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Cellular mechanics and gene expression in blood vessels

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Abstract

Blood vessels are permanently subjected to mechanical forces in the form of stretch, encompassing cyclic mechanical strain due to the pulsatile nature of blood flow, and shear stress. Alterations in stretch or shear stress invariably produce transformations in the vessel wall that will aim to accommodate the new conditions and to ultimately restore basal levels of tensile stress and shear stress. Vascular cells are equipped with numerous receptors that allow them to detect and respond to the mechanical forces generated by pressure and shear stress. The cytoskeleton and other structural components have an established role in mechanotransduction, being able to transmit and modulate tension within the cell via focal adhesion sites, integrins, cellular junctions and the extracellular matrix. Beyond the structural modifications incurred, mechanical forces can also initiate complex signal transduction cascades leading to functional changes within the cell. Many intracellular pathways, including the MAP kinase cascade, are activated by flow or stretch and initiate, via sequential phosphorylations, the activation of transcription factors and subsequent gene expression.

Keywords: Blood vessel; Endothelial cells; Smooth muscle cells; Mechanotransduction; Pressure; Flow; Shear stress

Blood vessels are permanently subjected to mechanical forces in the form of stretch, encompassing cyclic mechanical strain due to the pulsatile nature of blood flow and shear stress. Blood pressure is the major determinant of vessel stretch. It creates radial and tangential forces which counteract the effects of intraluminal pressure, and which 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 to ultimately restore basal levels of tensile stress and shear stress (Glagov, 1994; Tronc et al., 1996). Hence, while acute changes in stretch or shear stress correlate with transient adjustments in vessel diameter, mediated through the 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 these transformations that occur in vessels undergoing mechanical stresses.

1. Mechanical forces

1.1. Pressure, tension and tensile stress

Blood pressure produces strain on the vessel wall in a direction perpendicular to the endoluminal surface. This is counterbalanced by the intraparietal tangential forces in the longitudinal and circumferential directions exerted by different elements of the vessel wall, opposing the distending effects of blood pressure. The force per unit length of the vessel (the wall tension, T) is related to the blood pressure (P) and the vessel radius (r) by Laplace's law:

$$T = P\,r.$$

The relation between circumferential tension and deformation of the vessel as intraluminal pressure increases depends both on the geometry and the elastic characteristics of its wall. The circumferential tension is actually borne by the whole thickness of the arterial

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wall. Each element of the wall only bears part of this tension. The tension per unit of thickness represents the stress exerted on the wall in the circumferential direction. It is expressed as

$$T = P\,r/h,$$

where h is the thickness of the wall.

1.2. Blood flow and shear stress

As blood flows, it exerts a frictional force on the endothelial surface. This force is expressed as a shear stress (τ) on the endothelium, defined as the product of the blood viscosity and the blood velocity gradient measured at the vessel wall. The shear stress transmitted to the endothelium by the blood flow tends to displace the endothelium and the intimal layer in the direction of flow (one might equally say that it is because the endothelium is fixed that friction occurs). In the case of laminar flow (where the profile of blood velocity is parabolic), shear stress is expressed as

$$\tau = 4\mu Q/\pi r^3,$$

where μ is the viscosity, Q the flow rate and r the vessel radius. Note that the radius appears at the third power in the denominator. Thus, for a constant volume flow, a slight reduction in vascular diameter produces a much greater increase in shear stress.

Hence, vessels are normally exposed to two types of mechanical forces: (a) circumferential stress acting tangentially on the vascular wall and directly related to pressure and dimensions (diameter and thickness) of the vessel, and (b) shear stress acting in the longitudinal direction at the blood–endothelium interface and directly related to the flow-velocity profile.

2. Mechanical factors and vascular remodeling

On the basis of observations in chick embryos, Thoma in 1893 hypothesized that the diameter of blood vessels is regulated by the magnitude of blood flow, while the thickness of vessel walls depends on the magnitude of the forces of tension generated by blood pressure. This hypothesis has subsequently been experimentally confirmed. It has been demonstrated, for example, that the diameter of the abdominal aorta of a lamb undergoes a significant reduction between the 4th and 14th days post-partum (Langille et al., 1990). This reduction can be accounted for by a fall of approximately 70% in the blood velocity in the abdominal aorta at the time of delivery, due to the disappearance of the placenta circulation, and is associated with apoptosis of vascular cells (Cho et al., 1995). Concurrently, the diameter of the thoracic aorta increases in parallel with the rise in systemic blood flow. Similarly, the thicknesses of the pulmonary artery and aorta, which are almost identical at birth since pressures in utero are similar in both vascular territories, evolve differently after birth. The pulmonary artery atrophies during development, following the fall in pulmonary pressure post-partum, while the thoracic aorta thickens proportionately to the increase in systemic pressure (Leung et al., 1977).

2.1. Effects of pressure

Numerous studies have demonstrated a direct relationship between the circumferential stress to which the vessel wall is exposed and the structure of the wall itself. When the stress increases due to an increase in arterial pressure, smooth muscle cell hypertrophy and increase of collagen and elastin contents follow. Inversely, when the circumferential stress falls, the wall undergoes atrophy (Bomberger et al., 1980). Several physiologic and experimental arguments confirm the relationship between circumferential stress and the thickness and composition of the vessel wall:

(1) From one animal species to another, as the diameter of a particular blood vessel increases, the number of lamellar units and the total thickness of the wall increase proportionately, so that the circumferential stress remains constant irrespective of the size of the animal, from the rat to the horse. This "ideal "value is of the order of 2.10^6 dyn/cm^2 in the descending thoracic aorta (Wolinsky and Glagov, 1969). It varies according to the arterial territory and essentially depends on the structure of the blood vessel concerned.
(2) In all experimental models of arterial hypertension, a close correlation is observed between the level of arterial pressure and the frequency of polyploidy and hypertrophy of the smooth muscle cells of the arterial wall.
(3) Smooth muscle cell hypertrophy in the wall of the major arterial trunks only develops when the distending pressure has reached a threshold level, and never precedes the onset of hypertension, even when the neurohumoral abnormalities responsible for hypertension are already present.

The conceptually simplest experimental model used to underscore the effect of blood pressure on the arterial wall structure is arterial stenosis. In this model high blood pressure and wall stresses are observed proximal to the coarctation, whereas normal or low pressure and stresses occur distal to the stenosis. In rats and in monkeys with thoracic coarctations (Tedgui et al., 1992; Zarins et al., 1981), the arterial wall was reported to be thickened in the section submitted to high blood pressure and normal in the lower part of the arterial

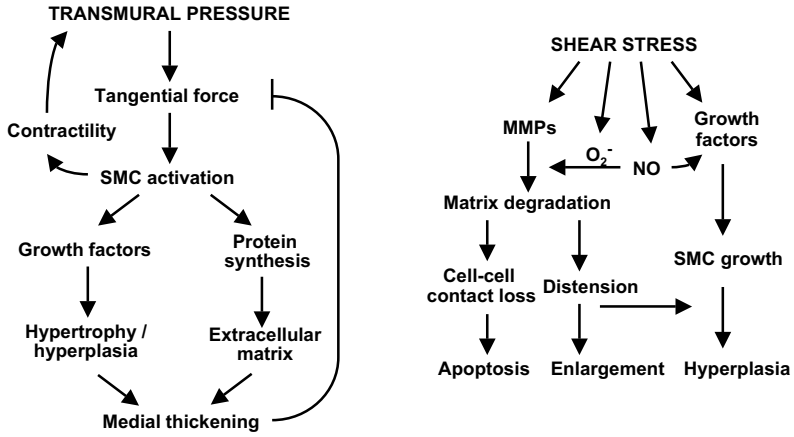

Fig. 1. Sequence of vascular responses stemming from increased transmural pressure or shear stress and leading, through sequential events, to vascular remodeling.

network of the same animals, in proportion to the level of blood pressure and the tensile wall stresses.

The effects of mechanical tensile stress on the arterial wall have been extensively described and have been applied to the understanding of hypertension. Tensile stress is a strong determinant of the vascular structure among other factors including sympathetic activity and autocrine and paracrine factors. In the early phase of essential arterial hypertension, it is generally admitted that the vessel wall is submitted to increased pressure because of abnormal peripheral resistances related to genetic, humoral, nervous and/or structural factors. At length, damage to the large arteries is clearly involved in the cardiovascular morbidity and mortality associated with hypertension. Numerous animal and human studies have shown that sustained hypertension is associated with structural and functional alterations to both large arteries and arterioles. There is good evidence that hypertension is associated with increased arterial wall thickness (Levy et al., 1988) mostly due to smooth muscle cell (SMC) hypertrophy, accompanied by polyploidism, hyperplasia, and proportional changes in contractile and matrix proteins, leading to altered arterial function (Intengan and Schiffrin, 2001).

According to Laplace's equation ($T = Pr/h$), the hypertrophy of the arterial wall compensates for the increase in blood pressure and contributes to maintain a normal level of circumferential stress. In elastic and large conduit arteries, the adaptive response to hypertension serves to reduce and eventually to normalize the tensile stress (Fig. 1).

2.2. Effects of blood flow

Shear stress arising from the mechanical effects of blood flow on the vascular endothelium is also a determinant of arterial growth. Under physiologic conditions, the mean shear stress to which the vascular endoluminal surface is exposed is remarkably constant, close to 10–15 dyn/cm^2, whatever the part of the arterial network considered, conductance or resistance arteries, and whatever the size of the animal (with the exception of the rat and the mouse in which the values are closer to 30–35 dyn/cm^2). Restricting blood flow by placing a stenosing ring around the common carotid artery in young rats produces a significant delay in growth of the carotid during the subsequent weeks, amounting to a 25% reduction in its diameter compared with the contralateral carotid (Guyton, 1985). The content of fibrous proteins in the arterial wall is also controlled by blood flow and shear stress. In rabbits, the reduction in caliber of the developing carotid associated with a reduction in its blood flow is accompanied by a reduction in the elastin content of the carotid arterial wall (Langille et al., 1989).

The most spectacular illustration of the phenomenon of flow-dependent growth is the arteriovenous fistula model. In carotid-jugular arteriovenous fistulas, the flow rate in the developing carotids can be multiplied by a factor of up to 8. As long as there is no excessive increase in blood flow, however, shear stress is normalized by a compensatory increase in carotid diameter (Tronc et al., 1996). The chronic increase in shear tends to enhance the L-arginine/NO pathway in endothelial cells, and chronic inhibition of NO production by L-NAME treatment inhibits, at least partially, the adaptive wall shear stress regulation in flow-loaded vessels (Tronc et al., 1996). However, simple relaxation of vascular smooth muscle cannot alone account for the very significant increase in vascular caliber observed, which may almost double in response to large increases in flow. Previous microscopic and ultrastructural studies of the arterial wall proximal to an arteriovenous fistula have shown extensive tears and fragmentation, as well as enlarged fenestrae, in the internal elastic lamina (IEL) (Jones and Stehbens, 1995; Tronc et al.,

1996; Wong and Langille, 1996). Disruption of the IEL in flow-loaded arteries suggests a potential role for matrix metalloproteinases (MMPs) in matrix digestion and reorganization leading to arterial wall remodeling. Indeed, increased blood flow in the rabbit carotid due to an arteriovenous shunt causes the release of MMP-2 and MMP-9 (Tronc et al., 2000). Furthermore, chronic MMP inhibition prevents IEL fragmentation and adaptive remodeling of the flow-loaded artery. Thus, MMP-induced IEL fenestrations are formed following increased blood flow, contributing to arterial distensibility and resulting in an enhanced arterial diameter. As arterial caliber gradually increases, wall shear stress diminishes and the stimulus for MMP production/activation fades. This process will continue until arterial caliber is such that wall shear stress is normalized (Fig. 1).

Arteries are subjected, during the cardiac cycle, to cyclic variations of pressure and blood speed. The dynamic characteristics of the flow and the cyclic movements of the wall which accompany the pulsatile flow have a crucial role in atherogenesis (Glagov et al., 1988). The pulsatile nature of the flow is all the more significant in certain areas, because of the existence of oscillatory shear (it is the case on the external face of the carotid sinus). In fact, many works highlight the protective quality of shear, showing an increased susceptibility to atherosclerotic lesions in territories where flow is pulsatile or turbulent, rather than linear (Traub et al., 1999). Hence the levels of flow and pulsatility influence both the degree and the localization of intimal lesions in predisposed territories.

In summary, significant variations in mechanical forces, of physiological or physiopathological nature, occur in vivo. These are accompanied by phenotypical modulation of the SMC and the endothelial cells, producing structural modifications of the arterial wall. In all the cases, vascular remodeling can be allotted to a modification of the tensional strain or shear, and underlie a trend to reestablish baseline mechanical conditions.

3. Membrane signal transduction

Vascular cells are equipped with numerous receptors that allow them to detect and respond to the mechanical forces generated by pressure and shear stress. The cytoskeleton and other structural components have an established role in mechanotransduction, being able to transmit and modulate tension within the cell via focal adhesion sites, integrins, cellular junctions and the extracellular matrix. The cytoskeleton is composed of three major types of protein filaments: microtubules, microfilaments, and intermediate filaments. Microfilaments are polymers of actin that together with a large number of actin-binding and associated proteins form a continuous, dynamic connection between nearly all cellular structures. The cytoskeletal network changes in response to extracellular stimuli and participates in transmembrane signaling, providing a scaffold for organizing or translocating signaling molecules and organelles. Beyond the structural modifications incurred, mechanical forces can thus initiate complex signal transduction cascades leading to functional changes within the cell, often triggered by activation of integrins but also by stimulation of other structures such as G-protein receptors, tyrosine kinase receptors or ion channels (Fig. 2).

3.1. Integrins

The extracellular matrix is an important contributor to the process of mechanotransduction, containing glycoproteins which are displaced by stretch or shear forces and interact with integrins. These latter proteins participate not only to cell attachment to the substrate but also to intracellular transmission of mechanical signals. Mechanical stresses stimulate conformational activation of cell integrins and increase cell binding to the extracellular matrix (Jalali et al., 2001). In fact, the dynamic formation of new integrin-ligand connections is required for stretch- or shear-induced mechanotransduction, since blocking unoccupied extracellular matrix

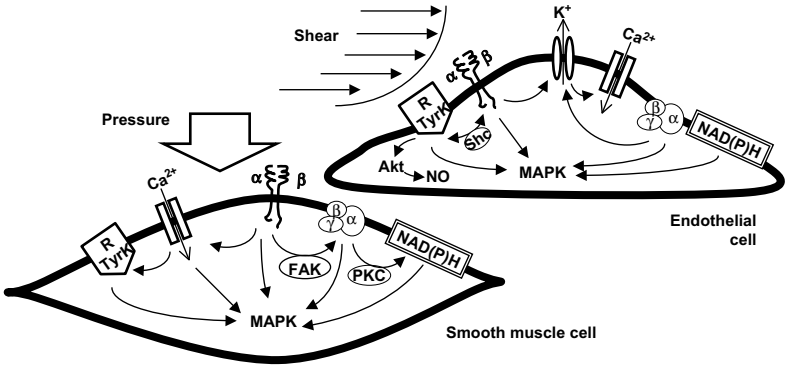

Fig. 2. Schematic representation of receptors involved in initiating signaling cascades in vascular cells stimulated by pressure (stretch) or shear stress.

ligand sites with isotype specific antibodies or RGD peptides (RGD being the principal amino acid sequence on extracellular matrix proteins to which integrins bind) inhibits intracellular signaling induced by mechanical forces (Jalali et al., 2001; Wilson et al., 1995) The cytoplasmic domain of integrins is functionally linked to various intracellular proteins that constitute the cytoskeleton and numerous kinases such as focal adhesion kinase (FAK), a key regulator of biochemical cascades initiated by mechanical forces. Integrins therefore form a signaling interface between the extracellular matrix and the cell.

Integrins exist as $\alpha\beta$ pairings that interact with extracellular matrix components including fibronectin (ligand for $\alpha5\beta1$ and $\alpha v\beta3$), vitronectin (ligand for $\alpha v\beta3$), and laminin (ligand for $\alpha6\beta1$). The capacity of cells to sense mechanical forces and the ensuing responses therefore depend on specific integrin-extracellular matrix interactions. For example, cyclic stretching of SMC grown on fibronectin or vitronectin induces cellular proliferation, which is prevented by anti-$\beta5$ or anti-$\alpha v\beta3$ antibodies, whereas SMC grown on elastin or laminin do not proliferate under the same conditions (Wilson et al., 1995). In comparison, cyclic stretch induces greater expression of the SM-1 isoform of myosin heavy chain in SMC plated on laminin than in SMC grown on collagen or fibronectin (Reusch et al., 1996). Finally, in SMC plated on type I collagen, serum induces expression of c-*fos* and cell proliferation equally in stretched cells and unstretched controls. However, in SMC grown on elastin matrix, both the serum-induced expression of c-*fos* and the ensuing cell proliferation are abated by stretch (Spofford and Chilian, 2001).

Shear stress also induces integrin-specific signaling cascades. In endothelial cells plated on fibronectin or vitronectin, but not on collagen or laminin, shear triggers $\alpha v\beta3$-dependent mechanotransduction and association of the integrin with the adapter protein Shc. In contrast, shear stress causes association of $\alpha6\beta1$ with Shc in cells plated on laminin, but not on fibronectin, vitronectin or collagen (Jalali et al., 2001). In cultured endothelial cells, shear stress activates the nuclear factor NFκB, which acting at the shear stress response element (SSRE) can promote the expression of mechanosensitive genes. Incubating endothelial cells with an anti-$\alpha v\beta3$ antibody prevents activation of NFκB by shear stress (Bhullar et al., 1998). Perhaps most importantly, in isolated coronary arteries, where endothelial cells lie on native extracellular matrix, flow-dependent dilation can be abrogated by addition of RGD peptides to the culture medium (Muller et al., 1997). Similar results are obtained when anti-$\beta3$ antibodies are used (Muller et al., 1997).

Integrins are therefore key sensing elements involved in mechanotransduction in vascular cells. The nature of the mechanical stimulus and the substrate components to which the cells are attached determine which integrin ligand pairs will be recruited and which downstream intracellular cascades will be activated, and hence the ensuing cell response. In this context, whole vessel preparations are particularly adapted to the study of the role of integrins in mechanotransduction, since cells are then in their original three-dimensional and complex extracellular matrix environment.

3.2. Ion channels

Two different mechanosensitive channels have been described in vascular cells: shear activated potassium channels and stretch-activated channels (Sackin, 1995). Stretch-activated ionic channels are cation-specific and have an electric activity mainly detectable at the time of their opening. The activation of these channels leads to calcium (Ca^{2+}) influx followed by membrane depolarization (Sackin, 1995). A role for stretch-activated cation channels in mechanotransduction in SMC was confirmed using the specific blocker gadolinium (Shirinsky et al., 1995). Stretch induced smooth muscle marker protein expression that was reduced by gadolinium, whereas other calcium channel blockers, such as verapamil, did not inhibit the stimulatory effect of stretch. Gadolinium also prevents cell proliferation observed in periodically stretched SMC (Standley et al., 1999).

Exposing endothelial cells in culture to shear stress leads to membrane hyperpolarization due to potassium channel opening (Nakache and Gaub, 1988). Because calcium entry in the cell is dependent on membrane potential, the increase in this potential induced by shear raises Ca^{2+} intake, resulting in an accumulation of calcium in endothelial cells and an enhancement of calcium-dependent signaling cascades. This interpretation is supported by experiments showing on the one hand that endothelial cells do not possess voltage-dependent calcium channels, on the other hand that high extracellular potassium concentrations reduce calcium entry in these cells (Nakache and Gaub, 1988).

Nevertheless, the mechanisms involved in the control of open/closed ion channel conformations by shear remain obscure. One likely contributor is the cytoskeleton, which by deformation could alter channel activation state. In support of this hypothesis, one study implicates cytoskeleton-G-protein coupling in shear-induced potassium channel opening (Ohno et al., 1993). Another recent work highlights a direct role for gadolinium-sensitive channels in endothelial endothelin-1 expression stimulated by rotating integrin-linking RGD peptide-covered ferromagnetic beads (Chen et al., 2001c), establishing a functional link between integrins, the cytoskeleton, and ion channels. As shown by Davies (1989), in areas where flow is alternately laminar and turbulent and where mechanical forces vary within short

distances, shear and stretch can induce synergistic or antagonistic effects through differential activation of ion channels. Ultimately, the physiological role of various ion channels, sensitive either to shear stress or to stretch, appears to depend on the balance between these hemodynamic forces in the circulation.

3.3. Heterodimeric G proteins

G proteins consist of three subunits, α, β, and γ, which couple membrane receptors with intracellular signaling cascades. If one considers the crucial role of G proteins in the regulation of the cardiovascular system, it is not surprising to find they participate in the transduction of mechanical forces in the endothelium. Indeed, it has been shown that shear-induced regulation of platelet-derived growth factor (PDGF) gene expression is regulated by a protein kinase C (PKC)-dependent mechanism requiring the presence of calcium and G-protein induction (Hsieh et al., 1992). The same authors also reported that shear induces the expression of c-*fos* via a complex mechanotransduction cascade involving PKC, phospholipase C, G proteins and calcium (Hsieh et al., 1993). Moreover, the direct effect of shear on the activation of Gαq/α11 and Gαi3/αo in endothelial cells was demonstrated (Gudi et al., 1996), and activation of both of these G proteins was found to be necessary for activation of downstream signaling cascades (Bao et al., 2001).

The γ subunit of heterodimeric G proteins is reported to be present at integrin-rich focal adhesion sites and adjacent to F-actin filaments stress fibers (Hansen et al., 1994). Co-localization of G proteins and integrins would even allow for a single signal to activate two transmembrane receptor families simultaneously, G protein coupled receptors and integrins. Thus, G proteins could be indirectly involved in integrin mediated signaling. Indeed, G protein inhibition prevents activation of potassium channels stimulated by cell adhesion to the extracellular matrix via integrins (Arcangeli et al., 1993). Acting on integrins, shear deforms the cytoskeleton and so activates a G protein that opens the potassium channels. Interestingly, there are thus far no indications that mechanical forces can activate G proteins in vascular SMC.

3.4. Receptor tyrosine kinases

Another class of membrane proteins, receptor tyrosine kinases, also take part in mechanotransduction. For example, activation and phosphorylation of PDGF receptor-α are observed in SMC exposed to cyclic stretch or shear stress (Hu et al., 1998). That could be explained by a disturbance of the cellular surface or an alteration of the receptor conformation by mechanical forces (Hu et al., 1998). However, the participation of gadolinium-sensitive Ca^{2+} channels cannot be excluded. Indeed, the latter are implicated in the phosphorylation of the EGF receptor by mechanical stimulation (Iwasaki et al., 2000). The role for the phosphorylation of EGF receptors in mechanotransduction was highlighted, since protein synthesis induced in stretched SMC was blocked when the cells are incubated with an EGF receptor antagonist (Iwasaki et al., 2000).

In endothelial cells, shear stress induces the transitory phosphorylation of the VEGF receptor Flk-1 and its association with Shc and $\alpha v\beta 3$ and $\beta 1$ integrins (Chen et al., 1999). If the role of Flk-1 in mechanotransduction has not yet been perfectly established, it remains that preventing the association of Shc with Flk-1, or with other proteins, attenuates downstream activation cascades as well as gene transcription stimulated by shear (Chen et al., 1999).

3.5. Oxygen free radicals

Recent data suggest that oxygen free radicals, as well as endogenous antioxidants, probably have critical signaling functions in cells (Hensley et al., 2000). A significant source of vascular oxygen free radicals is the membrane oxidase NADH/NADPH, whose activity is controlled by hormones, growth factors and mechanical forces. The basic product of this enzymatic system is the superoxide anion (O_2^-), which is transformed quickly into H_2O_2 by superoxide dismutase. The H_2O_2 is transformed in its turn by two enzymes, catalase and glutathione peroxidase. The breakdown products of the H_2O_2, including lipid hydroperoxides, are also biologically active. As a whole, oxygen free radicals thus comprise several potential second messengers.

The production of oxygen free radicals has been detected in endothelial cells exposed to a cyclic stretch of 10–12% (Cheng et al., 1998), and similarly applying a 10% cyclic stretch to human coronary artery SMC stimulates the production of O_2^-, while a stretch of 6% does not have any significant effect (Hishikawa et al., 1997). The activation of PKC, which is induced by stretch and which can activate NADPH oxydase, could in certain cases precede the generation of O_2^- (Hishikawa et al., 1997). However, 10% cyclic stretch stimulates generation of O_2^- and downstream signaling independently of PKC in whole vessel preparations (Lehoux et al., 2000). It has also been proposed that an increase in H_2O_2 in endothelial cells can induce the reorganization of F-actin, characterized by the formation of stress fibers and the recruitment of vinculin to focal adhesion sites (Huot et al., 1997). Furthermore, the endothelial oxidative response to stretch is matrix protein-dependent, and is reduced by coincubation with RGD peptides or blocking antibodies to $\alpha 2$- and β-integrins antibodies (Wang et al., 2001).

Interestingly, NADH oxidase activity is upregulated in endothelial cells exposed to oscillatory shear for 24 h, whereas steady laminar shear induces a more transient response (De Keulenaer et al., 1998). In fact, at 24 h steady shear induces superoxide dismutase, unlike oscillatory shear (De Keulenaer et al., 1998), consistent with the atheroprotective quality of laminar flow.

4. Intracellular signal transduction

4.1. NO and Akt

One of the early events which occurs in endothelial cells placed under flow is the activation of the endothelial NO synthase (eNOS) and the subsequent release of NO. Recent studies show that activation of eNOS by shear stress does not require Ca^{2+} influx in the cell, as is case for its activation by vasoactive agonists, but rather its phosphorylation by Akt (or protein kinase B) (Dimmeler et al., 1999), itself phosphorylated by phosphatidylinositol-3-kinase (PI3 K) (Dimmeler et al., 1998). The intracellular transduction pathways that link shear with eNOS activation are numerous. On the one hand, eNOS activation by shear can be prevented by a potassium channel blocker and necessitates an intact cytoskeleton. On the other hand, the phosphorylation of eNOS and of Akt in endothelial cells under flow is sensitive to tyrosine kinase inhibitors, indicating a possible implication of receptors for VEGF or insulin (Govers and Rabelink, 2001). Akt activation is also observed in cultured SMC subjected to a cyclic stretch (Chen et al., 2001a).

In addition to its role of vasodilator, NO intervenes in the regulation of the vascular remodeling induced by chronic shear stress, since inhibition of this pathway attenuates the increase in diameter observed in arteriovenous fistulas and thus prevents flow-dependent adaptation (Tronc et al., 1996). As a result the vessel loses its capacity for enlargement and shear levels stay at an abnormally high level. Under this condition, NO play the role of cofactor, facilitating metalloproteinase activation (Tronc et al., 2000). In addition, Akt activation and the production of NO support the survival of the vascular cells by stimulating anti-apoptotic pathways and inhibiting pro-apoptotic cascades (Dimmeler et al., 1998).

4.2. Focal adhesion kinase

During the stimulation of vascular cells by mechanical factors such as stretch or shear, several signaling events are associated with the formation of focal adhesions, which comprise integrin clusters and cytoskeletal proteins, as well as various tyrosine kinases, including FAK. There are in fact several different proteins that are known to bind the cytoplasmic domain of integrins and which may also be involved in mechanotransduction. Nevertheless, the role for FAK is particularly well established in the context of mechanotransduction. Indeed, a recent study shows that FAK is activated in stretched pulmonary vessels, in particular in the endothelium (Tanabe et al., 2000), and activation of this enzyme was also demonstrated in cultured endothelial cells exposed to shear stress (Ishida et al., 1996). The recruitment of integrins to focal adhesion sites is mediated by their cytoplasmic domains, which bind proteins of the cytoskeleton (Solowska et al., 1989). The proteins present at focal adhesions become phosphorylated on tyrosine when the cells are stimulated, and FAK activation is an indicator in focal adhesion formation, rather than the engine of their assembly (Gilmore and Romer, 1996). c-Src, a tyrosine kinase associated with the membrane, also plays a role in the process of FAK activation. Following its activation by stretch, c-Src is transferred to the focal contacts (Schlaepfer and Hunter, 1997), where it interacts with an autophosphorylation site on FAK and creates an acceptor for the Src-homology-2 (SH2) domain of Grb2 and thus supports association of FAK with the latter (Fig. 3). Though not shown yet in the context of mechanotransduction, activation of FAK could also involve RhoA, since inhibition of this small G protein by *Clostridium botulinum* C3 exoenzyme transferase disassembles focal adhesions and reduces phosphorylation of FAK in endothelial cells (Carbajal and Schaeffer, 1999) and vascular SMC (Bobak et al., 1997).

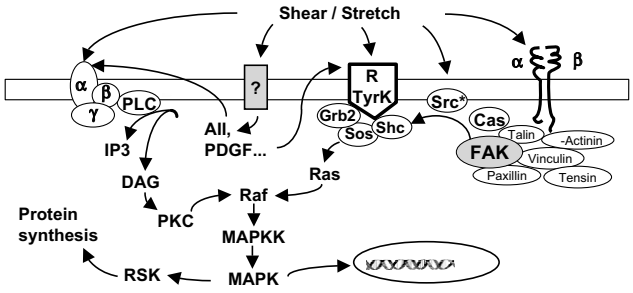

Fig. 3. Diverse pathways potentially involved in the activation of MAP kinases (ERK1/2 in this diagram) by mechanical factors.

4.3. The mitogen-activated protein (MAP) kinase cascade

The MAP kinase cascade is an important pathway whereby signals originating from mechanical forces can lead to gene expression and protein synthesis (Lehoux and Tedgui, 1998). This pathway implicates the sequential phosphorylation and activation of the cytoplasmic protein kinases MEKK, MEK, and finally MAP kinase. The MAP kinase cascade comprises in reality 3 different pathways which are triggered in response to various

stimuli and initiate distinct cellular responses. The phosphorylation of one of the MAP kinases, which lies downstream from Raf and is present under 2 isoforms, extracellular signal-regulated kinase (ERK) 1 and 2, leads to the activation of regulatory proteins in the cytoplasm and the nucleus. Other MAP kinases, called stress-activated protein kinases (SAPK) because they are activated by stimuli such as UV light, heat shock, hypoxia, or hyperosmolarity, include JNK kinases (which phosphorylate the amino-terminal of the transcription factor c-*jun*), and p38.

There is ample evidence that MAP kinases are activated in vascular cells exposed to mechanical forces, both in vivo and in vitro. Cyclic stretch activates ERK1/2 and JNK in cultured SMC (Reusch et al., 1997), and ERK1/2 and JNK are transiently activated in the arterial wall by acute hypertension (Xu et al., 1996). Using aortic segments in organ culture, it was shown that high intraluminal pressure (150 mmHg) induces a biphasic stimulation of ERK1/2, characterized by an acute activation peak with return to baseline at 2 h, and a second, more prolonged rise within 24 h and lasting at least 3 days (Birukov et al., 1997). A similar phenomenon, though slower in its acute phase, was also observed in vessels exposed to 10% cyclic stretch (Lehoux et al., 2000). In the latter model, cyclic stretch also activated p38 (Lehoux et al., 2000). Finally, MAP kinase activation pathways were also underscored in endothelial cells, in which shear forces of 12 dyn/cm^2 induced the phosphorylation of ERK1/2 and p38, but reduced activity of JNK (Surapisitchat et al., 2001).

The activation of MAP kinases most likely involves integrins as upstream mechanical sensors, and this for several reasons. Firstly, the in vitro response of vascular cells to stretch or shear varies considerably according to the nature of the substrate on which the cells are plated. For example, ERK1/2 and JNK are both activated by cyclic stretch in neonatal SMC grown on pronectine, but if the same cells are grown on laminin, only JNK is stimulated by cyclic stretch (Reusch et al., 1997). Secondly, in endothelial cells, ERK1/2 activation by shear or following adhesion to fibronectin occurs via a common integrin-dependent pathway sensitive to the c-Src kinase family inhibitor herbimycin A and dependent on PKC (Takahashi and Berk, 1996). Thirdly, overexpression of FAK increases fibronectin-dependent c-Src kinase activity and subsequent activation of ERK2, whereas a dominant negative Ras blocks activation of ERK1/2 without affecting phosphorylation of FAK or c-Src activity (Schlaepfer and Hunter, 1997). Finally, substituting of the c-Src acceptor on FAK blocks the transmission of signals between integrins and ERK1/2 (Schlaepfer and Hunter, 1997). Taken together, these observations highlight a pathway starting with integrin activation, focal adhesion assembly, FAK activation by c-Src, association with Grb2 driving c-Src-dependent activation of Ras, and ultimately activation of ERK1/2 via the MAP kinase cascade (Fig. 2).

Other pathways separate from that described above also participate in mechanotransduction. For instance, there is evidence that integrin-dependent activation of MAP kinases can in certain cases bypass FAK. Adhesion to matrix can activate ERK in cells expressing a mutant form of the β1 integrin lacking the cytoplasmic segment necessary for FAK interaction (Barberis et al., 2000). Furthermore, the MAP kinase cascade can also be activated by tyrosine phosphorylation of α, β, and γ GTP subunits of G proteins (Crespo et al., 1994), as well as by mechanosensitive phosphorylation of tyrosine kinase type receptors (Chen et al., 1999; Hu et al., 1998; Iwasaki et al., 2000). As described above, cyclic stretch induces the release of oxygen free radicals in cultured cells. The activation of Ras by oxygen free radicals, which in theory precedes activation of Raf and the MAP kinase cascade, was reported (Abe et al., 2000), in agreement with the observed activation of ERK1/2 by O_2^- in SMC (Baas and Berk, 1995). Finally, the inhibition of small G protein RhoA or its downstream kinase RhoA kinase (p160ROCK) completely prevents stretch-induced ERK1/2 activation (Numaguchi et al., 1999), or shear-induced JNK activation (Li et al., 1999).

Not surprisingly, different pathways can bridge the gap between mechanical stimulation and ERK1/2 activation in vascular cells. As an example, both high intraluminal pressure (150 mm Hg) and 10% cyclic stretch activate ERK1/2 in vessels in organ culture. Nonetheless, c-Src kinase inhibition prevents ERK1/2 activation only in vessels at high pressure, not in pulsatile vessels. On the other hand, activation of ERK1/2 by cyclic stretch is mediated by the release of oxygen free radicals (Birukov et al., 1997; Lehoux et al., 2000). In comparison, shear-induced ERK1/2 activation in cultured endothelial cells is prevented by inhibition or downregulation of PKC, or inhibition of tyrosine kinase activity, and is probably coupled with the activation of G proteins (Davies, 1995). Hence multiple MAP kinase activation pathways can be induced by stretch or shear in vessels, depending on the nature of the mechanical stimulus and the cell types and extracellular matrix environment involved.

The events that occur downstream of the activation of MAP kinases are numerous and varied. Once phosphorylated, ERK1/2 can transfer to the nucleus, where it interacts with and phosphorylates transcription factors, thus controlling gene expression. ERK1/2 and JNK can both lead to the ternary complex formation with the serum response element (SRE), present on several gene promoters, and thus increase transcriptional activity (Whitmarsh et al., 1995). Alternatively, phosphorylation of the protein PHAS-I (phosphorylated heat- and acid-stable protein), a translation regulation factor, supports the dissociation of the

PHAS-I-eukaryotic initiation factor (eIF)-4E complex, normally closely apposed when PHAS-I is relatively underphosphorylated, releasing eIF-4E which in turn initiates translation in the nucleus (Proud, 1994). Another downstream target of ERK1/2 in SMC is the 90-kDa ribosomal S6 kinase (RSK), which by activation of the transfer RNA-binding factor provides an additional pathway for initiation of translation (Proud, 1994). Finally, ERK1/2 activation leads to enhanced expression of c-*fos* and c-*jun* and to activation of the AP-1 transcription factor, and as such is likely to play a significant role in the regulation of cell cycle progression and in protein synthesis in SMC (Proud, 1994). The availability of downstream ligands could be a factor which determines the biological response to ERK1/2 activation.

5. Mechanical factors and gene expression profile in vascular cells

Although many pathways likely to lead to phenotypical modulation of vascular cells have been identified, the differential gene expression profiles induced by mechanical forces remain relatively unknown. As mentioned above, earlier works established that shear or stretch increase the expression of c-*fos* and c-*jun*, which form protein homo- or hetero-dimers, comprising the activator protein-1 (AP-1). Binding DNA transcription promoter sites on TRE (TPA response element) and CRE (cAMP response element), c-*fos* and c-*jun* act as activators or repressors of transcription. In addition, shear stress or stretch can stimulate gene expression through activation of the shear stress response element or SSRE, identified by Resnick et al (1993) in the transcription promoter region of PDGF-B, t-PA, TGF-β1, c-*fos*, and c-*jun*. Studies have shown that the gene of adhesion molecule ICAM-1 contains the SSRE in its promoter, but not the VCAM-1 gene. Interestingly, expression of ICAM-1 is upregulated by flow, transiently and independent of the shear value (Nagel et al., 1994), whereas VCAM-1 expression is negatively controlled by shear in a force-dependent manner (Ohtsuka et al., 1993). Other shear-sensitive transcription factors present in endothelial cells include NFκB, early growth response-1 (Egr-1), and Sp-1.

There are numerous reports describing cyclic stretch or shear stress-induced regulation of gene expression and protein synthesis in cultured SMC or endothelial cells. However, it is owing to molecular techniques developed lately, such as DNA microarray and differential display, that elucidating the widespread differential expression of genes has been possible. Thus, one recent study using human umbilical vein endothelial cells (HUVEC) identified 52 flow-sensitive genes, citing cytochrome p450 and the human prostaglandin transporter among the most potently upregulated genes, those for connective tissue growth factor, endothelin-1 and monocyte chemotactic protein among the most dramatically decreased (McCormick et al., 2001). Differential display revealed 13 downregulated (including angiopoietin-2 and growth-arrest specific mRNA-3) and 20 upregulated genes (featuring metalloproteinase METH-1) in sheared HUVECs (Bongrazio et al., 2000). Similarly, using DNA microarray on human aortic endothelial cells, metalloproteinase 1, as well as angiopoietin and VEGF receptors Tie2 and FLK-1, were found to be upregulated by shear, whereas genes related to inflammation and proliferation were underexpressed (Chen et al., 2001b). In an interesting study using HUVECs, 143 genes were found to be differentially expressed whether cells were exposed to static conditions, laminar shear stress or turbulent shear stress, and a number of them were classified according to their known relation to mechanosignaling, response to injury, or atherogenesis (Garcia-Cardena et al., 2001). In SMC, cyclic stretch stimulates gene expression of VEGF, cyclooxygenase-1, tenascin-C and plasminogen activator inhibitor-1 (PAI-1), but negatively regulates MMP-1 and thrombomodulin (Feng et al., 1999). A DNA microarray was also used to identify multiple genes associated with proteoglycan synthesis or organization, differentially regulated by stretch (Lee et al., 2001). These publications therefore reveal the biomechanical regulation of numerous genes, and the techniques used have the advantage of allowing for the discovery of unexpected mechanosensitive genes.

However, insofar as the matrix environment and the cellular phenotype are determinants of the response of vascular cells to mechanical stimuli, the gene expression profiles obtained in cultured endothelial or smooth muscle cells do not necessarily represent faithfully what occurs in vivo. One study even underlined the significance of distinguishing arterial preparations from venous preparations, since gene expression varies greatly from one network to another (Adams et al., 2000). Some recent works have in fact called upon models of vessels in culture or in vivo to elucidate differential gene expression under various conditions of pressure or flow. Hence in perfused organ culture of rat aorta, c-*fos* expression was found to be enhanced proportionally to the level of intraluminal pressure (Mangiarua et al., 1996). Increasing flow in arteries in vivo induced PDGF-A gene expression, transiently in SMC but over at least 7 days in endothelial cells, which also overexpressed PDGF-B (Tulis and Prewitt, 1998). High shear also stimulated furin and TGFβ expression in a rabbit model of carotid fistula (Negishi et al., 2001), whereas in carotids injured by balloon angioplasty, expression of urokinase plasminogen activator receptor, TGFβ and its receptors ALK-5 and TβR-II, integrins αv and β3, metalloprotease MDC9, and the hyaluronate receptor

D44V6, was accentuated in vessels in which flow was diminished by 30–35% compared with vessels at normal flow (Ward et al., 2001). In perfused umbilical veins, expression of c-*fos* (Gan et al., 2000a), eNOS and endothelin (Gan et al., 2000c) was stimulated by high intraluminal pressure, contrary to tPA that showed reduced expression in such conditions (Sjogren et al., 2000). Expression of c-*jun*, COX-1 and COX-2 is upregulated by high shear in umbilical veins (Doroudi et al., 2000; Gan et al., 2000a), but that of VEGF downregulated (Gan et al., 2000b). Combining whole vessel models with newer molecular techniques will be optimal to elucidate complex patterns of gene and protein expression regulated by mechanosensitive pathways.

6. Conclusion

Blood vessels have autocrine and paracrine hormonal mechanisms that enable them to react immediately to local hemodynamic modifications involving tangential mechanical stretch (which increases with pressure) or shear stress (which increases with blood flow). Vascular tone is modified almost immediately to compensate for changes in the environment and in most cases this efficiently restores mechanical forces to normal levels. Exceptionally, the variations in vasomotor tone are not sufficient to compensate for the new mechanical constraints, and the phenotype of the vascular cells is altered, causing local modifications in trophicity. At length (over a few days to a few weeks), these adaptative changes also tend to return mechanical forces to their physiological values. Vascular remodeling is observed in various situations where the local pressures and flows are modified, such as arterial hypertension, atherosclerosis, arteriovenous fistula, stenosis, and aneurysm.

Many receptors, present on the surface of endothelial cells and SMC, allow vessels to detect subtle changes in their physical environment. From that point, different mechanotransduction cascades can be initiated according to the nature of the mechanical stimulus perceived. Inside the vascular cells, cytoskeletal proteins transmit and modulate the tension between focal adhesion sites, integrins, and the extracellular matrix. In addition to the structural modifications induced by the mechanical forces, they may lead to changes in the ionic composition of the cells, mediated by ion channels, stimulate various membrane receptors, and induce complex biochemical cascades. Many intracellular pathways, such as the MAP kinase cascade, are activated by flow or stretch and initiate, via sequential phosphorylations, the activation of transcription factors and subsequent gene expression. Thus, by purely local mechanisms, the blood vessels are capable of a true autonomic regulation which enables them to adapt to their mechanical environment.

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