Flow-dependent Regulation of Gene Expression in Vascular Endothelial Cells

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SUMMARY

Vascular endothelial cells are constantly exposed to wall shear stress generated by blood flow. Endothelial cells act as mechanoceptors sensing and responding to shear stress, and play a role in flow-dependent phenomena such as angiogenesis, vascular remodeling and atherosclerosis. Numerous recent studies have demonstrated that endothelial cell functions change in response to shear stress, and that the responses are often accompanied by changes in related gene expression. More recently there has been evidence that genes known to be regulated by shear stress may have a common cis-element (shear stress responsive element; SSRE) in their promoter regions. A molecular mechanism for endothelial cell responses to mechanical stress is close to being elucidated. In this paper, shear-stress-mediated regulation of endothelial gene expression is reviewed. (Jpn Heart J 1996; 37: 19–32)

Key words: Shear stress Hemodynamic forces Adhesion molecules

PRESENTLY there is much interest in the functions of vascular endothelial cells lining the inner surface of blood vessels. This is because endothelial cells have been demonstrated to not merely constitute a barrier between the blood and other tissues, but also to serve a variety of important physiological functions. Endothelial cells play a significant role in regulating blood pressure, for instance, by producing vasodilating factors such as prostacyclin, nitric oxide and natriuretic peptide, and vasoconstricting factors such as endothelin, thromboxane A2 and angiotensin converting enzyme. Endothelial cells also regulate the transport of blood-borne substances into the vascular wall and display anti- and procoagulant activity. They also synthesize and secrete many different growth factors and play an important role in angiogenesis and vascular remodeling. In addition, they interact with other cells via the extracellular matrix and adhesion molecules. Their functions are too numerous to list here in their entirety.

Besides their variety of functions, endothelial cells have another characteris-
tic feature, i.e., they are constantly exposed to blood flow. This means that wall shear stress, a mechanical force generated by flowing blood, directly stimulates endothelial cells. Although endothelial cell functions have long been known to be regulated by chemical stimuli such as hormones, autacoids and neurotransmitters, it has only recently been demonstrated that endothelial cell function can be modulated by mechanical forces such as shear stress.1)

Endothelial cells subjected to fluid shear stress undergo shape modification from a polygonal to ellipsoidal shape and become uniformly oriented in the direction of flow.2) This change in shape is associated with the formation and redistribution of actin-containing microfilament bundles, or stress fibers.3) Focal contacts and the associated protein, vinculin, shift to the proximal (relative to flow direction) cell margins.4) In this way, endothelial cells respond to shear stress and alter their morphology and functions dynamically.

Recently, it has become apparent that shear-stress-induced alterations in endothelial cell functions are often accompanied by changes in associated gene expression.5) This suggests the presence of a mechanism by which shear stress modulates endothelial gene expression, resulting in alterations in cell functions. The main objectives of this review are to present data concerning the effect of shear stress on the gene expression of endothelial adhesion molecules, and to document the current state of knowledge concerning the shear-stress-mediated regulation of endothelial genes.

Shear Stress Effects on Leukocyte Adhesion to Endothelium

Blood flow affects leukocyte adhesion to endothelium. Intravital microscopy has shown that the rolling phenomenon of leukocytes occurs preferentially in venules at low flow rates, and that the rate of leukocyte adhesion to endothelium is reduced as the blood flow rate or shear stress is increased.6) In vitro studies have also shown that slight changes in shear stress can cause large changes in leukocyte adhesion to cultured endothelial cell monolayers.7) In these situations the higher flow rate may shorten the time for adhesive interactions, cause high shear forces that remove leukocytes from endothelial cells, or alter cell surface expression of adhesion molecules. In the past, however, there has been little work on the effect of shear stress on endothelial adhesion molecule expression.

Endothelial cells cultured from the venules of murine lymph node were exposed to controlled levels of shear stress in a specially designed flow-loading chamber and examined for changes in adhesiveness to lymphocytes. Fluorescence-labeled murine lymphocytes were incubated for 30 minutes with endothelial cells either exposed to shear stress or maintained in a stationary state, and the number of adherent lymphocytes was measured with a fluorescence spectropho-
tometer. The results showed that the lymphocyte adhesion rate of shear-stressed endothelial cells was significantly lower than that of static control cells. Treatment of static control endothelial cells with monoclonal antibody against intercellular adhesion molecule-1 (ICAM-1) or CD44 caused no changes in lymphocyte adhesion, while vascular cell adhesion molecule-1 (VCAM-1) antibody markedly suppressed adhesion. Treatment of lymphocytes with an antibody to very late activation antigen-4 (VLA-4), a ligand for VCAM-1, decreased the rate of lymphocyte adhesion to static endothelial cells to the same degree as did shear stress. These findings indicate that shear stress makes murine endothelial cells less adhesive to lymphocytes, and that VCAM-1 may be involved.8)

**Shear Stress Modulates Cell Surface Adhesion Molecule Expression**

The effect of shear stress on cell surface expression of adhesion molecules, including VCAM-1, ICAM-1 and CD44, has been investigated.9) Flow cytometric analysis using monoclonal antibodies against these adhesion molecules revealed that murine endothelial cells exposed to a shear stress of 1.5 dynes/cm² for 6 hours showed a marked decrease in cell surface VCAM-1 expression and a slight increase in CD44 (Figure 1). The level of ICAM-1 expression on static control cells was very low and did not change even after exposure to shear stress. The decrease in VCAM-1 expression was time-and shear force-dependent. When endothelial cells subjected to shear stress were returned to a stationary state, the decreased VCAM-1 expression recovered with time, indicating that the shear stress-induced changes in VCAM-1 were reversible. The decrease in cell surface VCAM-1 expression was comparable to the decrease in lymphocyte adhesion. Thus, the decrease in endothelial cell adhesiveness to lymphocytes produced by shear stress was concluded to be due to the decrease in VCAM-1 expression.

**Shear Stress Alters Adhesion Molecule mRNA Levels**

Changes in VCAM-1 mRNA levels were studied by the reverse transcriptase/polymerase chain reaction (RT/PCR) method to examine the mechanism of the shear stress-induced decrease in cell surface VCAM-1 expression.8) Total RNA was extracted from either shear-stressed or static endothelial cells, and reversely transcribed into DNA with Molony murine leukemia virus reverse transcriptase. The cDNA samples were then co-amplified using PCR with sense and antisense primers for VCAM-1, CD44, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Figure 2 shows the electrophoregram of the amplification product on polyacrylamide gel. The level of VCAM-1 mRNA decreased in endothelial cells subjected to shear stress (1.5 dynes/cm²) for 6 hours. In contrast,
Figure 1. Shear stress-induced changes in cell surface VCAM-1 and CD44 expression. Flow cytometric analysis revealed that VCAM-1 expression decreased in murine endothelial cells exposed for 6 hours to shear stress (1.5 dynes/cm²), but that CD44 increased slightly. The figure in each panel represents mean channel fluorescence.

Figure 2. Changes in VCAM-1 and CD44 mRNA levels induced by shear stress. Electrophoresis of the amplification products showed a marked reduction in VCAM-1 mRNA levels and increases in CD44 mRNA levels in murine endothelial cells exposed to shear stress (1.5 dynes/cm²) for 6 hours. The GAPDH mRNA level remained unchanged even after exposure to shear stress.

The CD44 mRNA levels in the shear-stressed endothelial cells increased. GAPDH mRNA levels remained constant even after exposure to shear stress. VCAM-1 mRNA levels began to decrease one hour after the start of shear stress, and further diminished with time. The time course of shear stress-induced mRNA
levels closely paralleled cell surface protein levels.

Changes in the mass transport of chemical mediators in perfusates need to be considered with regards to the effect of flow on cells in addition to the action of shear stress. The diffusional accumulation of chemical mediators on the endothelial cell surface is dependent on flow rate and shear rate. To identify the relative importance of each factor, two perfusates with different viscosities were applied to endothelial cells and the VCAM-1 mRNA levels measured. The results showed that an increase in shear rate induced a corresponding decrease in VCAM-1 mRNA levels, although this occurred to a greater extent with higher viscosity or higher shear stress. This means that the flow-induced decrease in VCAM-1 mRNA levels was shear stress- rather than shear rate-dependent.

The chromosomal VCAM-1 gene was cloned from murine endothelial cells. A reporter gene containing the murine VCAM-1 promoter sequences (1.7-kb upstream of the transcription initiation site) linked to the luciferase gene was constructed and transfected into murine endothelial cells. Murine endothelial cells transfected with the VCAM-1-luciferase reporter gene showed significantly lower promoter activity in response to shear stress. This finding indicates that the down-regulation of the VCAM-1 gene is transcriptionally mediated.

**A Cascade of Endothelial Response to Shear Stress**

A hypothetical cascade of endothelial cell response to shear stress can be constructed based on the example described above (Figure 3). When stimulated with shear stress, endothelial cells perceive the stress and transmit the signal into the cell interior, resulting in the activation of a certain transcription factor (shear stress-related transcription factor; SSTF). SSTF enters the cell nucleus and binds to a cis-element, shear stress responsive element (SSRE), in the promoter of the VCAM-1 gene, and suppresses its transcriptional activity and decreases the mRNA levels. The synthesis and cell surface expression of VCAM-1 protein decreased as a result. Finally, adhesion of the lymphocytes to endothelial cells via the binding of VLA-4 and VCAM-1 is suppressed.

Unlike the VCAM-1 gene, the CD44 gene was upregulated by shear stress. Thus, shear stress upregulates one gene and simultaneously downregulates another. Although the exact reason for this is unclear, different multiple transcription factors may be activated by shear stress. Even if the same transcription factor is activated, the binding site or the location of the enhancer and silencer may vary in individual genes, thereby having different effects on transcriptional activity.
Figure 3. A hypothetical cascade for the endothelial cell adhesion molecule response to shear stress. The endothelial cell recognizes a mechanical force, shear stress, and transmits the signal into the cell interior. The signal activates shear stress-related transcription factor (SSTF), which enters the nucleus and binds to shear stress-responsive element (SSRE). This binding suppresses transcription of the VCAM-1 gene into mRNA, and this is followed by a decrease in the biosynthesis and cell surface expression of VCAM-1 peptide. The result is suppression of lymphocyte adhesion to endothelial cells via VCAM-1/VILA-1 binding. $\mu =$ blood viscosity, $u =$ blood velocity, $r =$ vessel radius.

**Shear Stress-mediated Regulation of Endothelial Gene Expression**

Numerous cases other than that of the adhesion molecules described here have been reported in which shear stress alters endothelial cell function by modulating gene expression. Endothelial genes that respond to shear stress are listed in the Table.

Shear stress stimulates endothelial cells to release nitric oxide, a potent vasodilating factor. Nishida et al\textsuperscript{10} demonstrated that expression of constitutive nitric oxide synthase (NOS) mRNA is markedly increased by 24 hour exposure to shear stress (15 dynes/cm\textsuperscript{2}) in bovine aortic endothelial cells. It is unclear at present whether inducible nitric oxide synthase is influenced by shear stress. In regard to the vasoconstricting factor endothelin (ET), Yoshizumi et al\textsuperscript{11} first reported transient stimulation of ET-1 mRNA and peptide release with a peak time of 2 to 4 hours in porcine endothelial cells exposed to shear stress (5 dynes/cm\textsuperscript{2}). Recently, Morita et al\textsuperscript{12} demonstrated that shear stress (5 dynes/cm\textsuperscript{2}) stimulates F-actin conversion to G-actin within 5 minutes and that stabilization of F-
actin abolishes the shear stress-induced increases in ET mRNA, suggesting that actin depolymerization plays an important role in the induction of ET gene expression. On the other hand, Sharefkin et al.\(^{13}\) observed that the ET-1 mRNA levels of cultured human umbilical vein endothelial cells decreased after 24 hour exposure to shear stress (25 dynes/cm\(^2\)). Malek et al.\(^{14}\) also reported downregulation of ET-1 mRNA by shear stress. The downregulation of ET-1, which began within one hour after the application of shear stress and was complete 2 hours later, was shear force-dependent and saturated at 15 dynes/cm\(^2\). Although the reason for the disparities is not clear, the ET response to shear stress may be bi-phasic, i.e., shear stress may cause a transient elevation of ET mRNA and peptide release, and then induce highly significant inhibition.

Thrombomodulin (TM) is a membrane glycoprotein expressed in vascular endothelial cells which plays a central role in endothelial anti-thrombotic activity by inactivating thrombin. Expression of its gene can be affected by shear stress. We observed that human umbilical vein endothelial cells exposed to shear stress showed an increase in cell surface TM expression.\(^{15}\) TM mRNA levels also increased to around three times the static control level after 8 hour exposure to shear stress (15 dynes/cm\(^2\)). In contrast, Malek et al.\(^{16}\) reported downregulation of TM in response to shear stress (15 or 36 dynes/cm\(^2\)). The exact reason for this discrepancy is not known, but it may be due to the difference in origin of the endothelial cells (human umbilical vein endothelial cells versus bovine aortic endothelial cells) or experimental conditions used (parallel-plate type versus cone-plate type of flow chamber). With regard to the fibrinolytic activity of endothelial

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### Table. Shear Stress-mediated Regulation of Endothelial Genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>mRNA response</th>
<th>Reference no.</th>
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<tbody>
<tr>
<td>PDGF-A</td>
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<td>18, 19</td>
</tr>
<tr>
<td>PDGF-B</td>
<td>↑, ↓</td>
<td>18, 19, 20, 21</td>
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<tr>
<td>bFGF</td>
<td>↑, →</td>
<td>17, 21</td>
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<tr>
<td>HB-EGF</td>
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<td>22</td>
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<td>23</td>
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<tr>
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<td>TM</td>
<td>↑, →</td>
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<tr>
<td>ET-1</td>
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<tr>
<td>NOS</td>
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<td>MCP-1</td>
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<td>VCAM-1</td>
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</tr>
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<td>ICAM-1</td>
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</tr>
<tr>
<td>E-selectin</td>
<td>↓, →</td>
<td>25, 26</td>
</tr>
<tr>
<td>c-fos</td>
<td>↑</td>
<td>28</td>
</tr>
<tr>
<td>c-jun</td>
<td>↑</td>
<td>28</td>
</tr>
<tr>
<td>c-myc</td>
<td>↑</td>
<td>28</td>
</tr>
</tbody>
</table>

↑ = upregulation; ↓ = downregulation; → = no change.
cells, Diamond et al\textsuperscript{17} demonstrated that expression of tissue plasminogen activator (tPA) mRNA was increased about 10-fold by shear stress (25 dynes/cm\textsuperscript{2}) at 24 hours.

Hsieh et al\textsuperscript{18} performed Northern blot analysis to examine changes in platelet-derived growth factor (PDGF) mRNA levels in human umbilical vein endothelial cells exposed to shear stress to study growth factors released by endothelial cells. The mRNA levels of both the PDGF-A and B chains increased, reaching a peak 1.5 to 2 hours after the start of exposure to 16 dynes/cm\textsuperscript{2}. Mitsumata et al\textsuperscript{19} and Resnick et al\textsuperscript{20} also observed increases in PDGF-B chain mRNA expression when bovine aortic endothelial cells were exposed to shear stress (10–30 dynes/cm\textsuperscript{2}). In contrast, Malek et al\textsuperscript{21} observed a decrease in PDGF-B chain mRNA in response to laminar shear stresses of 15 and 36 dynes/cm\textsuperscript{2}. They reported in the same paper that the levels of basic fibroblast growth factor (bFGF) mRNA, which were previously reported to be unaffected by shear stress in human umbilical vein endothelial cells,\textsuperscript{17} increased due to shear stress. Heparin-binding epidermal growth factor (HB-EGF) is a mitogen for fibroblasts and vascular smooth muscle cells, and its potency is comparable to that of PDGF. HB-EGF mRNA levels in human umbilical vein endothelial cells were observed to increase in response to shear stress.\textsuperscript{22} Ohno et al\textsuperscript{23} have shown that application of shear stress (20 dynes/cm\textsuperscript{2}) to bovine aortic endothelial cells increases generation of transforming growth factor-beta-1 (TGF-\(\beta\)1), and that this is associated with a sustained increase in TGF-\(\beta\)1 mRNA levels.

In addition, we\textsuperscript{24} and other groups\textsuperscript{25,26} have observed that shear stress upregulates ICAM-1 gene expression in human umbilical vein endothelial cells. Shyy et al\textsuperscript{27} have shown that monocyte chemotactic protein-1 (MCP-1) mRNA levels in human umbilical vein endothelial cells subjected to a shear stress of 16 dynes/cm\textsuperscript{2} had increased 2- to 3- times by 1.5 hours, decreasing thereafter to basal levels at 4 hours, and declining further to quiescence at 5 hours. So-called “immediate early genes” such as c-fos, c-myc, and c-jun respond to shear stress. Hsieh et al\textsuperscript{28} reported that a shear stress of 16 dynes/cm\textsuperscript{2} induced a transient increase in c-fos mRNA levels in human umbilical vein endothelial cells 0.5 hours after the onset of flow, declining to basal levels within 1 hour. In contrast, both c-jun and c-myc mRNA levels increased slightly and the increases were sustained for at least 2 hours.

**Shear-Stress Responsive Element**

Resnick et al\textsuperscript{20} utilized the PDGF-B chain to investigate the mechanism of shear-stress-mediated gene regulation in cultured bovine aortic endothelial cells. They constructed a series of mutant human PDGF-B promoter-chloramphenicol
acyltransferase (CAT) reporter genes and transfected them into bovine endothelial cells. They identified a cis-acting element between −101 and −152 bp upstream from the translation initiation site of the PDGF-B gene that is necessary for the response to shear stress by analyzing the activity of CAT in shear-stressed endothelial cells. They then carried out a gel-shift assay using synthesized oligonucleotide probes which contain sequences homologous to the cis-acting element, and identified a 12-bp component (CTCTCAGAGACC) that formed complexes with nuclear proteins extracted from shear-stressed endothelial cells. They referred to it as Shear-Stress-Responsive Element (SSRE). Interestingly, the core-binding sequence (GAGACC) was also found to exist in the genes for feline and murine PDGF-B, human, rodent, and murine tPA, human and murine TGF-β1, and human ICAM-1, all of which were responsive to shear stress. Later, it became apparent that other shear stress responsive genes, the genes for NOS, TM, MCP-1, and HB-EGF, also have GAGACC in their promoter regions. This suggests a general mechanism for shear-stress-induced upregulation of gene transcription.

However, it has not been confirmed by luciferase or CAT assay that GAGACC actually works as an SSRE in genes other than the PDGF-B chain gene. Recently, Halnon et al.29) reported that shear stress upregulates the gene expression of PDGF-A chain, which has no GAGACC sequence in its promoter. Shear-stress responsive genes, including VCAM-1 and ET, lack a GAGACC sequence in their promoters. More recently, Ohno et al.23 indicated that the GAGACC sequence in the TGF-β1 promoter has no influence on the induction of the gene expression by shear stress, and that a region between −453 and +11 that does not contain GAGACC is involved. Therefore, multiple SSREs, not only a single one, appear to exist.

**Transcriptional Factors**

Transcriptional factors that bind to the promoters of genes are thought to play an important role in shear stress-mediated regulation of endothelial gene expression. Although no transcriptional factors that bind to PDGF-B SSRE have yet been identified, Lan et al.30) reported that the transcriptional factors nuclear factor kappa B (NFκB) and nuclear factor activator protein-1 (AP-1) are activated by shear stress in endothelial cells. NFκB binding was stimulated within 30 minutes, reaching a peak at 1 hour. AP-1 binding activity was biphasic, rising fourfold within 20 minutes and returning to basal levels before steadily increasing by 2 hours. Since these factors are known as protein kinase C (PKC)-coupled transcriptional factors, the data suggest the involvement of the PKC pathway in some endothelial gene responses to shear stress. The shear stress-induced upregulation
of PDGF-B chain mRNA was inhibited by the PKC inhibitor H7. Induction of HB-EGF gene expression by shear stress was also blocked by pretreating the endothelial cells with 12-O-tetradecanoylphorbol-13-acetate, which activates PKC, indicating that the induction of HB-EGF is mediated by PKC. PKC activation leads to the binding of AP-1 to tumor-promoting agent responsive elements (TRE). TRE have consensus sequences of TGACTCA. Shyy et al. have indicated that the promoter regions of genes known to be regulated by shear stress, including genes for MCP-1, PDGF-A, PDGF-B, tPA, ET, ICAM-1, TGF-β1, and c-fos and c-jun, contain sequences with homology to TRE. Interestingly, c-fos and c-jun are known to form protein dimers that bind to TRE, and the promoter regions of shear stress-inducible genes which include c-myc, interleukin-1, 6 and 8, E-selectin, VCAM-1, G-CSF, and tissue factor genes are also known to contain recognition elements for NFκB. Therefore, the activation of AP-1 or NFκB and the subsequent binding to the recognition elements such as the NFκB TRE site may be one of several mechanisms that mediate endothelial gene responses to shear stress.

**Shear-Stress Signal Transduction**

The fact that endothelial cell functions are regulated by shear stress strongly suggests the presence of a mechanism by which endothelial cells perceive shear stress, a mechanical force produced by blood flow, and transmit the signal into the cell interior. Various second messengers, including Ca++, inositol trisphosphate, diacylglycerol, cAMP, cGMP, phosphatidylinositol-3 kinase, protein kinase C, tyrosine kinase and so on, have been reported to be involved in the endothelial response to shear stress. However, no signal transduction pathway specific for shear stress has been identified. That is mainly because no sensor (receptor) for shear stress has ever been found. Although the existence of a shear stress sensor remains unclear, there are some hypotheses concerning the signal transduction pathway for shear stress: 1) the Ca++ theory, 2) the membrane hyperpolarization theory, and 3) the integrin theory.

Ca++, an important second messenger, enters endothelial cells exposed to shear stress. In the presence of extracellular Ca++, especially at concentrations around 500 nM, it has been shown that intracellular Ca++ concentrations increased shear force-dependently. We confirmed in a flow-loading experiment using two perfusates having different viscosities that the increase in Ca++ is due to mechanical shearing force and not due to the increase in ATP reaching the cell surface as a result of flow-dependent mass transport. An influx of extracellular Ca++, which is not influenced by the membrane potential and has nothing to do with the activation of stretch-activated ion channels, is the major source for the
Ca++ response. The exact nature of the ion channels in endothelial cells through which extracellular Ca++ passes in response to shear stress, however, remains unclear. Recently, Nilius et al\textsuperscript{35} used a patch clamp method and identified a Ca++ permeable channel which was activated by shear stress. On the other hand, while we failed to detect any Ca++ response in cultured endothelial cells exposed to shear stress in the absence of extracellular ATP, Shen et al\textsuperscript{36} and Geiger et al\textsuperscript{37} reported the induction of Ca++ transients by shear stress. The reason for this discrepancy is unclear and the current situation is confusing.

Morita et al\textsuperscript{12} showed that shear stress-induced ET-1 gene expression is completely inhibited by chelation of extracellular Ca++ with EGTA and partially blocked by intracellular Ca++ chelation with bis-(o-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid, tetra (acetoxymethyl)-ester (BAPTA/AM), indicating that Ca++ is involved in the ET-1 gene response to shear stress. They also showed that shear-induced upregulation of HB-EGF mRNA is partially inhibited by chelation of intracellular Ca++ by BAPTA/AM.\textsuperscript{22}

Olesen et al\textsuperscript{38} attached a patch-clamp pipette to bovine aortic endothelial cells cultured on the inner surface of a glass capillary tube and recorded whole-cell currents during perfusion of the glass tube with medium. A K+-selective ionic current was identified in the cells exposed to flow, and its intensity was dependent on the magnitude of the shear stress applied. They postulated that the shear-induced increases in opening frequency of the K+ channel caused hyperpolarization of the endothelial cell membrane, which transmitted the signal into the cell interior. Hyperpolarization may be transmitted electronically through myoendothelial junctions to vascular smooth muscle, or it may increase the driving force which facilitates Ca++ influx. It remains unknown, however, which second messengers actually function following shear-induced hyperpolarization. Recently, an instance in which K+ channels are involved in shear-mediated changes in endothelial gene expression was reported by Ohno et al.\textsuperscript{23} They showed that blockade of endothelial cell K+ channels with tetraethylammonium significantly inhibited the shear stress-induced increase in TGF β1 gene transcription.

Ingber hypothesized that cell-surface integrin, which mediates cell attachment and interlinks extracellular matrix with the intracellular cytoskeleton, acts as a mechanoreceptor and transmits shear stress signals to the cytoskeleton.\textsuperscript{39} Actually, they bound ferromagnetic microbeads to cell surface integrin β1 molecules and twisted the beads, thereby applying a controlled shear stress to integrin via a magnetic field.\textsuperscript{40} Endothelial cytoskeletal stiffness increased in proportion to the stress applied. Combination of a microfilament disrupting agent, cytochalasin D, with nocodazole, which disrupts microtubules and intermediate filaments, completely suppressed the shear-induced cytoskeleton stiffening. Interestingly,
Morita et al\textsuperscript{12)} reported that shear stress induces ET-1 gene expression in endothelial cells and that this induction is mediated by the depolymerization of actin fibers.

**CLOSING REMARKS**

Vascular endothelial cells perceive wall shear stress produced by flowing blood and transmit the signal into the cell interior, which results in the cell response. They extract information on environmental changes from blood flow and play a role in maintaining homeostasis by appropriately and adaptively responding to the changes. Endothelial cell function as a mechanoreceptor has not received much attention until recently. Studies concerning this issue have been performed in rapid succession because methods for culturing vascular endothelial cells and applying controlled levels of shear stress to cells have been established. This subject has also been taken up in new research fields such as bioengineering and biomechanics, which deal with problems concerning flow and deformation and mechanical phenomena in biological systems, respectively.

In this paper we have suggested that dynamic changes in gene expression are involved in the response of endothelial cells to shear stress. The research on the endothelial sensing and responding mechanism to shear stress has just started however, and many questions remain concerning how endothelial cells perceive shear stress, whether a shear-stress sensor actually exists, and if it does, where it is located and what it is. The answers to these questions require a great deal of further experimental work. Such research will not only help us to understand the molecular mechanisms by which endothelial cells respond to the mechanical stimulation shear stress, but will also provide us with knowledge concerning the role of shear stress in blood flow-dependent phenomena such as angiogenesis, vessel remodeling and atherosclerosis. Research on this problem is also expected to lead to the development of new therapy directed towards eliminating the causes of and preventing cardiovascular diseases such as atherosclerosis.

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