ANTIOXIDANTS & REDOX SIGNALING
Volume 15, Number 5, 2011

DOI: 10.1089/ars.2010.3361

FORUM REVIEW ARTICLE

Effects of Shear Stress and Stretch on Endothelial Function

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Abstract

Vascular endothelial cells (ECs) play a central role in the control of blood vessel function and circulatory system homeostasis. It is well known that that EC functions are regulated by chemical mediators, including hormones, cytokines, and neurotransmitters, but it has recently become apparent that EC functions are also controlled by hemodynamic forces such as shear stress and stretch (cyclic strain). ECs recognize shear stress and cyclic strain as mechanical stimuli, and transmit the signal into the interior of the cells, thereby triggering a variety of cellular responses that involve alterations in cell morphology, cell function, and gene expression. Impaired EC responses to shear stress and cyclic strain lead to vascular diseases, including hypertension, thrombosis, and atherosclerosis. A great deal of research has already been conducted on the mechanotransduction of shear stress and cyclic strain, and its molecular mechanisms are gradually coming to be understood. However, much remains unclear, and further studies of mechanotransduction should increase our understanding of the molecular basis of the hemodynamic-force-mediated control of vascular functions. *Antioxid. Redox Signal.* 15, 1389–1403.

Introduction

ENDOTHELIAL CELLS (ECs) lining vessel lumens play crucial roles in the control of vascular functions that maintain homeostasis of the circulatory system. ECs regulate blood pressure and blood supply to tissues by releasing smooth muscle relaxants and constrictors, and they prevent intravascular thrombus formation through mediators involved in anticoagulation and fibrinolysis. ECs also play important roles in angiogenesis and vascular remodeling by producing a variety of cell growth factors, and they are deeply involved in inflammatory responses and immune responses in the tissues via adhesive interactions with leukocytes. It is well known that EC functions are regulated by "chemical mediators", such as hormones, cytokines, and neurotransmitters, but it has recently become apparent that "mechanical stresses" generated by blood flow and blood pressure control EC functions. Since ECs are in contact with flowing blood, they are exposed to fluid shear stress and simultaneously stretched by pulsatile changes in blood pressure. Many recent studies have shown that shear stress and cyclic strain modulate EC morphology and function, and that they alter the expression of related genes (9, 59). Many advances have also been made in elucidating the mechanism by which ECs sense mechanical stresses and transmit the signal to the cell interior. EC responses to mechanical stresses have mainly been studied in research conducted in the field of biomechanics, and rapid progress has been made in analysis at cellular and molecular levels since fluid-dynamically designed devices in which cultured ECs are subjected to controlled levels of shear stress and cyclic strain were introduced. Based on the results of numerous studies, it appears that EC responses to shear stress and cyclic strain are essential to maintaining homeostasis of the circulatory system, and that once their responses are impaired, a variety of abnormalities occur in the circulatory system that, in turn, lead to vascular diseases, such as atherosclerosis, hypertension, and aneurysm. This article reviews the effects of fluid mechanical forces on EC function, focusing on EC responses to shear stress and their physiological roles in the circulatory system.

Fluid Mechanical Forces and Loading Devices

Blood flow generates shear stress in ECs, and its intensity (τ) can be calculated by using the formula, $\tau = 4\mu Q/\pi r^3$, where μ is blood viscosity, Q is blood flow volume, π is the ratio of the circumference of a circle to its diameter, and r is the radius of the blood vessel. Under physiological conditions, arterial ECs are exposed to a shear stress of around 20 dynes/cm^2, and venous ECs to shear stress ranging from 1.5 to 6 dynes/cm^2 (60). Pulsatile changes in blood pressure stretch the vessel wall circumferentially and create cyclic strain in ECs. The degree of stretch is around 9%–12% in the aorta, 1%–2% in the carotid arteries, 2%–15% in the femoral arteries, and 6%–10% in the pulmonary arteries (32).

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Various types of flow-loading devices, including a rotating-disk type (15, 87), a parallel-plate type (109), and a tube type (81), have been developed to apply controlled levels of shear stress to cultured ECs. In the rotating-disk type, rotation of a cone forces the medium between the cone and the bottom of a round dish to flow concentrically, exposing the cells attached to the bottom of the dish to shear stress. The rotating-disk type can also be used to apply laminar and turbulent shear stress selectively to cells by changing the cone angle and rotation rate. In the parallel-plate type, two flat plates, on one of which the cultured cells rest, are held a couple of hundred micrometers apart by a gasket, and perfusion of medium through the gap between the two plates generates laminar shear stress in the cells. In the tube type, cells are cultured on the inner surface of the tube, and medium is perfused through the tube. When a compliant tube is used, pulsatile flow stretches the tube, and the cells are simultaneously subjected to both shear stress and cyclic strain. Commercially available stretch-loading devices, including Flexercell (Flexcell International, Hillsborough, NC) and STREX (STREX Inc., Osaka, Japan), have been used to apply cyclic strain to cells (114, 116). In Flexercell, cultured cells rest on an elastic membrane that is stretched by negative pressure. In STREX, cells are cultured in a polydimethylsiloxane (PDMS) chamber, one end of which is attached to a fixed frame, and the other end of which is fixed to a movable frame connected to a motor-driven shaft. The amplitude and frequency of stretching is controlled by a programmable microcomputer.

Endothelial Cell Responses to Shear Stress

Morphology

When examined *in vivo*, the ECs lining segments of blood vessels in which blood flow is rapid and unidirectional are spindle-shaped and aligned with their long axis parallel to the direction of blood flow, whereas the ECs lining segments in which blood flow is turbulent or stagnant are much rounder in shape and do not have a uniform orientation (28, 67, 86). Because of these findings, shear stress is thought to determine the shape and orientation of ECs. When cultured ECs have been subjected to laminar shear stress in a flow-loading device *in vitro*, they have been found to become elongated and undergo a net cellular movement that orients them in the direction of the shear stress (Fig. 1).This morphological change is accompanied by cytoskeletal reorganization, with actin filaments becoming rearranged into bundles of stress fibers and aligned in the direction of the shear stress (76, 125, 127). The induction of stress fiber formation results in protection of ECs from detachment by the increased shear stress.

Proliferation and apoptosis

Shear stress plays a crucial role in the control of EC proliferation. When blood flow in canine carotid arteries was increased by surgically constructing an arteriovenous shunt, EC density markedly increased in response to the increased blood flow (77), and *in vitro* studies with ECs in flow-loading devices showed that steady laminar flow reduced EC proliferation (2), whereas disturbed flow increased EC turnover (24). Laminar flow is known to arrest ECs in the G0/G1 phase of the cell cycle by upregulating cyclin-dependent kinase inhibitor p21.

Vascular endothelium has the ability to repair EC areas denuded as a result of toxic injury or mechanical desquamation, and adjacent ECs migrate and proliferate to regenerate the injured endothelium. We examined the effect of laminar shear stress on endothelial regeneration *in vitro* by creating a partially denuded area in a confluent monolayer of cultured ECs and assessing proliferation and migration across the line of denudation in the presence or absence of shear stress (3). The results showed that shear stress stimulated EC migration and proliferation, thereby accelerating regeneration of the denuded area.

Shear stress has been shown to be essential for EC survival. After birth, the absence of the placenta leads to a marked decrease in blood flow in a variety of arteries, which causes the arteries of newborns to undergo postnatal remodeling, and high rates of EC and smooth muscle cell (SMC) apoptosis occur during the process (19). When human umbilical cord veins that had been removed immediately after birth were organ-cultured, massive apoptosis occurred during static culture, but no apoptotic cells were detected when the veins were perfused with culture medium (58). An apoptosis-suppressing effect of shear stress has also been demonstrated *in vitro*. Laminar shear stress was shown to inhibit apoptosis of cultured ECs induced by growth factor withdrawal or incubation with H_2O_2 or tumor necrosis factor α (TNFα) (30, 42). Several mechanisms have been proposed to account for the apoptosis-suppressing effects of shear stress, including upregulation of superoxide dismutase (SOD) and Akt-mediated activation of nitric oxide synthase, with subsequent inhibition of the caspase cascade. Turbulent shear stress, on the other hand, has been shown to induce apoptosis by cultured ECs (37). There is *in vivo* evidence suggesting that blood flow

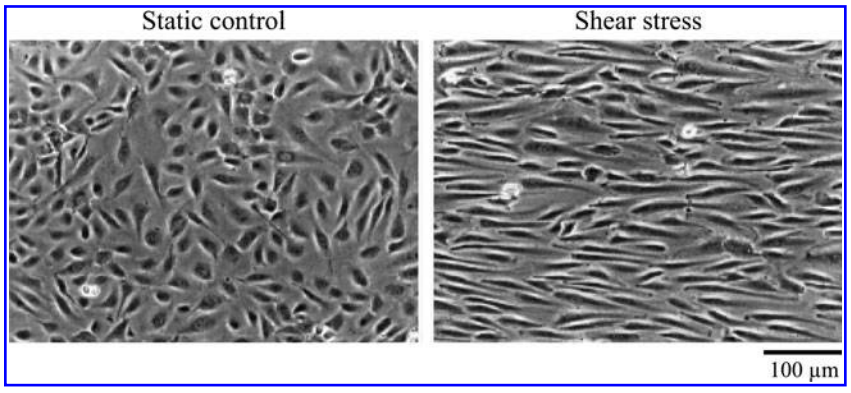

FIG. 1. Flow-induced EC morphological changes. Human umbilical vein ECs (HUVECs) that were polygonal under static culture conditions became elongated with their long axis oriented in the direction of flow when exposed to laminar shear stress (10 dynes/cm^2, 24 h). This response is reversible because the flow-induced morphological changes were no longer seen when the cells were placed under static conditions.

determines whether ECs survive or undergo apoptosis in human atherosclerosis (119). EC apoptosis was observed in 60% of the plaques examined, and apoptosis preferentially occurred in the downstream parts of the plaques, where low shear stress prevailed, rather than in the upstream parts. EC apoptosis may be involved in plaque erosion and thrombosis.

Vascular tone

Shear stress is involved in the regulation of vascular tone, because it stimulates ECs to produce SMC relaxing or constricting factors. Increases in laminar shear stress usually cause vasodilation. When blood flow in vessels increases, they acutely dilate, and the dilation is mainly mediated by nitric oxide (NO) released by ECs (100). A stimulatory effect of laminar shear stress on NO production has been demonstrated in cultured ECs (14, 63, 91), and laminar shear stress has been found to increase NO production via activation of endothelial NO synthase (eNOS) and upregulation of its gene expression (12). Shear stress induces an increase in the intracellular concentrations of Ca^{2+} and tetrahydrobiopterin (BH4), an essential cofactor of eNOS, and shear stress also activates protein kinases, which results in eNOS activation (22, 31, 35, 126). eNOS gene expression increases in response to shear stress, and the increase occurs as a result of an increase in transcription that involves transcription factor NFκB and a shear stress response element (GAGACC) located in the eNOS gene promoter, and as a result of eNOS mRNA stabilization through its 3′ polyadenylation (26, 122, 124). Production of other potential vasodilators, including prostacyclin, C-type natriuretic peptide, and adrenomedullin, also increases in ECs exposed to shear stress (20, 36, 95). By contrast, production of the vasoconstrictor endothelin-1 (ET-1) and cell surface expression of angiotensin-converting enzyme (ACE), which generates the potent vasoconstrictor angiotensin II, decreases in response to laminar shear stress (98, 103).

Antithrombotic activity

Laminar shear stress stimulates the antithrombotic activity of ECs. NO and prostacyclin, both of which are produced by ECs exposed to shear stress, have potent platelet aggregation inhibiting activity. ECs also express the antithrombotic membrane glycoprotein thrombomodulin (TM), which inactivates the procoagulant factor thrombin and activates the anticoagulant protein C, which retards the coagulation process by degrading activated coagulation factors. We have demonstrated that laminar shear stress increases expression of TM by cultured human umbilical vein ECs (HUVECs) time- and dose-dependently (Fig. 2) (115). Shear stress also contributes to maintaining ECs nonthrombogenic by increasing the production of heparan sulfate proteoglycans and tissue-type plasminogen activator (tPA) (10, 29).

Growth factors and cytokines

ECs produce a variety of growth factors and cytokines, and laminar shear stress has been shown to increase their production of platelet-derived growth factor (PDGF) (46, 80), heparin binding-epidermal growth factor-like growth factor (HB-EGF) (82), basic fibroblast growth factor (bFGF) (75), transforming growth factor-β (TGF-β) (92), interleukin-1 and

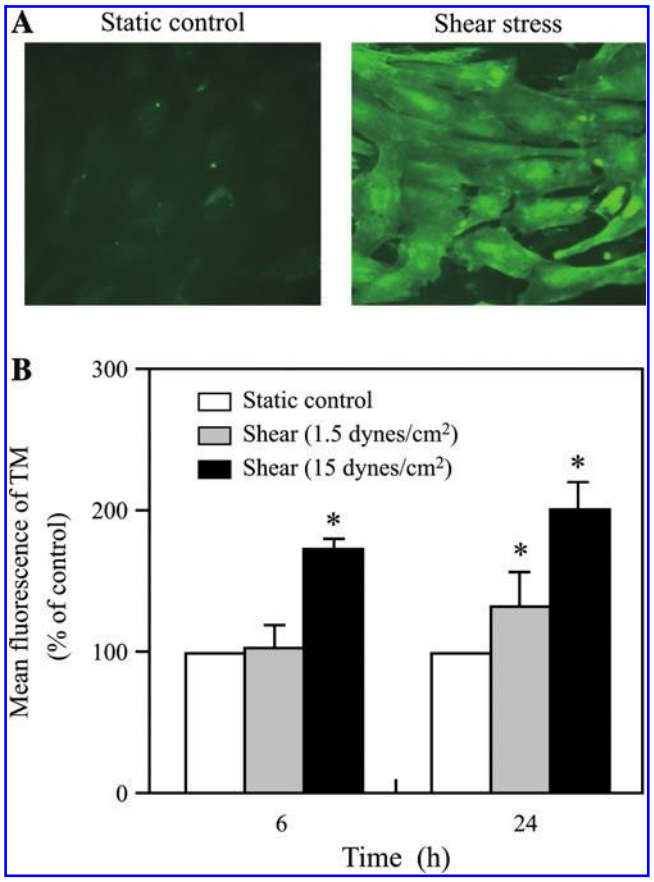

FIG. 2. Effect of shear stress on cell surface expression of the antithrombotic protein thrombomodulin (TM). (A) Immunofluorescence photomicrographs of HUVECs obtained with an antibody against TM. Expression of TM increased significantly in response to laminar shear stress (15 dynes/cm^2, 24 h). **(B)** Flow cytometric analysis of flow-induced changes in the amount of TM antigen. The mean TM fluorescence of the shear-loaded cells was calculated by normalizing it with that of matched static controls and expressed as a percentage (%) of the control value. Shear stress increased TM expression in a dose- and time-dependent manner. *Bars* represent the mean ± SD of five samples. *$p < 0.01$ vs. static control. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

−6 (IL-1 and IL-6) (110), and granulocyte/macrophage colony stimulating factor (GM-CSF) (66).

Adhesive interactions with leukocytes

It is well known that local blood flow conditions affect the adhesive interactions that occur between ECs and leukocytes (70). Laminar shear stress has been shown to modulate adhesion of leukocytes to ECs by altering EC expression of adhesion molecules. When murine lymph node venule ECs, which express abundant vascular cell adhesion molecule-1 (VCAM-1), were exposed to laminar shear stress in a flow-loading device, VCAM-1 expression decreased markedly (Fig. 3A), and the decrease resulted in a significant reduction in the number of lymphocytes that adhered to the cells (6, 93). The decrease in VCAM-1 protein expression was due to a decrease in gene expression, because the VCAM-1 mRNA levels decreased time-dependently in response to laminar shear stress

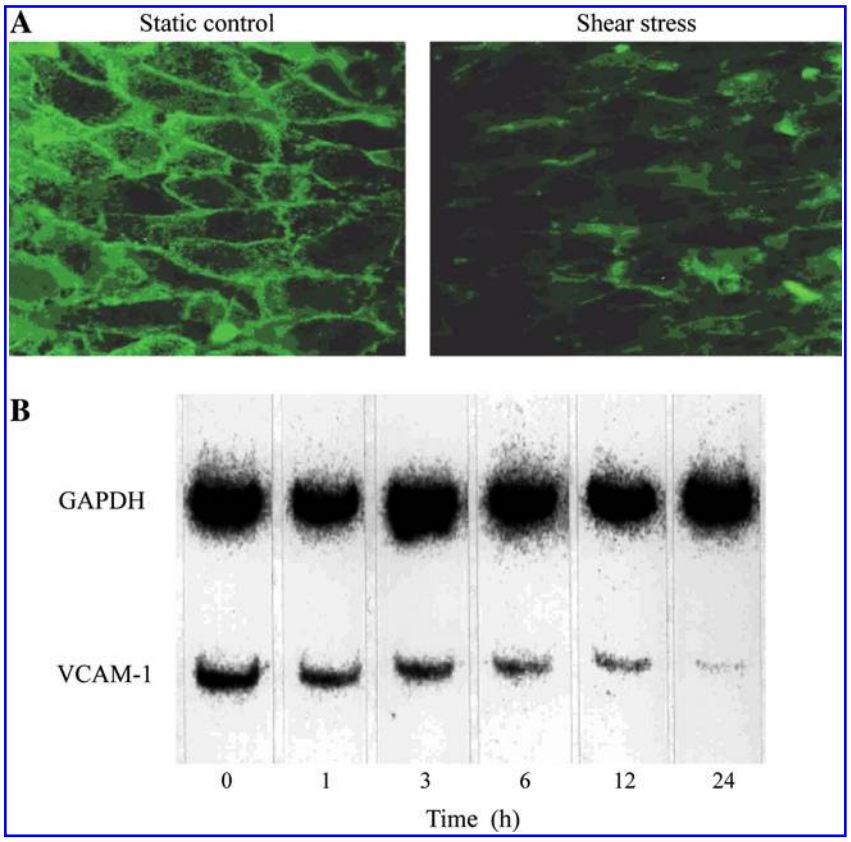

FIG. 3. Effect of shear stress on cell surface expression of vascular cell adhesion molecule-1 (VCAM-1), a key adhesion molecule involved in leukocyte binding to the vascular endothelium. (A) Immunofluorescence photomicrographs of murine lymph node venule ECs obtained with an antibody against VCAM-1. VCAM-1 expression markedly decreased in response to laminar shear stress (1.5 dynes/cm^2, 24 h). A large number of lymphocytes adhered to the stationary control cells, but not to cells exposed to shear stress. **(B)** Temporal changes in VCAM-1 mRNA levels. VCAM-1 mRNA levels decreased time-dependently in ECs exposed to laminar shear stress (1.5 dynes/cm^2). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

(Fig. 3B) (64). The effect of shear stress on adhesion molecules varies among EC lines. Expression of intercellular adhesion molecule-1 and E-selectin increases in HUVECs exposed to shear stress, but expression of VCAM-1 does not (120).

Reactive oxygen species

Reactive oxygen species (ROS), including the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), are produced in ECs and act as intracellular second messengers or mediators of human diseases such as atherosclerosis and reperfusion injury (34). Shear stress affects the production and elimination of ROS in ECs (69). Exposure of ECs to oscillatory shear stress has been demonstrated to markedly increase ROS production through activation of the O_2^--producing NAD(P)H oxidase (27, 49), whereas laminar shear stress has been shown to increase SOD, which inactivates ROS (52). In addition, both increased mRNA and protein levels of Cu/Zn SOD and increased Cu/Zn SOD activity have been observed in cultured human aortic ECs exposed to laminar shear stress. A recent study showed that laminar shear stress, but not oscillatory shear stress, increased expression of antioxidant enzyme peroxiredoxins by ECs (83).

Cell differentiation

Recent studies have demonstrated that shear stress affects the differentiation of immature cells, including endothelial progenitor cells (EPCs) and embryonic stem (ES) cells, into ECs. Bone marrow-derived EPCs circulating in peripheral blood migrate toward their target tissue, where they differentiate and contribute to the formation of new vessels (11). During this process, they are exposed to shear stress generated by interstitial fluid flow and blood flow, and when cultured EPCs are subjected to shear stress in a flow-loading device, their differentiation into mature ECs accelerates significantly (134).

ES cells have the potential to differentiate into every cell type in the body, and they are attracting interest as a promising source of cells for use in regenerative medicine. ES cells are exposed to fluid mechanical forces, including shear stress and the cyclic strain generated by the beating heart during the process of embryonic development (57). However, we have demonstrated that shear stress and cyclic strain have very different effects on ES cell differentiation: shear stress induces differentiation of ES-cell-derived VEGF receptor 2 (VEGFR2)-positive cells (138) into the EC lineage (135), whereas cyclic strain induces differentiation of VEGFR2-positive cells into the SMC lineage (105). Interestingly, differentiation into the EC lineage and differentiation into the SMC lineage are mediated by ligand-independent phosphorylation of VEGFR2 and PDGF receptors, respectively. Moreover, our recent study has shown that shear stress increases expression of an arterial EC marker, ephrinB2, in EPCs and murine ES cells, suggesting that shear stress can affect the arteriovenous differentiation of ECs (78, 90). It seems likely that fluid mechanical forces act as regulators of EPC-mediated neovascularization and of ES cell-mediated early embryonic vascular development.

Gene expression

When EC functions change in response to shear stress, expression of related genes also usually changes. We conducted a DNA microarray analysis to identify genes in HUVECs and

Table 1. Percentage of Shear Stress-Responsive Genes

Cell line	*Shear stress (dynes/cm^2)*	*Up (SD) > 2-fold*	*Down(SD) > 50%*	*%*
HUVEC	Laminar 15	50 (21)	131 (33)	3.2
HUVEC	Laminar 1.5	32	86	2.1
HCAEC	Laminar 15	50 (1)	120 (4)	3.0
HCAEC	Laminar 1.5	25 (22)	88 (14)	2.0
HCAEC	Turbulent 1.5	23 (3)	40 (8)	1.1

$n = 3$ for HUVEC; $n = 2$ for HCAEC.

human coronary artery ECs (HCAECs) whose expression is altered by shear stress (94). Expression of approximately 3% of the 5600 genes increased more than two-fold or decreased to less than half of the level of expression in the static control in response to an arterial level of laminar shear stress (15 dynes/cm^2 for 24 hours) (Table 1). If ECs are assumed to express a total of around 20,000 genes, this finding suggests that more than 600 EC genes are shear stress responsive. Our previous study using mRNA differential display showed that approximately 4% of all mRNAs detected in HUVECs were

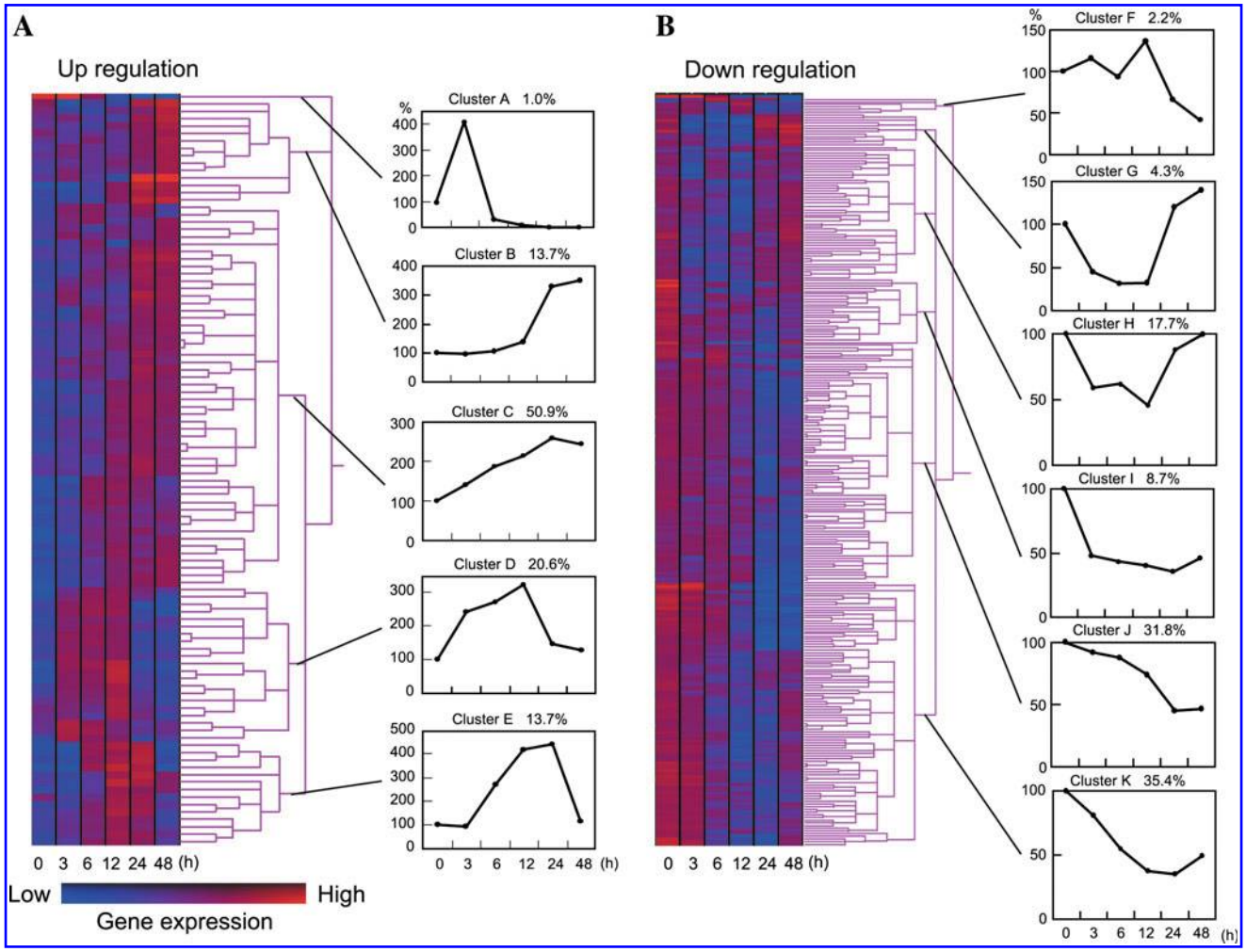

FIG. 4. Cluster image showing the different classes of gene expression profiles. HUVECs were exposed to a laminar shear stress of 15 dynes/cm^2 for 3, 6, 12, 24, and 48 h, and 379 genes whose expression increased more than two-fold or decreased to less than half at least at one time point were selected. Genespring software (Silicon Genetics, Redwood City, CA) was used to cluster these genes hierarchically into groups on the basis of similarities between their expression profiles. The similarity tree has been divided into 8 levels of branching depth. Division of the tree at branching level 3 divides the genes into eleven clusters of gene expression. **(A)** Upregulated genes; **(B)** downregulated genes. The expression pattern of each gene is displayed as a *horizontal strip*. The ratio of the level of expression of a gene at the time indicated after shear stress stimulation to its level in the static control is represented in *color*. The *graphs* show the average normalized expression pattern over the time points for all genes in each cluster indicated by the letters A to K and the frequency of each cluster. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

responsive to laminar shear stress (7). A review of our data and data reported by others revealed that the ratio of shear stress-responsive EC genes appeared to range from 1.3% to 6% (17, 38, 79). The ratio decreased to around 2% at a venous level of laminar shear stress (1.5 dynes/cm^2), and decreased further to 1.1% under turbulent shear stress, indicating that the number of genes that respond to flow depends on the magnitude or nature of the shear stress. Cluster analysis showed that the temporal profiles of the gene response to shear stress are variable instead of uniform, and they were classified into eleven clusters (Fig. 4). Thus, EC responses to shear stress seem to consist of complex cascades of gene responses having different temporal profiles.

Shear stress regulates endothelial gene expression transcriptionally and/or post-transcriptionally (8). The transcriptional regulation is mediated by transcription factors, including AP-1, NFκB, Egr-1, SP-1, GATA 6, and Kruppel-like factor 2 (KLF2), each of which binds to its consensus motif in the gene promoter (48, 61, 65, 72, 106, 108). The post-transcriptional regulation is mediated by RNA-binding proteins that bind to mRNA and control its degradation rate. We have shown that the expression of genes encoding GM-CSF and urokinase-type plasminogen activator (uPA) is upregulated by laminar shear stress through mRNA stabilization (66, 108).

Differences between the effects of laminar and turbulent shear stress on EC function

EC responses differ according to whether the shear stress is laminar or turbulent. Turbulent shear stress increased DNA synthesis in bovine aortic ECs, but laminar shear stress did not (24). Laminar shear stress increased eNOS gene expression in HUVECs, leading to an increase in NO release, whereas turbulent shear stress had no effect (88). Recent studies using DNA microarrays have revealed striking differences between the numbers of kinds of endothelial genes that respond to laminar shear stress and turbulent shear stress (13). We have shown that uPA expression is differentially regulated by laminar and turbulent shear stress *in vitro* (108), and application of laminar shear stress to HCAECs significantly decreased the secretion of uPA protein as well as uPA mRNA levels (Figs. 5A and 5B). By contrast, turbulent shear stress markedly increased uPA protein secretion and gene expression (Figs. 5C and 5D). The downregulation of uPA gene expression by laminar shear stress was due to the suppression of gene transcription via transcription factor GATA6 and the acceleration of mRNA degradation. The upregulation by turbulent shear stress was attributable to mRNA stabilization. The effects of turbulent shear stress on uPA expression may contribute to atherosclerotic vascular remodeling, because uPA has the activity to stimulate SMC migration and proliferation in addition to stimulating proteolysis of the extracellular matrix. Increased expression of uPA has actually been observed in atherosclerotic lesions (62). Based on the results of numerous studies, it appears that laminar shear stress has an anti-atherosclerotic effect on arteries, whereas turbulent shear stress acts as a pro-atherosclerotic factor.

Endothelial Cell Responses to Cyclic Strain

ECs undergo changes in morphology in response to cyclic strain. When ECs cultured on flexible membranes were subjected to cyclic strain, they became elongated and aligned perpendicularly to the direction of stretch (55). They pro-

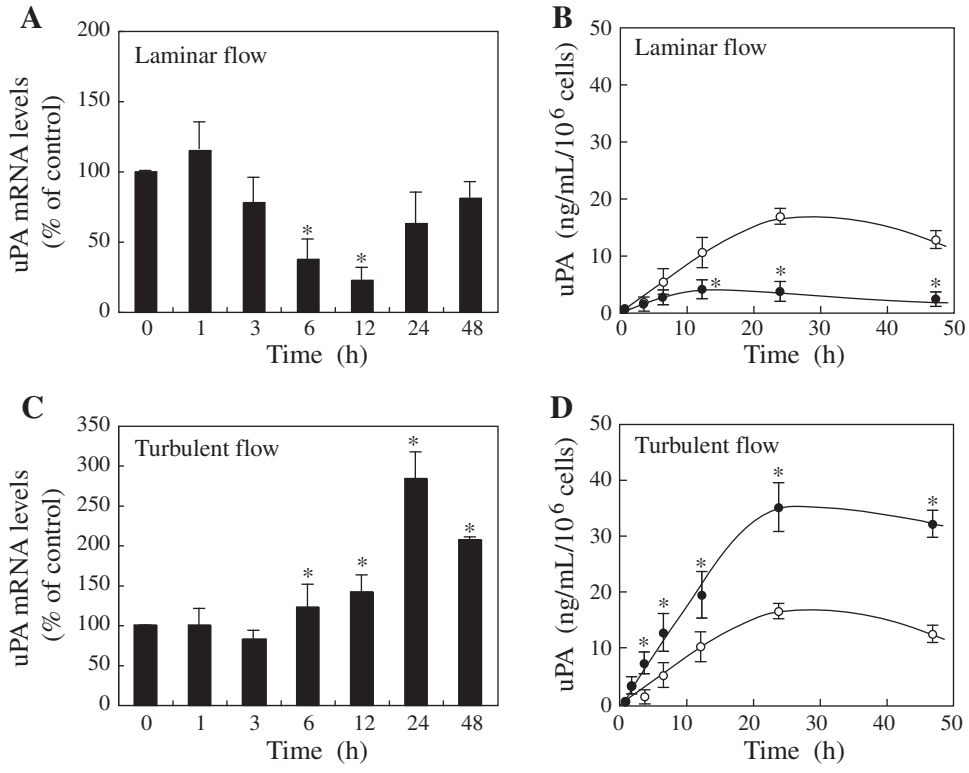

FIG. 5. Effects of shear stress on uPA mRNA levels and uPA protein release by human coronary artery ECs. Total RNA was isolated from ECs that had either been exposed to laminar or turbulent shear stress for the period of time indicated or maintained as a static control, represented by "time 0"; the samples were then analyzed by real-time PCR and ELISA. **(A)** and **(C)** mRNA levels; **(B)** and **(D)** amount of uPA protein released by cells. The *open circles* represent static control cells, and the *solid circles* represent flow-loaded cells. **(A)** and **(B)** laminar flow; **(C)** and **(D)** turbulent flow. uPA mRNA and uPA release decreased in response to laminar shear stress (1.5 dynes/cm^2), whereas they increased in response to turbulent shear stress (1.5 dynes/cm^2). The mRNA values and protein levels are means $\pm$ SD of the results of five separate experiments and three separate experiments, respectively. *$p < 0.01$ vs. static control.

duced actin stress fibers, whereas ECs cultured under static conditions did not produce actin stress fibers (23, 113). The effects of cyclic strain on EC proliferation vary with the cell type and level of cyclic strain, and while some studies have reported an increase in proliferation rate in response to cyclic strain (111), other have reported an opposite effect (128). Physiological levels of cyclic strain (6%–10%) were found not to affect the apoptosis rate in comparison with a static control, but they inhibited the apoptotic effect of TNFα and of serum deprivation (73). Cyclic strain has been shown to cause an increase in production of various vasoactive substances, including prostacyclin, ET-1, tPA, ROS, and monocyte chemotactic protein-1 (MCP-1) (44, 45, 50, 74, 112, 129). Cyclic strain also affects EC gene expression, and many transcription factors, including AP-1, cAMP response element binding protein (CREB), and NFκB, are known to be involved in the cyclic-strain-mediated regulation of gene expression (33, 130).

In vivo, vascular ECs are exposed to both shear stress and cyclic strain simultaneously, but little is known thus far about the combined effects of shear stress and cyclic strain. We recently developed a system in which ECs cultured on the inner surface of an elastic tube can be exposed to both laminar shear stress and cyclic strain at the same time, and we have examined their combined effects on gene expression of ET-1 and eNOS (Figs. 6A and 6B) (118). The ET-1 mRNA levels were found to increase in response to cyclic strain, but to decrease in response to shear stress. However, there was no significant change in ET-1 mRNA levels when cyclic strain and shear stress were applied simultaneously. By contrast, exposure to shear stress resulted in an increase in eNOS mRNA levels, while cyclic strain had no effect. Combined application increased the eNOS mRNA level to a level equivalent to that induced by shear stress alone. These results indicate that the response of EC genes to shear stress or cyclic strain depends on whether the two forces are applied alone or in combination.

Shear Stress Mechanotransduction

The fact that ECs respond to shear stress by undergoing changes in morphology, function, and gene expression indicates the existence of a mechanotransduction mechanism by which ECs sense shear stress as a signal and transmit it into the cell interior. Numerous studies have shown that multiple pathways are involved in shear stress signal transduction (Fig. 7) (25). However, which pathways are primary and which are secondary remains unclear, because the initial sensing mechanism or sensors that recognize shear stress have not been identified. Various membrane molecules and cellular microdomains, including ion channels (39, 89, 96, 132), growth factor receptors (18, 56, 104), G protein-coupling receptor (GPCR) (16, 40, 41), caveolae (54, 99, 139), adhesion proteins (53, 71, 97, 123), the cytoskeleton (51), the glycocalyx (107, 117), and primary cilia (1, 43, 85), have been shown to play important roles in the shear stress sensing mechanism.

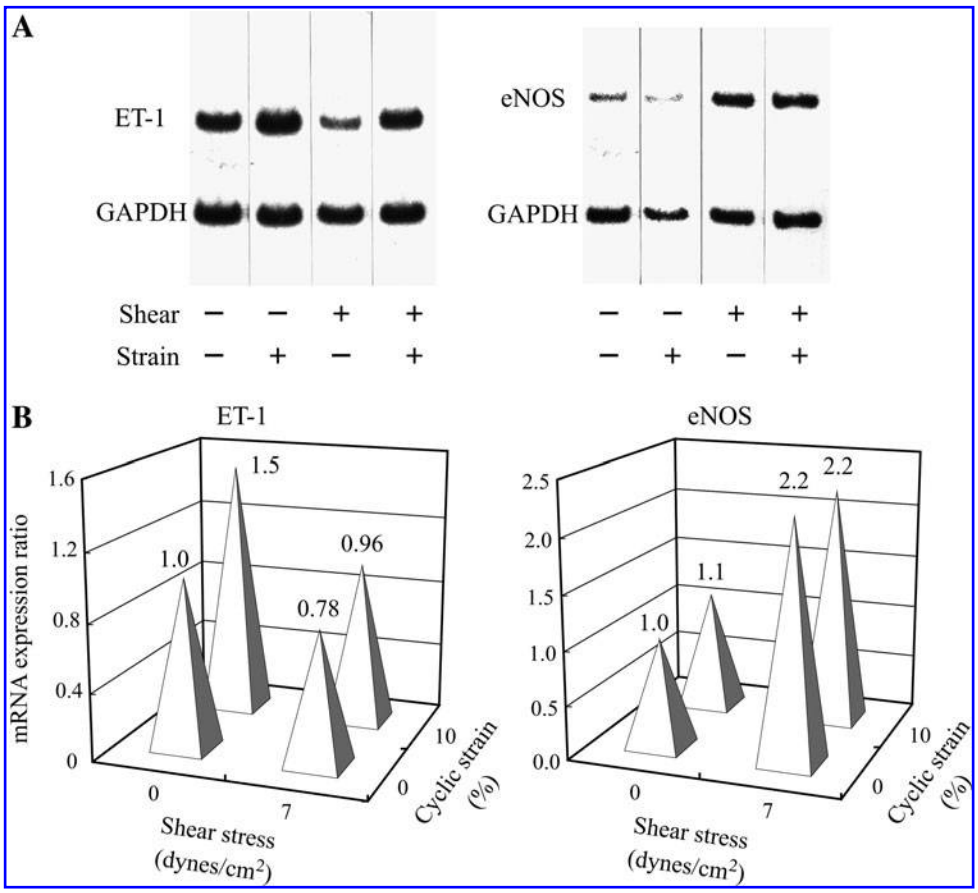

FIG. 6. Endothelial gene responses to shear stress and/or cyclic strain. HUVECs were incubated under static conditions or exposed to cyclic strain alone, to laminar shear stress alone, or to both shear stress and cyclic strain simultaneously. **(A)** RT/PCR bands. Total RNA was extracted from these cells and reverse-transcribed into cDNA. The cDNA was amplified by PCR with specific primers and resolved on a polyacrylamide gel. **(B)** Densitometry analysis. The *bar graph* shows the changes in ET-1 and eNOS mRNA levels relative to those of cells incubated under static conditions whose levels were set equal to 1.0. ET-1 gene expression by ECs increased in response to cyclic strain, decreased in response to shear stress, and remained unchanged when ECs were exposed to both cyclic strain and shear stress at the same time. By contrast, shear stress increased eNOS gene expression, whereas cyclic strain had no effect. Simultaneous application of shear stress and cyclic strain increased eNOS gene expression to almost the same level as induced by shear stress alone.

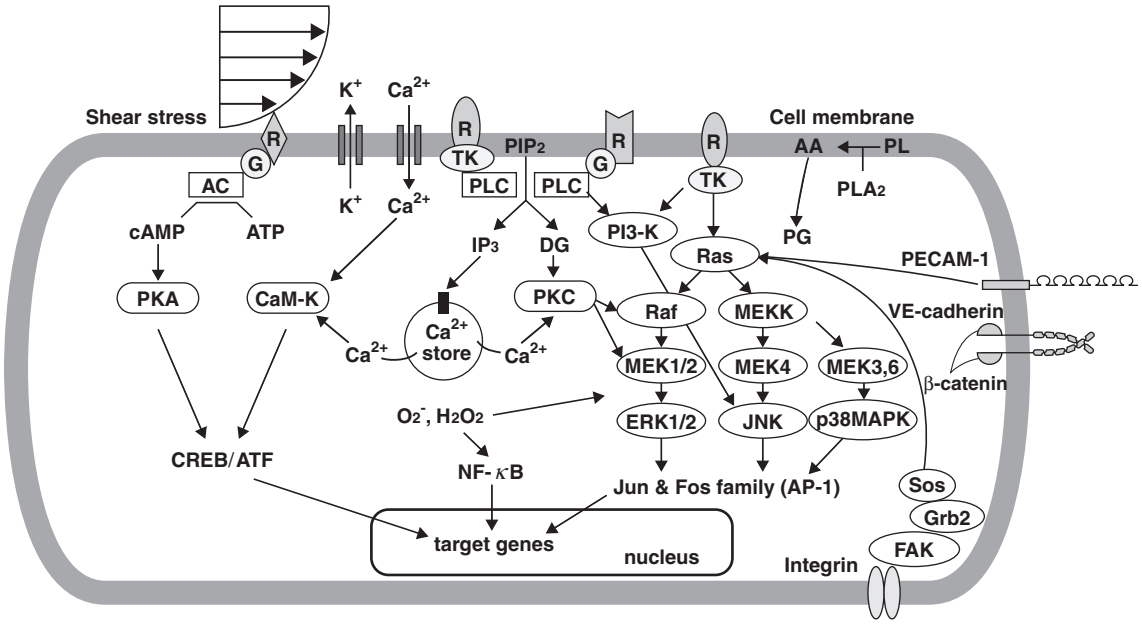

FIG. 7. Signal transduction factors that are capable of being activated by shear stress. Multiple pathways are involved in the shear stress signal transduction that leads to alterations in EC morphology and function and to activation of various transcription factors. Which pathways are primary and which are secondary remains unclear and the initial sensor that recognizes shear stress has not been identified. Shear stress may activate several pathways simultaneously. AA, arachidonic acid; AC, adenylate cyclase; ATP, adenosine 5′-triphosphate; CaM-K, calmodulin kinase; cAMP, cyclic adenosine 3′,5′-monophosphate; CREB/ATP, cAMP-responsive element-binding protein/activating transcription factor; DG, 1,2-diacylglycerol; ERK, extracellular-signal-regulated kinase; FAK, focal adhesion kinase; G, G protein; IP3, inositol,4,5-triphosphate; JNK, c-jun N-terminal kinase; MEK, MAP kinase-ERK kinase; MEKK, MAP kinase-ERK kinase kinase; NFκB, nuclear factor kappa B. Jun & Fos family (AP-1), transcription factors; p38 MAPK, mitogen-activated kinase; O_2^- and H_2O_2, reactive oxygen species; PECAM-1, platelet endothelial cell adhesion molecule-1; PG, prostaglandin; PI3-K, phosphatidylinositol 3-phosphate; PKA, protein kinase A; PKC, protein kinase C; PL, phospholipids; PLA2, phospholipase A2; PLC, phospholipase C; Ras and Raf, small G proteins; Sos and Grb2, adaptor proteins. R, receptor; TK, tyrosine kinase.

Recently, it has been proposed that vascular endothelial cadherin (VE-cadherin), which is a major protein of adherens junctions that mediate cell–cell adhesions, comprises a mechanosensory complex with PECAM-1 and VEGFR2 to play a critical role in shear stress signal transduction (121). In this system, PECAM-1 is assumed to directly transduce mechanical forces, VEGFR2 to activate PI3 kinase that mediates integrin activation, and VE-cadherin to function as an adaptor to form signaling complexes.

Stretch-activated (SA) ion channels have been found to play a crucial role in cyclic strain sensing. When the cell membrane is stretched, SA channels open, and extracellular Ca^{2+} passes through the channels into the cell, thereby activating downstream signaling pathways (84). Other molecules besides SA channels, including integrin (102), PDGF receptor (47), and GPCR (21), have been shown to be involved in the cyclic strain signal transduction.

Ca^{2+} signaling is one of the pathways involved in shear stress mechanotransduction (4, 5). When ECs cultured from human pulmonary arteries were subjected to laminar shear stress, the intracellular Ca^{2+} concentration increased in a dose-dependent manner (Fig. 8A) (131). The Ca^{2+} response was due to an influx of extracellular Ca^{2+} 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 Ca^{2+} influx (Fig. 8B), and activation of P2X4 required ATP, which was supplied in the form of endogenous ATP released by the ECs (133). The ECs released ATP dose dependently in response to laminar shear stress (Fig. 8C), and suppression of ATP release with the ATP synthase inhibitor angiostatin abolished the shear stress-induced Ca^{2+} responses (Fig. 8D). These findings suggest that ECs are capable of converting information accurately regarding shear stress intensity into changes in intracellular Ca^{2+} concentrations through ATP release and P2X4 activation. We recently discovered that ATP synthase is localized in caveolae and involved in the ATP release induced by shear stress (137). This finding means that ATP synthase not only produces ATP as an energy source in the mitochondria but also generates ATP as a signal transduction factor on the cell surface. The mechanism responsible for the cell surface ATP synthase-mediated ATP release in response to shear stress, however, remains unclear

Roles of Shear Stress Ca^{2+} Signaling in the Control of the Circulatory System

In order to identify the physiological roles of P2X4-mediated shear stress signal transduction in the circulatory system, we generated P2X4 gene knockout mice (KO mice) (136). The KO mice did not exhibit normal EC responses to shear stress, such as a Ca^{2+} influx and subsequent production of NO. When the ECs of wild-type (WT) mice were exposed to

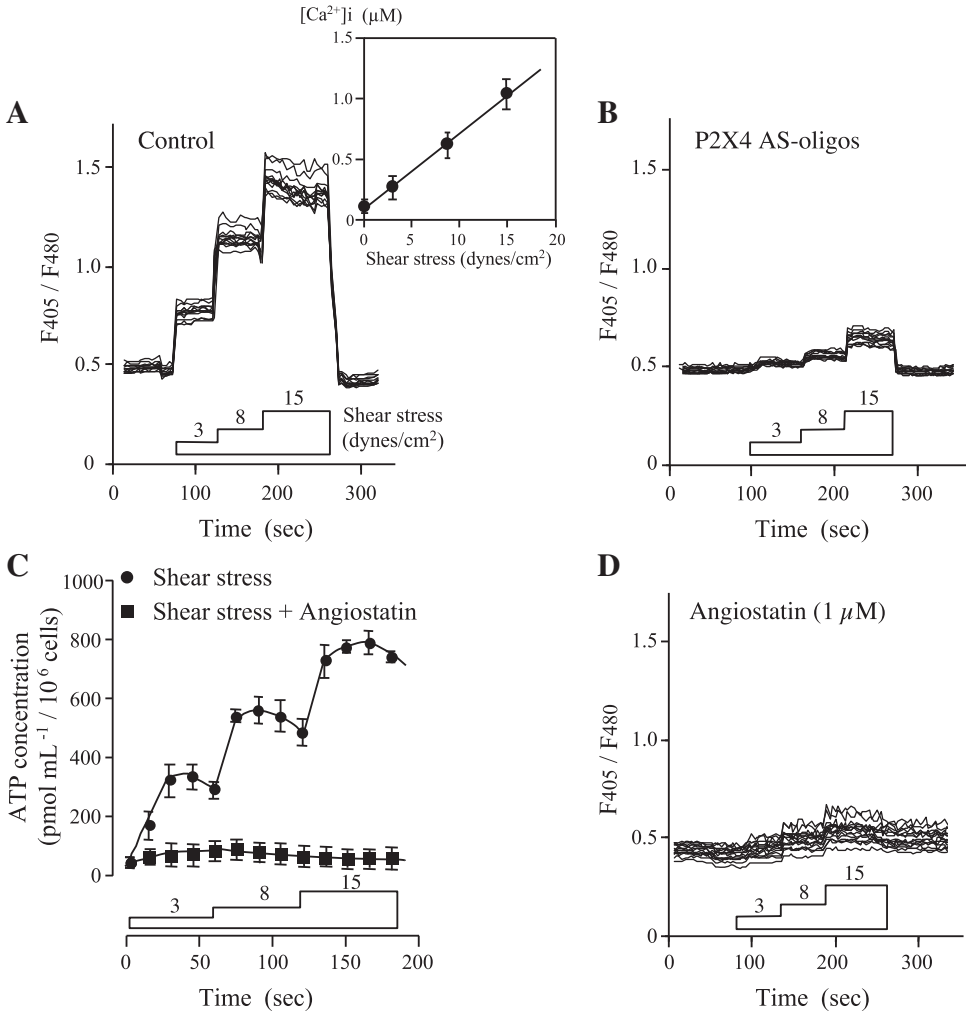

FIG. 8. Ion channel-mediated shear stress signaling. (A) Shear-stress-induced Ca^{2+} response. Intracellular Ca^{2+} concentrations ($[Ca^{2+}]i$) increased in a stepwise manner when cultured human pulmonary artery ECs were exposed to stepwise increases in laminar shear stress. $[Ca^{2+}]i$ is expressed as the ratio of the fluorescence of the Ca^{2+} indicator Indo-1 measured at 405 nm to its fluorescence measured at 480 nm. As shown in the *graph in the inset*, a linear relationship was found between $[Ca^{2+}]i$ and shear stress, indicating that ECs are capable of accurately converting information on shear stress into changes in $[Ca^{2+}]i$. The Ca^{2+} response was attributable to an influx of extracellular Ca^{2+}, because it did not occur in the absence of extracellular Ca^{2+}. **(B)** Involvement of the ATP-operated cation channel P2X4 in the Ca^{2+} influx. Antisense-oligonucleotides (AS-oligos) targeted against P2X4 markedly suppressed the shear-stress-dependent Ca^{2+} responses. **(C)** ATP release in response to shear stress. ECs released ATP in a shear stress-dependent manner, and the ATP-releasing response was completely blocked by angiostatin, a membrane-impermeable ATP synthase inhibitor, suggesting the involvement of cell surface ATP synthase in the shear stress-induced ATP release. **(D)** Involvement of ATP release in shear stress-dependent Ca^{2+} influx. Anigostatin almost completely blocked the Ca^{2+}response to shear stress, suggesting that ECs have shear stress mechanotransduction mechanisms in which shear stress stimulates ECs to release ATP via cell surface ATP synthase, which leads to P2X4 purinoceptor activation followed by a Ca^{2+} influx.

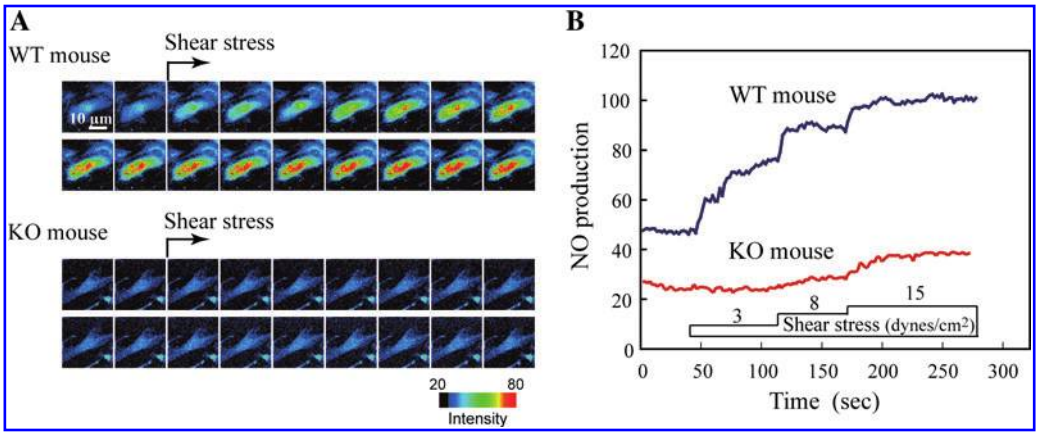

FIG. 9. Impaired NO production in response to laminar shear stress in P2X4 KO mice. (A) Pseudo-color images of DAF-2, a fluorescent NO indicator. Images were taken at intervals of 10 seconds. **(B)** Changes in DAF-2 intensity of 15–20 cells. NO production by the ECs of wild-type (WT) mice increased in a shear stress-dependent manner, whereas the ECs of P2X4 KO mice did not show any evidence of flow-induced NO production. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

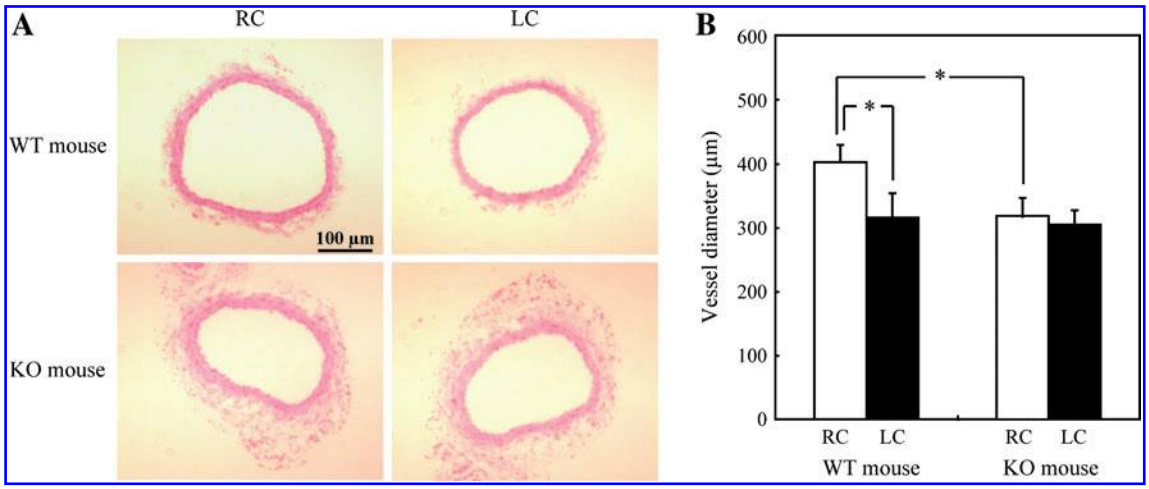

FIG. 10. Impaired flow-induced vascular remodeling in P2X4 KO mice. (A) Hematoxylin-eosin-stained cross sections of the right common carotid artery (RC) and left common carotid artery (LC). The left external carotid artery was ligated for 2 weeks, and at the end of the 2 weeks the RC and LC were perfusion-fixed and analyzed. The diameter of the lumen of the LC in the wild-type (WT) mice became smaller than that of the RC, whereas the diameter of the LC in the P2X4 KO mice remained unchanged. **(B)** Quantitative analysis of changes in lumen diameter. After ligation, the diameter of the LC decreased significantly in the WT mice, but not in the KO mice. The diameter of the RC of the KO mice was significantly smaller than that of the WT mice. Data are expressed as the mean ± SD. $*p < 0.01$. WT mice, $n = 10$; KO mice, $n = 12$. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

laminar shear stress, the intracellular Ca^{2+} concentration increased in a dose-dependent manner, whereas no flow-induced Ca^{2+} responses occurred in the ECs of the KO mice. Since increases in cytoplasmic Ca^{2+} concentration directly lead to the production of NO, we examined the ECs for changes in NO production with a fluorescence indicator, diaminofluorescein (DAF-2). NO production by the ECs of WT mice increased in response to laminar flow, and the response was shear stress-dependent (Figs. 9A and 9B). The ECs of KO mice, however, did not show any evidence of flow-induced production of NO. Because of this impairment of NO production, the vasodilation induced by acute increases in blood flow *in situ* was much weaker in the KO mice, and the KO mice had higher blood pressure than the WT mice.

Chronic changes in blood flow through large arteries induce structural remodeling of the vascular wall: increases in blood flow cause vessel diameter to enlarge, while decreases in blood flow have the opposite effect. When the left external carotid artery was ligated for 2 weeks, thereby decreasing blood flow through the left common carotid artery (LC), the diameter of the LC had significantly decreased at the end of the 2 weeks in the WT mice, but not in the KO mice (Fig. 10). The impaired vascular remodeling in the KO mice was similar to that observed in eNOS KO mice (101). 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.

Concluding Remarks

Biomechanical studies in the past 30 years have revealed the cellular and molecular mechanisms by which ECs sense and respond to fluid mechanical forces, and they have confirmed that fluid mechanical forces actually play important roles in the control of vascular structure and function. However, many topics remain to be investigated in future research, including fluid mechanical force sensors, the roles of fluid mechanical forces in the pathogenesis of vascular disease, such as atherosclerosis, hypertension, and aneurysm, and the relationships between fluid mechanical forces and the effects of physical exercise on human health (68).

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Date of first submission to ARS Central, August 16, 2010; date of acceptance, September 19, 2010.

Abbreviations Used

ACE = angiotensin converting enzyme
ATP = adenosine triphosphate
bFGF = basic fibroblast growth factor
BH4 = tetrahydrobiopterin
ECs = endothelial cells
eNOS = endothelial nitric oxide synthase
EPCs = endothelial progenitor cells
ET-1 = endothelin-1
GM-CSF = granulocyte/macrophage colony stimulating factor
GPCR = G protein-coupling receptor
HB-EGF = heparin binding-epidermal growth factor
HCAECs = human coronary artery endothelial cells
HUVECs = human umbilical vein endothelial cells
IL-1 = interleukin-1
KO mice = knockout mice
MCP-1 = monocyte-chemotactic protein-1
NO = nitric oxide
PDGF = platelet-derived growth factor
ROS = reactive oxygen species
SMC = smooth muscle cell
SOD = super oxide dismutase
TGF-β = transforming growth factor-β
TM = thrombomodulin
tPA = tissue-type plasminogen activator
uPA = urokinase-type plasminogen activator
VCAM-1 = vascular cell adhesion molecule-1
VEGF = vascular endothelial growth factor

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