Forum Minireview

New Molecular Mechanisms for Cardiovascular Disease: Blood Flow Sensing Mechanism in Vascular Endothelial Cells

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Abstract. Endothelial cells (ECs) lining blood vessels have a variety of functions and play a critical role in the homeostasis of the circulatory system. It has become clear that biomechanical forces generated by blood flow regulate EC functions. ECs are in direct contact with blood flow and exposed to shear stress, a frictional force generated by flowing blood. A number of recent studies have revealed that ECs recognize changes in shear stress and transmit signals to the interior of the cell, which leads to cell responses that involve changes in cell morphology, cell function, and gene expression. These EC responses to shear stress are thought to play important roles in blood flow–dependent phenomena such as vascular tone control, angiogenesis, vascular remodeling, and atherogenesis. Much research has been done on shear stress sensing and signal transduction, and their molecular mechanisms are gradually becoming understood. However, much remains uncertain, and many candidates have been proposed for shear stress sensors. More extensive studies of vascular mechanobiology should increase our understanding of the molecular basis of the blood flow–mediated control of vascular functions.

Keywords: endothelial cell, shear stress, mechanotransduction, calcium signaling, circulatory system, cardiovascular disease

1. Introduction

The endothelial cells (ECs) lining blood vessels have a variety of functions and play a central role in the homeostasis of the circulatory system. Biochemical mediators, including hormones, cytokines, and neurotransmitters, have long been thought to control EC functions. Recently, however, it has become clear that biomechanical forces generated by blood flow and blood pressure regulate EC functions. ECs are constantly exposed to shear stress, a frictional force generated by flowing blood, and to cyclic strain, which is caused by pulsatile changes in blood pressure. A number of recent studies have demonstrated that ECs have the ability to sense shear stress and cyclic strain as signals and transmit them into the cell interior, where they cause the ECs to change their morphology, functions, and gene expression (1–3). The EC responses to biomechanical forces are critical to maintaining normal vascular functions, and impairment of EC responses leads to the development of vascular diseases, including hypertension, thrombosis, aneurysms, and atherosclerosis. This paper will focus on fluid shear stress and summarize the data from recent studies in the molecular mechanisms underlying shear stress mechanotransduction in ECs.

2. Endothelial cell responses to shear stress

Blood flow generates a frictional force, shear stress, in ECs, and the following formula can be used to calculate its intensity (τ): \( \tau = \mu \frac{du}{dr} \), where \( \mu \) is blood viscosity, \( u \) is blood flow velocity, \( r \) is the radius of the blood vessel, and \( \frac{du}{dr} \) is the flow velocity gradient. Under physiological conditions, arterial ECs are exposed to a shear stress of around 20 dynes/cm² and venous ECs, to shear stress ranging from 1.5 to 6 dynes/cm² (4).
It has well been established that ECs are sensitive to shear stress. When cultured ECs are exposed to shear stress in fluid-dynamically designed flow-loading devices, the ECs change their morphology and functions. ECs are polygonal under static culture conditions, but become elongated with their long axis oriented in the direction of flow in response to shear stress (5, 6). EC functions also change in response to flow (7). For example, ECs increase production of various vasodilating substances, including nitric oxide (NO) (8, 9), prostacyclin, C-type natriuretic peptide, and adrenomedulin in response to shear stress, and decrease the production of vasoconstricting factors, including endothelin and angiotensin-converting enzyme. Shear stress also results in an increase in the antithrombotic activity and fibrinolytic activity of ECs by stimulating production of thrombomodulin and plasminogen activators. Shear stress also affects EC synthesis of growth factors, cytokines, and reactive oxygen species (ROS), which are involved in EC apoptosis and adhesive interactions with leukocytes.

When shear stress modulates EC functions, it usually affects the expression of related genes. Our DNA microarray analysis showed that approximately 3% of all EC genes examined showed some kind of response to shear stress (10). Assuming that ECs express around 20,000 genes, this finding suggests that more than 600 genes are shear stress–responsive. Many studies, including our own, have demonstrated that shear stress regulates endothelial gene expression transcriptionally and/or posttranscriptionally (11); and various transcription factors, including AP-1, NFκB, Sp1, GATA6, Egr-1, and KLF2, and their binding sites in gene promoters (shear stress response elements) have been shown to be responsible for shear stress–mediated gene responses (12–16). On the other hand, shear stress also regulates gene expression through mRNA stabilization, e.g., in the genes encoding endothelial nitric oxide synthase (eNOS) (17), cyclooxygenase 2 (18), granulocyte-macrophage colony stimulating factor (19), and urokinase-type plasminogen activator (16). Shear stress also regulates gene expression by inducing epigenetic modification including DNA methylation, histone acetylation, and chromatin remodeling in ECs (20, 21). Shear stress modifies core histone H3 and H4 in the 5′-regulatory regions of genes, which is strongly associated with transcriptional activation. It has been reported that shear stress–dependent histone H3 phosphorylation and acetylation appeared linked to the activation of distinct signaling pathways, controlled by PI3K, ERK, p38, PKA, and MAPK/ERK, and regulated transcriptional activation of c-fos gene (22).

3. Shear stress mechanotransduction

The fact that ECs respond to shear stress by changing their morphology, function, and gene expression indicates that ECs recognize shear stress as a signal and transmit it into the cell interior. A vast number of studies have been conducted to clarify the mechanisms underlying shear stress mechanotransduction, but much remains unclear (23, 24). The following section will address shear stress signaling pathways and candidates for shear stress sensors.

3.1. Shear stress signaling pathways

Based on the results of numerous studies, multiple pathways appear to be involved in the shear stress signal transduction. When ion channels are activated, a variety of ions, including Ca2+, K+, Cl−, and Na+, enter or exit cells, and mechanical signals are transduced into changes in membrane potential and intracellular ion concentrations. Activation of G protein–coupling receptors increases the activity of adenylate cyclase (AC) and phospholipase C (PLC), which induces second messengers, including cAMP, Ca2+, inositol 1,4,5-triphosphate (IP3), and diacylglyceride (DG). cAMP then activates cAMP-dependent protein kinase (PKA: protein kinase A), which catalyzes phosphorylation of a variety of proteins. Cytosolic Ca2+ binds to calmodulin and activates calmodulin-dependent kinase. IP3 binds to its receptors expressed on the endoplasmic reticulum, where Ca2+ is stored, and the binding triggers Ca2+ release into the cytoplasm, and DG activates protein kinase C (PKC). Tyrosine kinase–type receptors transmit signals through phosphatidyl inositol-3 (PI3) kinase and phosphorylation of small G proteins, such as Ras, and leads to activation of MAP kinases. On the other hand, shear stress signals can enter via adhesion molecules, such as integrins located at focal contacts, and phosphorylate focal adhesion kinase (FAK). There is also a signaling pathway mediated by ROS, which are produced in ECs exposed to shear stress. Activation of these signal transduction pathways leads to activation of transcription factors and alterations of EC functions.

3.2. Shear stress sensors

Although shear stress activates multiple signal transduction pathways, it remains unclear which of the pathways are primary and which are secondary because the initial sensing mechanism or sensors that recognize shear stress have not been identified. Thus far, various membrane molecules and cellular microdomains, including ion channels, growth factor receptors, G proteins, caveolae, adhesion proteins, the cytoskeleton, the glyocalyx, and primary cilia, have been shown to play important roles in the shear stress–sensing mechanism (Fig. 1).
3.2.1. Ion channels

Various types of ion channels have been listed as candidates for shear stress sensors. Potassium ion channels open in response to shear stress, and their opening results in hyperpolarization of the plasma membrane, whereas activation of chloride ion channels by shear stress induces membrane depolarization (25–27). Some types of Ca²⁺-permeable cation channels have been shown to be shear stress–responsive. For example, P2X purinoceptors and transient receptor potential (trp) channels, both of which are Ca²⁺-permeable channels expressed by ECs, open in response to shear stress and mediate the influx of extracellular Ca²⁺ across the plasma membrane (28, 29). The Ca²⁺ influx triggers subsequent Ca²⁺-dependent signaling pathways that lead to EC responses to shear stress. The next section will review what has been discovered about P2X purinoceptor–mediated Ca²⁺ signaling of shear stress.

3.2.1.1. P2X4 channel–mediated Ca²⁺ signaling of shear stress

When cultured ECs were subjected to shear stress, the intracellular Ca²⁺ concentration increased in a strength-dependent manner (30–32). The Ca²⁺ response was due to an influx of extracellular Ca²⁺ via P2X4, a subtype of ATP-operated cation channel P2X purinoceptor. Treatment of ECs with an antisense oligonucleotide targeted to their P2X4 channels blocked the shear stress–induced Ca²⁺ influx. Activation of P2X4 required ATP, which was supplied in the form of endogenous ATP released by the ECs (33). Endogenous ATP was released at caveolae/lipid rafts in ECs strength-dependently in response to shear stress, and suppression of the ATP release with the ATP synthase inhibitor angiostatin abolished the shear stress–induced Ca²⁺ responses. These findings suggest that ECs are capable of accurately converting information regarding shear stress intensity into changes in intracellular Ca²⁺ concentrations through ATP release and P2X4 activation. Although the mechanism responsible
for the ATP release in response to shear stress remains unclear, several possibilities have been suggested: shear stress may increase ATP release through vesicular exocytosis or ATP binding cassette transporters or it may activate cell surface ATP synthase to catalyze the synthesis of ATP (34, 35).

3.2.1.2. Roles of shear stress Ca\(^{2+}\) signaling in control of circulatory system

Our study in \(P2X4\) gene knockout mice (\(P2X4\) KO mice) revealed physiological roles of \(P2X4\)-mediated shear stress signal transduction in the circulatory system (36). The \(P2X4\) KO mice did not exhibit normal EC responses to shear stress such as a Ca\(^{2+}\) influx and subsequent production of NO (Fig. 2: A – D). The vasodilation induced by acute increases in blood flow in situ was much weaker in the \(P2X4\) KO mice, and the \(P2X4\) KO mice had higher blood pressure than wild-type mice. No adaptive vascular remodeling, that is, decrease in vessel size in response to a chronic decrease in blood flow, was observed in the \(P2X4\) KO mice. The impaired vascular remodeling resembled that observed in eNOS KO mice. These findings suggest that Ca\(^{2+}\) signaling of shear stress via \(P2X4\) plays a crucial role in the control of vascular tone and in blood flow–dependent vasodilation and vascular remodeling through endothelial NO production (Fig. 2E).

3.2.2. Tyrosine kinase receptors

Activation of tyrosine kinase receptors, including vascular endothelial growth factor receptor 2 (VEGFR2) and angiopoietin receptor Tie-2, occurs in ECs exposed to shear stress, and the activation is assumed to be ligand-independent, because it occurs in the absence of VEGF or angiopoietin (37, 38). We showed that shear stress induces a ligand-independent phosphorylation of VEGFR2 in ES cell–derived VEGFR2-positive cells. Although the mechanisms by which mechanical forces activate tyrosine kinase receptors are not well understood, mechanical forces may trigger dimerization of VEGFR2 monomers by affecting their spatial distribution in the cell membrane, or they may activate the receptors by changing their conformation and promoting the binding of tyrosine kinases, such as Src, that are capable of phosphorylating the receptors. Phosphorylation of these tyrosine kinase receptors leads to activation of various protein kinases, including ERK, JNK, PI3-kinase, and Akt, which result in eNOS activation and inhibition of apoptosis.

3.2.3. G proteins

G protein–coupled receptors (GPCRs) have been postulated to play a role in shear stress signal transduction (39). GPCR conformational dynamics in a single EC were detected by real-time molecular imaging using fluorescence resonance energy transfer (FRET), and shear stress was found to cause a conformational transition of bradykinin B\(_2\) GPCRs that led to activation of the receptors (40). Shear stress was also demonstrated to activate purified G proteins reconstituted in liposomes in the absence of receptor proteins, suggesting that G proteins themselves act as a primary mechanotransducer (41).

3.2.4. Caveolae

Caveolae are membrane microdomains measuring around 50 – 100 nm in length that are visible as flask-shaped invaginations below the surface of cells (Fig. 1), and they contain many signaling molecules, including receptors, ion channels, and protein kinases (42). It has been well documented that caveolae play an important role in shear stress signal transduction (43). We have observed that flow-induced Ca\(^{2+}\) responses in ECs start at caveolae and propagate through the entire cell in the form of a Ca\(^{2+}\) wave (44). The Ca\(^{2+}\) increase occurring in the vicinity of caveolae causes caveolae to rapidly liberate eNOS in the cytoplasm, where the activated eNOS catalyzes the production of NO. Treatment of ECs with an antibody against caveolin-1, a constitutive protein of caveolae, has been shown to prevent shear stress–mediated ERK activation. It was also demonstrated that shear stress activates sphingomyelinase located in caveolae, causing them to produce ceramide, leading to ERK activation and Akt-mediated eNOS activation. The roles of caveolae and caveolin-1 in shear stress–mediated regulation of vascular functions have been assessed in caveolin-1 KO mice, which exhibit complete absence of caveolae in vessel walls (45). The caveolin-1 KO mice were characterized by impaired blood flow–dependent vasodilation and vascular remodeling in comparison with wild-type mice. These impairments were rescued by reconstituting caveolin-1 into the endothelium of the KO mice, suggesting that caveolae and caveolin-1 are involved in shear stress–mediated control of vascular functions.

3.2.5. Adhesion proteins

It has been proposed that shear stress is transmitted from the apical surface of ECs through the cytoskeleton to points of attachment at cell–cell and cell–matrix adhesions, and if that is true, adhesion proteins may serve as mechanotransducers. Integrins are transmembrane glycoproteins composed of \(α\) and \(β\) subunits (Fig. 1). Their extracellular domain binds directly to extracellular matrix proteins, and their cytoplasmic domains interact with many proteins aggregated at focal contacts, including both signaling molecules, such as focal adhesion kinase (FAK), Src...
**Fig. 2.** Impaired shear stress–dependent Ca\(^{2+}\) influx and NO production in P2X4 KO mice. A: shear stress–induced Ca\(^{2+}\) response in the ECs of wild-type mice (WT). Intracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]) increased in a stepwise manner when exposed to stepwise increases in shear stress, indicating that ECs are capable of accurately converting information on shear stress into changes in Ca\(^{2+}\) concentration. The ratio of the emitted light of the fluorescent Ca\(^{2+}\) indicator Indo-1/AM at 405 nm (F405) and 480 nm (F480) reflects [Ca\(^{2+}\)]. B: involvement of P2X4 in the Ca\(^{2+}\) influx. ECs of P2X4 knockout mice (KO) markedly suppressed the shear stress–dependent Ca\(^{2+}\) responses. C: shear stress–induced NO production in the ECs of WT and P2X4 KO. Fluorescent images of DAF-2, a fluorescent NO indicator. Images were taken at intervals of 10 s. D: changes in DAF-2 intensity of 15–20 cells. NO production by the ECs of WT increased in a shear stress–dependent manner, whereas the ECs of P2X4 KO did not show any evidence of flow-induced NO production. E: a schematic drawing of the roles of P2X4 receptors in shear stress–mediated signal transduction and physiological significance. Ca\(^{2+}\) signaling of shear stress via P2X4 plays a crucial role in the control of vascular tone, and in shear stress–dependent vasodilation and vascular remodeling, through endothelial NO production.
family protein kinases, Fyn, and p130CAS, and cytoskeletal proteins, such as α-actinin, vinculin, talin, tensin, and paxillin (46). Evidence has been found that shear stress activates integrins. When integrins are activated by shear stress, FAK, paxillin, ε-Src, Fyn, and p130CAS are rapidly activated, thereby leading to the activation of Ras–ERK pathways (47). The results of experiments in which a magnetic twisting device was used to apply shear stress directly to cell surface integrins suggested that integrins are capable of functioning as mechanosensors, thereby transmitting shear stress signals to the cytoskeleton (48). Indeed, when integrins were twisted by magnetic microbeads coated with antibodies against integrins, cytoskeletal filaments became reoriented and a force-dependent cell stiffening response occurred. It has also been demonstrated that activation of integrins by shear stress induces microfilament reorganization through activation of RohA small GTPase and phosphorylation of an actin-regulatory protein cofillin and that shear stress causes integrins to translocate to caveolae, leading to activation of caveolin-1, Src, and myosin light chain kinase, which results in the formation of stress fibers (49).

In addition, there is evidence implicating integrin signaling in the activation of VEGFR2 induced by shear stress but not by VEGF (50). Platelet endothelial cell adhesion molecule-1 (PECAM-1), a member of the immunoglobulin superfamily, is localized to the cell-cell borders of ECs, where it mediates the leukocyte extravasation during the inflammatory response. A novel mechanosignaling pathway via PECAM-1 has been proposed (51). PECAM-1 is tyrosine phosphorylated within 30 s of the start of exposure to shear stress, and as a result, the Ras signaling pathway is activated, leading to ERK activation. Similar signaling events occurred when magnetic beads coated with antibodies against PECAM-1 were used to directly apply tugging force to PECAM-1 molecules on the EC surface. These results seem to indicate that PECAM-1 is a mechanosensitive molecule.

Cadherins are the major proteins of the adherens junctions that mediate cell–cell adhesions. The cytoplasmic domains of cadherins are linked to the actin cytoskeleton via catenin proteins, including β-catenin and plakoglobin (52). It has recently been proposed that vascular endothelial cadherin (VE-cadherin), which is specific for ECs, forms a mechanosensory complex with PECAM-1 and VEGFR2 that plays a critical role in shear stress signal transduction (53). In this system, it is assumed that PECAM-1 directly transduces mechanical forces, that VEGFR2 activates PI3 kinase, which in turn, mediates integrin activation, and that VE-cadherin functions as an adaptor to form signaling complexes. ECs in which VE-cadherin or PECAM-1 had been knocked out failed to show normal responses to shear stress such as activation of PI3 kinase, Akt, and integrin, or an alignment of actin filaments in the direction of flow.

3.2.6. Tensegrity

Living cells stabilize their structure and shape by means of an interconnected network of cytoskeleton components that include microfilaments, microtubules, and intermediate filaments. The tensegrity cell model has been proposed to explain how mechanical forces are transduced into a biochemical response (Fig. 1) (54). The cell model is constructed with a series of isolated compression-resistant sticks that resist the pull of surrounding tensile strings and thereby create an internal pre-stress that stabilizes the entire network. When mechanical forces are applied to the tensegrity model, the structural elements rearrange without undergoing any topographical disruption or loss of tensional continuity, which may directly activate signaling molecules that associate with the cytoskeleton. The cytoskeleton has been shown to play an important role in shear stress–induced NO production and ICAM-1 gene expression by ECs (55).

3.2.7. Glycocalyx

The surface of ECs is covered with a layer of membrane-bound macromolecules that constitute the glycocalyx. The thickness of the EC surface glycocalyx ranges from 0.05 to 1 μm as vessels decrease in size from arterial and venous macrovessels to microvessels (56). The glycocalyx has been considered a possible shear stress sensor because it is located between flowing blood and the cell membrane (57). Involvement of the glycocalyx in EC responses to shear stress has been demonstrated by the finding that degradation of hyaluronic acid glycosaminoglycans within the glycocalyx with hyaluronidase significantly decreases flow-induced NO production in isolated canine femoral arteries (58) and by the finding that enzymatic removal of heparan sulfate with heparinase completely inhibits NO production in response to shear stress in bovine aortic ECs (59). Two possible mechanisms have been proposed to explain how the EC glycocalyx mediates shear stress mechanotransduction. One possible mechanism is that heparan sulfate proteoglycan is present as a random coil under no-flow conditions, but with increasing flow becomes unfolded into a filament structure (Fig. 1) (60). This conformational change is accompanied by an increase in binding sites for Na+ ions, and the Na+ binding may trigger signal transduction. In addition to glycocalyx-mediated regulation of the local concentration gradient and transport of ions, amino acids, and growth factors, it is also possible that shear stress is transmitted to the cell interior through the actin cytoskeleton or intracellular signaling molecules.
that directly associate with the core protein of the glycocalyx.

3.2.8. Primary cilia

The presence of primary cilia having a rod-like, non-motile structure and protruding from the apical cell membranes has been reported in embryonic ECs, HUVECs, and human aortic ECs (61). Recent studies provide evidence that the primary cilia mediate the mechanism by which ECs sense and respond to shear stress (62). Since primary cilia are physically connected to cytoskeletal microtubules, their bending by flow is assumed to transmit shear stress signals into the cells through the cytoskeleton. The bending of the primary cilia may also activate Ca²⁺-permeable ion channels and trigger Ca²⁺ signaling. It has recently been shown that polycystin-1, an 11-transmembrane protein with a long extracellular domain, and polycystin-2, a member of a superfamily of trp channels, are localized on the cilia of ECs and together involved in shear stress sensing (Fig. 1) (63). ECs in which polycystin-1 and polycystin-2 have been knocked out are unable to transduce shear stress into changes in intracellular Ca²⁺ concentration or to produce NO in response to shear stress.

4. Conclusion

There has been a large amount of work on shear stress–induced endothelial mechanotransduction during the past several decades and it has revealed how ECs recognize shear stress and respond to it. A striking feature of shear stress mechanotransduction is that shear stress activates a variety of membrane molecules leading to signal transduction through multiple pathways. It should be noted in regard to shear stress sensing by ECs that they are simultaneously exposed to shear stress in vivo. Elucidation of shear-stress-sensing mechanisms should lead to a better understanding of how blood flow regulates the circulatory system, how physical exercise exerts a beneficial effect on the human body, and how hemodynamic factors are involved in the pathophysiology of vascular diseases, including hypertension, thrombosis, aneurysms, and atherosclerosis. The invention of novel means of manipulating endothelial shear stress sensing may lead to the development of new therapies for the above vascular diseases. Furthermore, the increase in knowledge about shear stress mechanotransduction should be helpful for a better understanding of the critical phenomena that occur based on interactions between genetic information and environmental factors, including mechanical forces.

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