasic response to stretch.

Effects of Shear

The fact that shear acts mainly on endothelial cells, while stretch has repercussions on the entire vessel wall, does not rule out the likelihood that long-term changes in blood flow will bring about vascular remodeling, characterized by altered vessel wall thickness, matrix composition, and wall organization.[13] Accordingly, in endothelial cells subjected to oscillatory flow, fibronectin and laminin content was found to be greater than that in static cultures.[14] However, although laminar shear stress may lead to a reorganization of cytoskeletal proteins and change of cell shape, it apparently does not change protein levels in cultured endothelial cells.[15] On the other hand, a variety of genes encoding for growth factors (PDGF, transforming growth factor),[16,17] vasodilators (NO, prostacyclin),[3,18–20] vasoconstrictors (endothelin),[21] and adhesion molecules (intercellular adhesion molecule)[22] are regulated on shear stimulation. While a number of these inductions are transient, some persist and may mediate long-term alterations in vessel structure and function that occur through regulation of protein and gene expression.[3] Finally, pressure-induced circumferential cyclic strain increases endothelial cell sensitivity to shear stress, resulting in a lowered threshold level of shear to provoke structural responses.[23] Ultimately, concomitant stimulation of vascular cells by both stretch and shear stress may produce maximal remodeling responses in the vessel.

Transmission of Mechanical Stresses at the Cell Membrane

Integrins

Strategically situated at the boundary between the ECM and the cytoskeleton, integrins may act not only as mediators of cell adhesion but they can also transduce biochemical signals across the cell membrane. Indeed, integrins are present at sites of close apposition of the cell surface and the ECM, and they form a bridge between matrix proteins and the cytoskeleton, mediating binding and attachment of the cell to components of the ECM (such as fibronectin, vitronectin, and collagen) and creating focal adhesions.[24,25] A role for integrins as mediators of vascular strain is supported by observations that shear stress–induced tyrosine phosphorylation of endothelial cells in isolated arterioles exposed to intraluminal flow is abolished by inhibition of integrin binding to ECM proteins containing the RGD amino acid sequence,[26] RGD being the key combination via which ECM proteins are bound by

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integrins. Response to strain is also abrogated by antibodies to $\beta3$ or $\alpha v\beta5$ integrins, which bind fibronectin,[12] whereas flow-dependent vasodilation is RGD- and $\beta3$ integrin–sensitive[26] and is blocked by removal of the glycocalyx with neuraminidase.[27] $\beta3$ Integrin expression may actually be enhanced subsequent to cyclic stretching of endothelial cells.[28] In addition, mechanical strain of VSMCs grown on fibronectin or vitronectin induces cell proliferation, whereas elastin- or laminin-grown cells in the same conditions do not proliferate.[12] On the other hand, neonatal VSMCs grown on laminin express SM-1 and SM-2 smooth muscle myosin heavy chains in response to mechanical strain, unlike cells grown on the poly RGD substrate pronectin,[29] and cyclic stretch–induced SM-1 expression is greater in cells grown on laminin than on collagen or fibronectin.[9] It therefore appears that both the ability of cells to sense mechanical strain and the ensuing biochemical response depend on the nature of the interaction of specific integrins with the ECM. Ultimately, the hyperplasic versus hypertrophic response to stretch of VSMCs exposed to ECM will depend on their phenotype, as suggested by organ culture experiments in which stretch produces matrix synthesis without inducing cell proliferation.[10]

A recent report brings evidence that some ECM proteins actually serve as ligands to receptors. This is true at least in the case of collagen, which binds and activates the receptor-like tyrosine kinase discoidin domain receptor 1 (DDR1), a first example of a ligand shared by integrins and receptor tyrosine kinases. In this case, the receptors are phosphorylated with a protracted time course.[30] Collagen might thus signal via its 2 distinct receptor classes to regulate cellular responses to changes in the surrounding environment.[30] Hence, integrin-mediated cell signaling could implicate either a cytoskeletal alteration removing a constraint on the signal transduction machinery, or a direct action on the signaling systems, or a combination of both.[31]

Ion Channels

Submitting endothelial cells to either shear stress or stretch spurs a transient increase in intracellular calcium and divalent cations.[32,33] Endothelial cells possess stretch-activated ion channels that are relatively nonselective across cations.[34] These were identified using patch-clamp techniques. In isolated endothelial cells, membrane stretching by applying suction through the patch electrode increased the opening frequency of channels permeable to calcium.[35] Mechanosensitive ion channels were further characterized by their sensitivity to gadolinium but not to classic calcium channel blockers such as nifedipine.[33] The presence of mechanotransducing ion channels in endothelial cells may help explain how the endothelium mediates vascular responses to hemodynamic stresses. For example, the mechanism of stretch-activated phospholipase C activity in VSMCs was found to involve influx of calcium via gadolinium-sensitive ion channels but not via nifedipine-sensitive ion channels.[36] MAP kinase activation by Ang II also shows a calcium dependency in VSMCs.[37] On the other hand, potassium channels distinct from the nonselective gadolinium-sensitive cation channels may also participate in transduction of mechanical stress.[38] This was demonstrated by the observation that shear-induced increases in gene expression and cGMP concentrations are inhibited by tetraethylammonium ion, a nonspecific potassium channel blocker.[39] Furthermore, membrane stretch and fatty acids directly activate large conductance calcium-activated potassium channels in VSMCs.[40] Finally, RSK, a downstream target of ERK1/2, phosphorylates or may actually itself be the NHE-1 isoform of the Na^+-H^+ exchanger.[41] This is particularly interesting in light of the observation that cellular sodium entry via a tetrodotoxin-inhibited mechanosensitive channel modulates ERK1/2 activation by shear.[42] Either way, there appears to be a definite role for ion channels in the response of vascular cells to mechanical forces.

Intracellular Transmission of Mechanical Stresses

Focal Adhesion Kinases

At the cellular level, subjecting endothelial cells to oscillatory flow spurs a clustering of $\alpha5\beta1$ integrins and a concomitant gathering of the cytoskeletal proteins talin and vinculin.[14] In fact, during cell stimulation by mechanical factors such as stretch or shear stress, several signaling events are associated with the formation of focal adhesions, which consist of clustered integrins and accumulated cytoskeletal proteins. The recruitment of integrins into focal adhesions is mediated by the cytoplasmic domains of the bridging proteins, as deletion of the $\beta1$ subunit cytoplasmic domain inhibits integrin association.[43] In turn, the cytoplasmic domains bind cellular cytoskeletal proteins that are present in the focal adhesions α-actinin and talin.[44,45] α-Actinin is directly connected to actin microfilaments,[46] whereas talin is linked via vinculin,[47] which in turn binds α-actinin or tensin,[48] both of which associate with actin.[49]

Proteins present at focal adhesions, in particular the 125-kDa cytoplasmic tyrosine kinase FAK, become tyrosine phosphorylated when cells are stimulated by integrin antibodies, cell adhesion, or RGD-containing compounds.[50–52] Shear and adhesion activate FAK, but stimuli are not additive.[53–55] FAK associates with paxillin[56] and talin,[57] and both FAK and paxillin can bind to the cytoplasmic tail of integrins independently.[58] Focal adhesions containing talin, vinculin, and paxillin have been reported to form in endothelial cells despite the absence of FAK association and in conditions of reduced tyrosine phosphorylation; these findings suggest that FAK activation is downstream of focal adhesion and stress fiber formation and that its role is one of a signaling protein in focal adhesions rather than of focal adhesion assembly.[59] In confirmation, there is evidence that aggregation of FAK with $\alpha5\beta1$ integrin, RGD, or fibronectin occurs even in the presence of tyrosine kinase inhibition or actin filament assembly disruption by cytochalasin D. However, cytoskeletal protein recruitment and activation of downstream kinases is prevented.[60,61] Rho, a small G protein, is also implicated in the regulation of formation of stress fibers and focal adhesions, through phosphorylation of FAK, p130Cas (an adaptor protein bound by FAK), and paxillin, and, independently, actin polymerization.[62] FAK may be downstream of Rho, and Rho may be involved in activation of FAK by 7 transmem-

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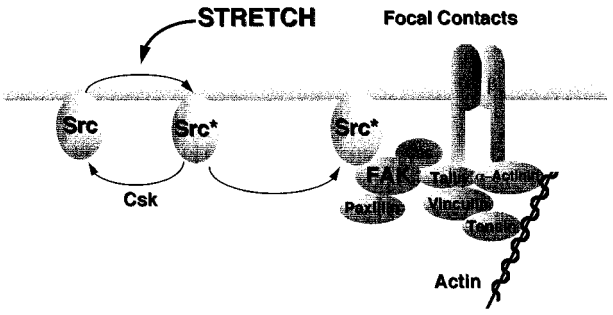

Figure 2. Schematic representation of c-Src activation by stretch. Once stimulated, activated c-Src (c-Src*) translocates to focal contacts where it interacts with FAK. C-Src is phosphorylated, and therefore returned to its inactive state, via the action of C-terminal Src kinase (Csk).

brane domain receptors of Ang II, bombesin, and lysophosphatidic acid.[31]

Shear in endothelial cells increases the tyrosine phosphorylation and activity of FAK and its association with Grb2.[53] In fact, it is the attachment of c-Src, a membrane-associated nonreceptor tyrosine kinase, at a key region of autophosphorylation on FAK that creates a binding site for the Src-homology-2 (SH2) domain of Grb2.[63] C-Src is active in its dephosphorylated state, which seems to be modulated by stretch,[64] and is inactivated by C-terminal Src kinase (Csk). After its activation, c-Src is translocated to focal contacts (Figure 2).

MAP Kinase Cascade: Upstream Events

The MAP kinase cascade is a major pathway through which signals coming from growth factors and mechanical strain are transduced into regulation of gene expression and protein synthesis. Involved are the sequential phosphorylation and activation of the cytoplasmic protein kinases MEKK, MEK, and finally MAP kinase.[65] The MAP kinase cascade actually comprises 3 separate pathways that respond to different stimuli and instigate distinct cellular responses. Phosphorylation of 1 MAP kinase, which lies downstream of the MEKK Raf and is present in 2 isoforms termed ERK 1 and 2, leads to the activation of regulatory proteins both in the cytoplasm and the nucleus.[65] A second branch of the MAP kinase family, termed stress-activated protein kinases (SAPK) because they are activated by such stimuli as UV light, heat shock, hypoxia, or high osmolarity, includes kinases that phosphorylate the amino terminal of transcription factor c-*jun* (JNK).[66,67] Finally, a third branch of the MAP kinase family comprises p38, also activated by osmotic stress.[68] In endothelial cells, physiological levels of shear stimulate ERK1/2,[54,69,70] whereas cyclic mechanical strain activates both ERK1/2 and JNK in VSMCs.[29] Furthermore, applying a high intraluminal pressure to aortas in organic culture induces a biphasic ERK1/2 stimulation, characterized by an acute peak in activity, subsequent reversal, and a second more lengthy activation[71] (Figure 3). In vivo, ERK1/2 is transiently activated by acute hypertension[72] and by vessel wall injury with a balloon catheter.[73,74]

Diverse pathways link mechanical strain to MAP kinase activation in vascular cells. Hence, G-protein and calcium-

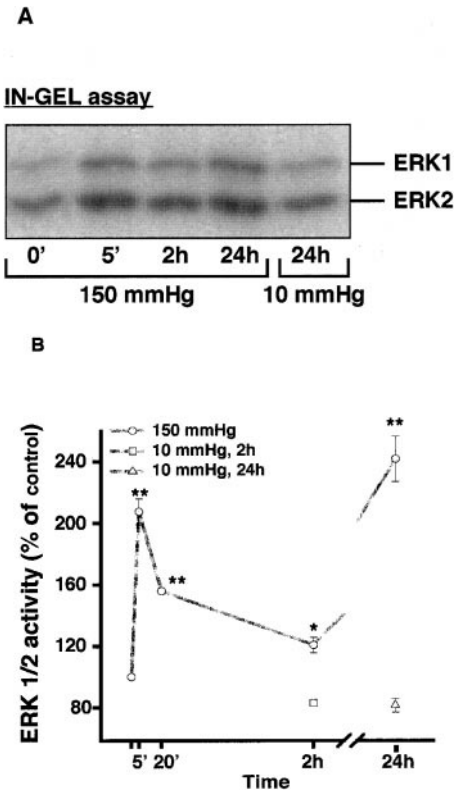

Figure 3. Time course of ERK1/2 activation in aortic organ culture by intraluminal pressure. Rabbit aortic segments were pressurized at 150 mm Hg during different times. A, Autoradiogram of in-gel kinase assays of ERK1 and ERK2 activity using myelin basic protein as the substrate. B, Quantification of in-gel assays. *$P<0.05$; **$P<0.01$ vs 0 minutes. Modified from Birukov et al.[71]

independent PKC activation is involved in ERK1/2 activation by shear stress,[70] whereas in certain conditions JNK may be more activated by shear than ERK1/2, through sequential phosphorylation of Sos, Ras, and MEKK.[69] Furthermore, integrins are likely to be among the actors involved in the transmission of mechanical forces to the MAP kinase cascade, for several reasons. First, cellular response to stretch or shear stress in vitro varies widely depending on the nature of the substrate on which the cells are grown. For example, ERK1/2 and JNK are both activated by cyclic mechanical strain in pronectin-grown neonatal VSMCs, whereas in their laminin-grown counterparts, only JNK is stimulated by cyclic strain.[29] Second, ERK1/2 activation by shear stress and by integrin-mediated adhesion to fibronectin occurs via a common herbimycin A–sensitive, PKC-dependent pathway in endothelial cells.[54]

Src-family tyrosine kinases, which are inhibited by herbimycin A, have also been implicated in intraluminal pressure–induced ERK1/2 activation in vascular organ culture, although a PKC-independent pathway was involved under these conditions.[71] Recent in vitro experiments report a role for c-Src in pressure-induced contraction of rat cerebral arteries.[75] Furthermore, both c-Src and Grb2 SH2 binding motifs have been involved in MAP kinase signaling pathways.[63] Accordingly, it was demonstrated that FAK overexpression enhances c-Src kinase activity and fibronectin-

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induced ERK2 activity, whereas a Ras dominant negative, which blocked ERK activation, did not affect FAK phosphorylation or Src activity. Also, replacing the c-Src binding site on FAK prevented integrin signaling to ERK.[63] Finally, fibronectin-induced ERK1/2 activation is Shc-dependent, and the Shc-ERK pathway is bridged by Ras.[76] Hence, these cumulative observations describe a pathway originating with integrin activation, focal adhesion assembly, activation of FAK by c-Src, association with Grb2 leading to Shc-dependent stimulation of Ras, and subsequent activation of ERK1/2 via the MAP kinase cascade (Figure 1). Accordingly, a dominant negative mutant of FAK was shown to attenuate shear-induced ERK2 and JNK activity in endothelial cells, as did a dominant negative mutant of Sos and an anti-vitronectin receptor antibody.[53] Likewise, both flow and β1-integrin activation stimulated ERK1/2 and tyrosine phosphorylation of proteins. However, ERK1/2 activation by β1-integrin activation occurred more slowly and to a lesser degree, and fewer proteins were tyrosine phosphorylated, than under flow conditions.[55] Multiple pathways are then likely to be recruited in the mechanotransduction of flow.

There is indeed evidence that integrin-mediated MAP kinase activation may in some cases bypass FAK, as demonstrated by the observation that a single-chain tailless mutant of integrin α1 recruited Shc and activated ERK but not FAK, whereas an activating $\alpha 6\beta 1$ antibody activated FAK but did not induce its association with Shc and did not activate ERK.[76] Furthermore, the increase in adhesion-mediated ERK activation by shear was only partially affected by actin filament disruption,[54] and JNK, but not ERK, remains activated by fibronectin despite the presence of cytochalasin D.[61]

MAP Kinase Cascade: Downstream Events

Events downstream to MAP kinase activation are numerous and varied. Once phosphorylated, ERK1/2 may translocate to the nucleus to phosphorylate transcription factors and thereby regulate cell cycle gene expression.[77] Both ERK1/2 and JNK can lead to ternary complex formation at the serum response element, present on several gene promoters, and to increased transcriptional activity.[78] Alternatively, phosphorylation of the translation regulator protein PHAS-I (phosphorylated heat- and acid-stable protein) promotes the dissociation of the PHAS-I–eukaryotic initiation factor (eIF) 4E complex, normally tightly bound when PHAS-I is relatively underphosphorylated, releasing eIF-4E that will facilitate initiation of translation in the nucleus.[79,80] Another downstream target of ERK in VSMCs is the 90-kDa ribosomal S6 kinase RSK, which through activation of the transfer RNA-binding factor may provide a pathway essential for the initiation of translation.[80] Ultimately, ERK1/2 activation coincides with enhanced c-*fos* and c-*jun* expression, and activation of the AP-1 transcription factor,[72] and it is likely to play a significant role in regulating cell cycle progression of VSMCs[81] as well as protein synthesis.[80]

The fact that ERK1/2 can also induce cyclooxygenase-2 in VSMCs[82] may explain why activation of this MAP kinase does not necessarily result in increased cellular proliferation. Indeed, cytosolic phospholipase A_2 is among the substrates of ERK1/2.[83] Phospholipase A_2 catalyzes the release of arachidonic acid from phospholipids in the cell membrane,[84] which will be transformed by the action of cyclooxygenase-2 into prostaglandins. The resulting elevated levels of prostaglandin E_2 and protein kinase A activation could counteract ERK1/2-induced proliferation.[82] A further target of activated ERK1/2 has been reported to be the contractile regulatory protein h-caldesmon, the high-molecular-weight form of caldesmon, indicating that ERK is involved in the regulation of contractile properties of the vascular wall.[85] Hence, in the end, the availability of downstream ligands may be a significant determinant of the biological outcome of ERK activation.[82] At length, ERK1/2 activity is modulated by MAP kinase phosphatase (MKP-1), which dephosphorylates the enzyme.[86] Alternatively, the activation of ERK1/2 may be terminated through a feedback loop, implicating Ras/Raf-mediated suppression of integrin activation.[87]

Role of the Renin-Angiotensin System and Growth Factors

Induction of protein synthesis by stretch may occur in many cases via increased synthesis of growth factors or mitogenic agonists, among which Ang II plays an important role. The pathways involved in the increased synthesis of these factors by mechanical strain are not yet clearly understood, although there is evidence that the AP-1 transcription factor downstream of ERK1/2 activation may regulate growth factor expression.[88] Stimulation of protein and fibronectin synthesis by high intraluminal pressure in aortic organ culture was found not only to result from augmented angiotensin levels but also to be further enhanced by addition of Ang II to the culture medium.[10] In a similar fashion, the rise in transforming growth factor-β mRNA expression brought about by Ang II and stretch is additive,[89] stretch induces parathyroid hormone–related peptide mRNA and secretion synergistically with Ang II,[90] and both stretch- and Ang II–induced DNA synthesis in collagen-plated VSMCs occurs in synergy.[11] Moreover, this latter effect is attenuated by PDGF antibodies (PDGF-AB), whereas Ang II and PDGF increase DNA synthesis in synergy,[11] demonstrating that more than 1 factor at once may be implicated in the remodeling process. In addition, a role for ECM proteins cannot be excluded. Indeed, attachment of cells to fibronectin and antibody-induced aggregation of $\alpha 5\beta 1$ integrins enhances PDGF-induced increase in cytoplasmic pH,[32] suggesting that integrins and growth factor receptors may act cooperatively.

In several circumstances, mechanical activation of vessels or vascular cells instigates the release of vasoactive factors that will be implicated in the ensuing changes in vessel structure and function. In organ culture, for example, angiotensin mediates the enhanced total protein and fibronectin synthesis induced by high intraluminal pressure.[91] Appropriately, Ang II is potentially involved in the stimulation of a number of intracellular pathways, leading in aortic VSMCs to hypertrophy, through enhanced protein synthesis, but not to hyperploidy.[10,92] Synthesis-promoting activities of Ang II are transduced via the angiotensin II subtype 1 receptor,[91] and the downstream signaling cascades include activation of phospholipases C and D, increased calcium, and inhibition of adenylyl cyclase.[93,94] Ang II may also induce protein synthe-

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sis, in part via activation of the 70-kDa S6 kinase, by an ERK1/2-independent pathway.[95] This is particularly interesting in light of the fact that intraluminal pressure induces both ERK1/2 activation and protein synthesis, but only the latter effect is mediated by Ang II.[10,71] Alternatively, Ang II activates c-Src,[96] which constitutes a probable pathway by which Ang II phosphorylates both FAK and paxillin.[93] In fact, not only Ang II but also epidermal growth factor and thrombin activate tyrosine phosphorylation of paxillin, as demonstrated in rat aortic VSMCs.[93] Not surprisingly, growth factors (fibroblast growth factor, PDGF-BB, epidermal growth factor) and integrins can activate the MAP kinase cascade in synergy, provided the integrins are both aggregated and occupied.[97] Like Ang II, growth factors (insulin) may activate relatively downstream events in the signaling cascade, such as 70-kDa S6 kinase stimulation and phosphorylation of PHAS-I, via MAP kinase–independent pathways.[79]

Growth factors may bypass the MAP kinase cascade and function instead by activating NFκB.[98] This family of transcription factors regulates the expression of genes encoding growth factors, inducible surface proteins, and molecules involved in ECM remodeling.[99] NFκB is present in the cytosol in association with 1 of several inhibitors (generally identified as IκB), forming an inactive heteromeric complex. NFκB is released after phosphorylation and subsequent degradation of the IκB, allowing the active NFκB dimers to translocate to the nucleus and promote transactivation of target genes.[57,100]

In parallel, recent reports propose a role for reactive oxygenated species in mechanical stress signal transduction. Indeed, stretch of VSMCs activates PKC, which presumably acts on NADPH oxidase, and thereby forms reactive oxygen species that then sequentially activate NFκB and DNA synthesis.[101] Alternatively, H_2O_2 in endothelial cells also induces f-actin reorganization, characterized by stress fiber formation and recruitment of vinculin to focal adhesions. These changes are modulated by activation of p38, followed by phosphorylation of heat shock protein HSP27.[102] Furthermore, observations of shear stress–induced oxygen free radical production and downstream HSP27 activation are combined in a single study describing sustained phosphorylation of HSP27 in endothelial cells subjected to shear stress and consequent reorganization of cytoskeletal proteins and change of cell shape.[15] Finally, a recent study identified 2 members of the MAD protein family (for mothers against decapentaplegic), Smad6 and Smad7, as unique among the MAD-related proteins, being expressed selectively in the endothelium in vivo and activated by physiological levels of flow in endothelial cell cultures.[103] MAD proteins traditionally act as second messengers distal to the transforming growth factor-β family of receptors.[103]

Conclusion

Understanding which signaling pathways are involved in the transduction of mechanical forces in the vascular wall should allow for a better approach to vascular remodeling. This may be of help in the development of novel therapeutic strategies for the treatment of cardiovascular diseases, including hypertension, atherosclerosis, and restenosis after angioplasty.

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