Flow detection and calcium signalling in vascular endothelial cells

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Received 17 December 2012; revised 13 February 2013; accepted 1 April 2013; online publish-ahead-of-print 9 April 2013

Abstract

Blood vessels alter their morphology and function in response to changes in blood flow, and their responses are based on blood flow detection by the vascular endothelium. Endothelial cells (ECs) covering the inner surface of blood vessels sense shear stress generated by flowing blood and transmit the signal into the interior of the cell, which evokes a cellular response. The EC response to shear stress is closely linked to the regulation of vascular tone, blood coagulation and fibrinolysis, angiogenesis, and vascular remodelling, and it plays an important role in maintaining the homeostasis of the circulatory system. Impairment of the EC response to shear stress leads to the development of vascular diseases such as hypertension, thrombosis, aneurysms, and atherosclerosis. Rapid progress has been made in elucidating shear stress mechanotransduction by using in vitro methods that apply controlled levels of shear stress to cultured ECs in fluid-dynamically designed flow-loading devices. The results have revealed that shear stress is converted into intracellular biochemical signals that are mediated by a variety of membrane molecules and microdomains, including ion channels, receptors, G-proteins, adhesion molecules, the cytoskeleton, caveolae, the glycocalyx, and primary cilia, and that multiple downstream signalling pathways become activated almost simultaneously. Nevertheless, neither the shear-stress-sensing mechanisms nor the sensor molecules that initially sense shear stress are yet known. Their identification would contribute to a better understanding of the pathophysiology of the vascular diseases that occur in a blood flow-dependent manner and to the development of new treatments for them.

Keywords

Endothelial cell • Shear stress • Mechanotransduction • Caveolae • Calcium (cellular)

This article is part of the Spotlight Issue on: Biomechanical Factors in Cardiovascular Disease.

1. Introduction

Blood flow delivers oxygen and nutrients to the tissues and removes waste products. In addition to these roles, blood flow has been shown to play roles in the regulation of circulatory functions via the frictional force that develops between flowing blood and the vascular endothelium. Blood vessels are not simply conduits through which blood passes but are composed of cells that are metabolically active. Endothelial cells (ECs) covering the inner surface of blood vessels are constantly exposed to shear stress, the frictional force generated by flowing blood, and they have the property of sensitively changing their morphology and function in response to changes in shear stress. For example, when shear stress increases, ECs increase the production of vasodilator substances, such as nitric oxide (NO) and prostacyclin, and they increase the level of the expression of the antithrombotic protein thrombomodulin on their cell membranes. The impact of shear stress extends to the gene level, where it induces increases or decreases in the expression of many genes (more than several per cent of all genes) through activation of transcriptional factors or stabilization of mRNA. The EC response to shear stress is essential to maintain the homeostasis of the circulatory system, and impairment of the response leads to the development of vascular diseases, such as hypertension, aneurysms, thrombosis, and atherosclerosis. As a result of the numerous studies that have been conducted thus far, progress has been made in elucidating how ECs sense shear stress and transmit the information to the interior of the cell, and the mechanotransduction of shear stress has been shown to be characterized by almost simultaneous activation of multiple intracellular signalling pathways that are mediated by a variety of molecules and microdomains associated with the cell membrane. However, the mechanism responsible for this characteristic is still not well understood. In this article, we review blood flow detection by the vascular endothelium, with particular focus on shear stress mechanotransduction in ECs.
2. Fluid shear stress on the vascular endothelium

Shear stress arises in ECs when blood flow brushes against the vascular endothelium. The intensity of shear stress ($\tau$) is calculated by using the formula: $\tau = \frac{\mu Q}{\pi r^3}$, where $\mu$ is the blood viscosity, $Q$ the blood flow, $\pi$ the ratio of the circumference of a circle to its diameter, and $r$ the radius of the blood vessel. Under physiological conditions, shear stress in the human aorta is $\approx 10-20$ dynes/cm$^2$, whereas shear stresses of $\approx 1-6$ dynes/cm$^2$ act on the walls of veins. Since blood flow changes in a pulsatile manner as the heart contracts and relaxes, shear stress rises and falls within each heartbeat. Blood flow is laminar in the straight portions of blood vessels, but it becomes turbulent in curved portions and at branch points, where blood flow stagnates, recirculates, and generates whirlpools. Atherosclerotic lesions preferentially occur at places where the shear stress acting on the vascular endothelium is weak and its direction and strength are unsteady. Laminar flow is thought to prevent atherosclerosis, whereas turbulent flow is thought to trigger atherogenesis. In this article, we will confine the discussion to laminar flow.

3. Shear stress mechanotransduction

When shear stress acts on ECs, it is converted to biochemical signals through various membrane-associated molecules and microdomains and transmitted into the interior of the cell. Multiple downstream pathways are involved in shear stress signalling, and they lead to changes in gene expression through the activation of a variety of transcriptional factors, which results in alterations in EC functions. However, the mechanisms and sensors by which ECs initially recognize shear stress have yet to be identified. Here, we will describe the following as candidates for shear stress sensors: (i) ion channels, receptors, adhesion molecules, and the glycocalyx, which are expressed in the cell membrane; (ii) primary cilia and caveolae, as membrane microdomains; and (iii) the cytoskeleton and lipid bilayer membrane, which support cell structures (Figure 1).

3.1 Ion channels

Shear stress is known to activate a variety of ion channels that are expressed on EC plasma membranes. When bovine aortic ECs were grown on the inner surface of glass capillary tubes and whole-cell patch-clamp recordings of single cells were made while the tubes were perfused with medium, flow was found to induce a K$^+$-selective ionic current that increased as a function of shear stress. The shear-stress-induced current represents the increase in the probability of K$^+$ permeable ion channel opening, which leads to the hyperpolarization of EC membranes. Experiments using a voltage-sensitive dye confirmed that EC membrane hyperpolarization occurs in response to shear stress. The cell membrane hyperpolarization is able to function as a driving force for extracellular Ca$^{2+}$ to enter into the cell. When shear stress hyperpolarizes EC membranes, depolarization follows, and Cl$^-$ channels are involved in the depolarization. Shear stress also activates the transient receptor potential (TRP) channels through which Ca$^{2+}$ is able to pass. When the TRPV4 gene was introduced into human embryonic kidney cells, which naturally exhibit no Ca$^{2+}$ influx in response to shear stress, they exhibited a Ca$^{2+}$ response to shear stress. However, it has yet to be determined whether these ion channels open directly in response to shear stress in the same way as the large-conductance mechanosensitive channel directly respond to stretching tension in the cell membrane.

On the other hand, there are ion channels that are indirectly activated by shear stress. P2X4 receptors, a subtype of ATP-operated cation channels, are constitutively expressed on EC plasma membranes. When exposed to shear stress, ECs release endogenous ATP in an intensity-dependent manner, and the ATP released activates P2X4 channels through which extracellular Ca$^{2+}$ enters into the cells. As soon as human pulmonary artery ECs were exposed to shear stress in a flow-loading device, the intracellular Ca$^{2+}$ concentration increased, and the increases correlated almost linearly with shear stress intensity (Figure 2A), suggesting that ECs have the ability to accurately convert information on shear stress intensity into changes in intracellular Ca$^{2+}$ concentration. The shear-stress-dependent increase in Ca$^{2+}$ concentration disappeared after removing extracellular Ca$^{2+}$ with EGTA, indicating that the Ca$^{2+}$ increase was due to a Ca$^{2+}$ influx (Figure 2B). Antisense-oligonucleotides (AS-oligos) designed to knock out P2X4 expression almost abolished the shear-stress-dependent Ca$^{2+}$ influx (Figure 2C). Moreover, angiotensin, an ATP synthase inhibitor that prevents shear-stress-induced ATP release, markedly suppressed the Ca$^{2+}$ response (Figure 2D). These findings indicate that shear stress activates P2X4 channels indirectly via ATP release. Mechanotransduction that involves cellular ATP release and subsequent activation of purinoceptors has been shown to play important physiological roles in a variety of cells besides ECs, including chondrocytes, biliary epithelial cells, gut epithelial cells, renal epithelial cells, and bladder urothelium.

3.2 Cell membrane receptors and G proteins

When ECs are exposed to shear stress, receptor-tyrosine kinases (RTKs), including the vascular endothelial growth factor receptor (VEGFR) and angiopoietin receptor (Tie-2), which are expressed on their cell membranes, become activated, but their activation does not require the presence of their ligands, VEGF, and angiopoietin. When these receptors are phosphorylated, protein kinases, including extracellular signal-regulated kinase (ERK), c-Jun NH2-terminal kinase, PI3-kinase, and protein kinase B (Akt), are activated through the small G-protein Ras, which leads to activation of NO synthase and inhibition of apoptosis.4–11 α,β3 and β1 integrins have been reported to be involved in this ligand-independent activation of RTKs by shear stress.29 Ligand-independent phosphorylation of VEGFR by shear stress has been observed in ES-cell-derived vascular progenitor cells as well as in adult ECs, and it plays an important role in inducing vascular progenitor cells to differentiate into mature ECs.26

GTP binding protein-coupled receptors (GPCRs) have also been shown to be involved in shear stress mechanotransduction. Molecular imaging by fluorescence resonance energy transfer has demonstrated that bradykinin B2 GPCRs change their conformation when bovine aortic ECs are stimulated by shear stress, hypotonic stress, or membrane fluidizing agents.29 In addition, the GTPase activity of G-protein has been shown to increase in a shear-stress-dependent manner when phospholipid vesicles (liposomes) containing purified heterotrimeric G-proteins are exposed to shear stress. This means that the membrane-bound G-protein itself can be activated by shear stress even in the absence of the receptor structure.
3.3 Adhesion molecules

Integrins are transmembrane glycoproteins composed of α and β subunits, and each subunit has an extracellular domain that binds directly to extracellular matrix proteins, such as collagen, fibronectin, vitronectin, and laminin, and the cytoplasmic domains that interact with cytoskeleton via focal adhesion proteins, such as vinculin, talin, and actinin. There is a large volume of evidence for the involvement of integrins in shear stress mechanotransduction. When shear stress acts on ECs, tension develops in the integrins, and the signal is transmitted to the cytoskeleton via focal adhesions. Tyrosine kinases in the focal adhesions, e.g., focal adhesion kinase and c-Src, are rapidly activated by shear stress, leading to ERK activation through multiple kinases, adaptor proteins, and small GTPases. When magnetic beads were bound to integrins on the cell surface and shear stress was directly applied to the integrins by changing the magnetic field, thereby twisting the beads, a reaction occurred in which the cells became hard. The reaction was strongly inhibited by tyrochlasan, nocodazole, and acrylamide, which degrade actin filaments, microtubules, and intermediate filaments, respectively. These findings indicate that the cytoskeleton is involved in the integrin-mediated cell response to shear stress. Integrin signalling has been shown to mediate actin filament reassembly in response to shear stress through the activation of the small G-protein RhoA, and to play a critical role in the shear-stress-induced activation of a transcription factor sterol regulatory element-binding protein that modulates the multiple genes involved in cholesterol and fatty acid biosynthesis and the uptake of low-density lipoproteins. Furthermore, it has been reported that β1 integrin moves to the membrane microdomain caveolae in response to shear stress, and that it causes tyrosine phosphorylation of the caveolar structural protein caveolin-1, which leads to the formation of stress fibres through the activation of Src and myosin light chain kinase.

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is concentrated in the region of cell–cell contact and is thought to establish homophilic interactions between neighbouring cells. When cultured ECs in a confluent monolayer were exposed to shear stress, tyrosine phosphorylation of PECAM-1 rapidly occurred, and then SHP-2 tyrosine phosphatase and a SHP-2 binding protein Gab-1 were recruited to the cell–cell association site and bound to PECAM-1. PECAM-1-SHP-2 binding activates the phosphatase activity of SHP-2, which positively regulates the Ras signalling pathway, leading to ERK activation. Down-regulation of PECAM-1 expression with AS-oligos significantly reduced shear-stress-induced ERK activation. To determine whether PECAM-1 is directly activated by mechanical force, magnetic beads were attached to the external domains of PECAM-1 and placed in a strong magnetic field. PECAM-1 bound to the beads was phosphorylated, but PECAM-1 not bound to beads was not phosphorylated, suggesting that PECAM-1 is capable of responding directly to mechanical force.

Vascular endothelial cell cadherin (VE-cadherin), which is specifically expressed in ECs, is the main protein at adherence junctions, and the cytoplasmic domain of VE-cadherin is connected to the cytoskeleton through β-catenin. Since adherence junctions are subjected to changes in mechanical tension, it seems possible that VE-cadherin may...
serve as mechanotransducer. VE-cadherin has recently been shown to form a mechanosensory complex with PECAM-1 and VEGFR2 in which PECAM-1 directly transmits mechanical force, VE-cadherin functions as an adaptor, and VEGFR2 activates PI3-kinase. Neither ECs lacking VE-cadherin nor ECs lacking PECAM-1 showed shear-stress-induced activation of integrins, PI3-kinase, or Akt or alignment of actin filaments in the direction of flow. These responses to shear stress were recovered in both VE-cadherin-reconstituted ECs and PECAM-1-reconstituted ECs, indicating that VE-cadherin and PECAM-1 are required for the shear-stress-induced activation of the integrin pathway and downstream events.

### 3.4 Glycocalyx

The inner surface of the vascular endothelium is lined with a layer of membrane-bound glycocalyx, which contains glycosaminoglycans (GAGs) including heparan sulfate, chondroitin sulfate, and hyaluronan. The thickness of the glycocalyx layer is estimated to be ranging from

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**Figure 2** Ca\(^{2+}\) signalling of shear stress in ECs. (A) Shear-stress-induced Ca\(^{2+}\) response. Cultured human pulmonary artery ECs loaded with Indo-1/AM were exposed to serial stepped rises in shear stress. Cells were excited with light of 351 nm wavelength, and the emitted light was divided into 480 nm (F480) and 405 nm (F405) by a beam-splitter. The ratio of these two values (F405/F480), which reflects changes in intracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\)). Each graph represents six Ca\(^{2+}\) responses of a group of 8–10 cells. [Ca\(^{2+}\)]\(_i\) increased in a stepwise manner in response to stepwise increases in shear stress, and a linear relationship was found between the Ca\(^{2+}\) concentration and shear stress (inset). (B) Disappearance of Ca\(^{2+}\) response to shear stress in the absence of extracellular Ca\(^{2+}\). When extracellular Ca\(^{2+}\) was removed with EGTA, the shear-stress-induced Ca\(^{2+}\) response completely disappeared, indicating that the Ca\(^{2+}\) response is due to the influx of extracellular Ca\(^{2+}\) across the cell membrane. (C) Involvement of P2X4 channels in the Ca\(^{2+}\) influx. AS-oligos targeted against P2X4 that KO P2X4 expression in HPAECs markedly suppressed the shear-stress-dependent Ca\(^{2+}\) responses. (D) Involvement of ATP release in shear-stress-induced Ca\(^{2+}\) influx. Angiostatin that blocks ATP release significantly suppressed the Ca\(^{2+}\) response to shear stress, suggesting that endogenous ATP released by ECs in response to shear stress activates P2X4 channels, which leads to a Ca\(^{2+}\) influx. (A–C) Modified from Yamamoto et al.\(^{18}\)
0.4 to 4.5 μm in microvessels and large blood vessels. Changes in blood flow affect the conformation of the glycocalyx, and the signal is transmitted to the cytoskeleton through the intracellular domain of the glycocalyx, or transduced into biochemical signals through changes in the local concentration gradients and transport of ions, amino acids, and cell growth factors. GAGs have been extensively studied in regard to their ability to function as a mechanotransducer.

Flow-induced NO production in isolated canine femoral arteries has been shown to significantly decrease to ~20% of the control level as a result of pre-treatments with hyaluronidase, which degrades the hyaluronic acid within the glycocalyx layer. A marked decrease in NO production in bovine aortic ECs in response to shear stress was also demonstrated when they were treated with heparinase, which selectively degrades heparan sulfate. These experimental results suggest that the EC glycocalyx is responsible for the mechanotransduction that mediates shear-stress-induced NO production.

### 3.5 Primary Cilia

Primary cilia are membrane-covered rod-like organelles that protrude from the surface of cells. The core of a cilium consists of nine doublet microtubules, and they are connected to microtubules in the cytoplasm through the intracellular microtubule organizing centre. The presence of primary cilia has been confirmed on the endothelium of the human aorta and umbilical veins, and on ECs obtained from embryos. A great deal of attention has recently been given to primary cilia as a possible shear stress sensor. Expression of the zinc-finger transcriptional factor Kruppel-like factor-2 (KLF-2) has been observed to markedly increase in response to shear stress in ECs that possessed primary cilia, whereas the KLF-2 response did not occur in ECs that never had cilia or had lost their cilia as a result of chemical treatment. However, the cilium itself does not seem to be the sole mechanosensor in ECs, because some ECs that lack primary cilia are still responsive to shear stress. Two mechanisms have been proposed to explain how primary cilia recognize shear stress. One proposed mechanism is that bending of a cilium by shear stress induces cytoskeletal deformation, with the cilium functioning as a lever, and the other is that bending of a cilium activates a cation channel localized in the cilium, which leads to an influx of extracellular Ca$^{2+}$. The cation channel polycystin-2 (PC2) functions in the second proposed mechanism. PC2 belongs to the TRP channel family and is known to require the proper ciliary localization and function of the transmembrane protein polycystin-1 (PC1) to sense and transduce shear-stress-induced phosphorylation of myosin light chain in cultured bovine aortic ECs.

When ECs were treated with a caveolin-1 antibody and no longer capable of forming caveolar structures, the ERK activation in response to shear stress was significantly suppressed. Arterial remodelling during chronic changes in blood flow in caveolin-1 knockout (KO) mice has been observed to be impaired and the arterial diameter narrowing response to a decrease in blood flow did not occur. In addition, flow-mediated dilation in isolated carotid arteries of caveolin-1 KO mice has been shown to be markedly reduced. These impairments in the response to flow were rescued by reconstituting caveolin-1 into the endothelium, suggesting that endothelial caveolin-1 and caveolae are necessary for shear stress mechanotransduction in ECs.

ECs are known to release ATP in response to shear stress. A novel ATP imaging method has recently revealed that highly concentrated ATP release occurs locally in caveola-rich regions of the plasma membrane. The results of that study showed that, upon shear stress stimulation, ATP was immediately released from the entire surface of the cell membranes, and the ATP concentration was, particularly, high in localized regions at the edge of the cell (Figure 3A). The localized ATP release reached a concentration of > 10 μM, which is sufficient to activate nearby purinoceptors, and the sites of localized ATP release coincided with caveolin-1-rich regions (Figure 3B). Ca$^{2+}$ imaging combined with ATP imaging showed that shear stress evoked an increase in intracellular Ca$^{2+}$ concentration, and that the subsequent Ca$^{2+}$ wave originated at the same sites as the localized ATP release and propagated throughout the entire cell. These findings suggest that localized ATP release at caveolae triggers shear-stress-dependent Ca$^{2+}$ signalling in ECs (Figure 3C). Since abundant ecto-enzymes that degrade ATP are present on the surface of ECs, these ectoATPases may modify Ca$^{2+}$ signalling by affecting the local concentration of ATP released by ECs. By releasing NO synthase from caveolae, the Ca$^{2+}$ increase in the vicinity of caveolae leads to activation of NO synthase, which results in the increase in NO production. Although it remains unclear how shear stress induces ATP release at caveolae, several mechanisms that function via vesicular transport of ATP, ATP-permeable channels, or caveolar ATP synthase have been proposed.

### 3.7 Cytoskeleton

Living cells stabilize their structure and shape by means of an interconnected network of cytoskeleton components that includes microfilaments, microtubules, and intermediate filaments. A ‘tensegrity’ cell model has been proposed to explain how mechanical forces are transduced into a biochemical response. The cell model is constructed of 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 signalling molecules that are associated with the cytoskeleton. The tensegrity model has been shown to play important...
roles in shear-stress-induced NO production and endothelin-1 gene expression.67,68

3.8 Lipid bilayer membrane

Plasma membranes are composed of a continuous phospholipid bilayer in which various lipids and proteins are embedded. They have a dynamic, fluid structure, because most of their lipids and proteins are capable of moving rapidly in the plane of the membrane. Plasma membranes are assumed to be in a liquid-crystalline-like state, and their physical properties as liquid crystals change in response to a variety of factors, including changes in lipid composition, density of lipid packing, membrane cholesterol, relative water content, ion concentration, pH, and temperature. Since the physical properties of the plasma membrane affect the conformation and function of membrane-associated molecules, it seems possible that shear stress activates a variety of membrane molecules by changing the physical properties of EC plasma membranes. Actually, measurements of membrane fluidity with the fluorescent dye 4-(dicyanovinyl)julolidine have shown that shear stress increased membrane fluidity in an intensity-dependent manner in human umbilical vein ECs,71 and fluorescence recovery after photobleaching methods have shown that shear stress rapidly also increases membrane fluidity in bovine aortic ECs.72 Changes in membrane fluidity as a result of treating liposomes containing G-proteins with benzyl alcohol or cholesterol have been shown to modulate the activation of G-proteins by shear stress.30 The plasma membrane itself may act as a shear stress sensor.

4. Physiological significance of flow detection by the vascular endothelium

At the end of the nineteenth century, Thoma noted in observations of chick embryos that many branches developed in blood vessels in which blood flow was rapid, but that no branches developed in blood vessels in which blood flow was slow.73 A number of subsequent studies confirmed that the growth of blood vessels is regulated by mechanical factors, such as velocity of blood flow and/or pressure.74 The impact of flow on angiogenesis is not just related to growing or pruning or the maintenance of blood vessels but to adjust the

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**Figure 3** Caveolae as a site of shear-induced ATP release and initiation of Ca$^{2+}$ wave. (A) Visualization of ATP release in response to shear stress. Biotin-luciferase protein prepared by genetic engineering was attached to a biotinylated cell surface with streptavidin. When ATP is released, it triggers luciferin–luciferase reaction, which emits luminescence. The luminescent ATP signals at the cell surface were monitored with a CCD camera and the signal was transformed into pseudo-colour images. As soon as cultured human pulmonary artery ECs were exposed to shear stress (10 dynes/cm$^2$) in a flow-loading device, relatively low concentrations of ATP were released diffusely from the entire surface of the cells, and at the same time highly concentrated ATP release occurred at localized regions at the edge of the cell. The image (shear stress) was obtained from 5 to 10 s after application of shear stress. (B) Comparison between the localized ATP release regions and caveolin-1 distribution. Cells were immunostained with an antibody against caveolin-1, a marker protein for caveolae. Broken lines represent the cell outlines obtained from the differential interference contrast images. Three pairs of images demonstrate that the regions of localized ATP release coincided with the caveolin-1-rich cell edge regions. (C) Imaging of shear-stress-induced ATP release and Ca$^{2+}$ responses. Ca$^{2+}$ imaging with Fluo-4 showed that shear stress evoked an increase in intracellular Ca$^{2+}$ concentrations that started at a single site and propagated throughout the entire cell in the form of a Ca$^{2+}$ wave. The first Ca$^{2+}$ image was obtained 1 s after application of shear stress, and the rest of the images shown were captured at intervals of 140 ms. ATP imaging was performed in the same cells after the Ca$^{2+}$ imaging. The localized ATP release was co-localized with the site of initiation of the Ca$^{2+}$ wave. (A–C) Modified from Yamamoto et al.62
architecture of the blood vessel network to flow conditions in order to keep the cardiovascular system running at low cost in a nevertheless optimized state.8,75 In addition, blood flow has been found to determine vessel size: increases in blood flow have been found to cause the vessel diameter to increase, and decreases in blood flow to have the opposite effect.76 Since these flow–diameter relationships do not occur in vessels whose endothelium has been removed,77 they are assumed to be an adaptive response of ECs that maintains the level of shear stress acting on them constant.78 The above findings suggested the presence of a mechanism by which ECs detect blood flow and regulate vascular growth and remodelling. However, there have been only a few in vivo studies on the physiological significance of blood flow detection by ECs.

The role of shear stress Ca^{2+} signalling in the regulation of circulatory system function was investigated using P2X4 gene KO mice.79 When vascular ECs cultured from P2X4 KO mice were exposed to shear stress, the increase in intracellular Ca^{2+} concentration failed to occur, and the NO production that depends on it also failed to occur (Figure 4A). Bio-microscopic observation of arterioles in the cremaster muscle of mice revealed that a flow-induced vasodilation occurred in WT mice, but that the response was markedly suppressed in P2X4 KO mice. Blood pressure was clearly higher in the P2X4 KO mice than in the WT mice. Moreover, when blood flow was reduced in the common carotid artery for 2 weeks, its diameter significantly decreased in the WT mice, whereas no reduction in arterial diameter occurred in the P2X4 KO mice (Figure 4B). These abnormalities resembled the abnormalities that were seen in NO synthase KO mice.80 Thus, blood flow detection by the vascular endothelium via P2X4-mediated Ca^{2+} signalling appears to play a crucial role in the regulation of blood pressure, the blood flow-dependent vasodilator response, and vascular remodelling through endothelial NO production.

5. Concluding remarks

Over the past two decades, the molecular mechanism by which the vascular endothelium detects changes in blood flow has gradually become clearer as a result of research on the mechanobiology of blood vessels. It
has also been confirmed that when the mechanism does not operate normally, various impairments of blood pressure, blood flow regulation in the tissues, and blood vessel remodelling develop at the whole-body level. However, many aspects of how ECs sense shear stress are still not understood. A unique feature of shear-stress sensing is that it seems to involve many different types of membrane-associated molecules and microdomains, but the machinery responsible for it remains to be elucidated. In vivo, ECs are simultaneously subjected not only to shear stress but to the stretching tension generated by pulsatile changes in blood pressure. Stretching tension is a powerful physical force that deforms several per cent of the ECs, whereas shear stress is a weak force, that is, several orders of magnitude smaller than stretching tension. Thus, a highly sensitive, special sensing mechanism would be necessary to sense shear stress while cells are being greatly deformed by stretching. There are differences between the EC responses to constant laminar flow, as in veins, and to pulsatile laminar flow with high acceleration rates, as in arteries, and there may be sensing mechanisms for acceleration rates. Most data regarding mechanotransduction have been obtained during the first several hours of flow exposure, and different pathways may be involved at later time points. More complete time–response curves and force–response curves for most shear-stress-regulated molecules and mechanisms are missing, and these characteristic lines are needed for running system-biology approaches, otherwise, it will not be possible to interpret the data with respect to the cardiovascular system. Moreover, research on shear stress mechanotransduction to date has focused on laminar flow, and hardly any research has been conducted on the turbulent flow, that is, closely related to atherosclerosis. If these unknown aspects of how ECs sense shear stress are clarified in the future, not only will it be possible to understand the mechanism by which the vascular endothelium detects blood flow, but also it will lead to a better understanding of the roles it plays in blood flow-dependent phenomena, including angiogenesis, vascular remodelling, and atherosclerosis. Furthermore, if measures that are capable of modifying blood flow detection by the vascular endothelium are found, they will contribute to the development of new therapies for vascular diseases, such as hypertension, thrombosis, aneurysms, and atherosclerosis.

Acknowledgements

We wish to acknowledge Dr Akira Kamiya for his invaluable support in our work.

Conflict of interest: none declared.

Funding

This work was partly supported by Grants-in-Aid for Scientific Research (S 21220011 and B223300150) from the Ministry of Education, Culture, Sports, Science and Technology to J.A. and K.Y.

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