Endothelial Communication
State of the Art Lecture

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SUMMARY By virtue of its location at the interface of flowing blood and vascular tissue, the endothelial cell monolayer is in a unique position for interactions with soluble and cellular elements of the blood on one side and with component cells of the vascular tissue on the other. This brief review outlines humoral and contact-mediated endothelial communication with other cells, particularly the resident cells of the vessel wall. Evidence for gap junctional communication channels between endothelium and vascular cells is summarized and discussed in relation to endothelial ion channel activity. Myoendothelial gap junctional communication is proposed as a mechanism involved in vasorelaxation, either independent of or in concert with secreted endothelium-derived relaxing factor(s). (Hypertension 11: 563-572, 1988)

KEY WORDS • ion channels • endothelium • artery • vascular cell interactions • vasorelaxation

By morphological, biochemical, and functional criteria, endothelial cells interact by two general mechanisms with neighboring cells. First, the synthesis and secretion of endothelial products into the extracellular fluid are an effective humoral mechanism for interactions by diffusion and convection to other cells. Second, direct contact between endothelium and other vascular cells occurs in several morphologically defined ways, the most important of which appears to be the communicating gap junction. The types of cells involved in such communication, together with possibilities for their humoral and contact-mediated interactions, are illustrated in Figure 1 and Table 1.

Humoral Mechanisms of Communication

In humoral communication, endothelial cells synthesize and secrete soluble molecules that are carried by diffusion or convective flow (or both) to a target cell. In many cases, there is a specific receptor for the endothelial product on the target cell's surface. Some endothelial proteins (e.g., platelet-derived growth factor [PDGF], interleukins) are secreted into the extracellular medium following a series of well-defined steps regulated at the transcriptional, translational, and posttranslational levels. Other, smaller endothelial products (e.g., prostaglandins, relaxing factors) are synthesized at or near the cell surface and may diffuse out of the cell. The effectiveness of the intercellular signal once secreted into the extracellular space depends on its rate of production, the diffusion coefficient in interstitial fluid, and the distribution of the molecule by convective transport throughout the vessel wall. It may be rendered inactive by an inherent molecular instability or by adsorption, degradation, or antagonism to its activity. Since the endothelium is a polarized cell with distinct differences between apical (luminal) and basal surfaces, there are probably preferred sides for its secretory activity; thus, secretion from the apical surface into the blood will have less effect on vessel wall cells than will secretion into the interstitial fluid from the abluminal side. The reverse is true for endothelial products interacting with platelets and leukocytes.

A summary of documented humoral communication...
FIGURE 1. Humoral (A) and contact-mediated (B) endothelial communication (see Table 1). Mø = monocyte; EC = endothelial cell; SMC = smooth muscle cell; Mac = macrophage; Ly = lymphocyte; Gj = gap junction.

Involving endothelium is included in Table 1. Important endothelial products that influence circulating elements and vascular wall smooth muscle cells include prostaglandins, chemoattractants, and mitogens. Prostacyclin, the most potent antagonist of platelet aggregation yet discovered, also influences vascular tone, stimulating cyclic adenosine 3',5'-monophosphate (AMP) formation in smooth muscle cells and resulting in vasodilation. Endothelium-derived relaxing factors (EDRFs), originally described by Furchgott and Zawadzki, are the subject of intensive study. Nitric oxide has recently been shown to be released from endothelial cells and to mimic the biological activity of EDRF, suggesting that at least one form of EDRF is nitric oxide. It appears likely that other EDRFs will be identified and, as will be discussed, direct gap junctional communication between endothelium and smooth muscle cells is also likely to play a role in vasorelaxation.

In the process of entering the vessel wall, monocytes adhere to the apical surface of the endothelium in response to their recognition of specific binding proteins on the endothelial cell surface. The monocytes subsequently migrate between endothelial cells entering the subendothelial interstitial space as macrophages. While it is likely that they actively communicate with other vascular cells through humoral mechanisms, it is as yet unclear whether they can form contact-mediated communication channels with endothelium or smooth muscle. The release of leukocyte chemoattractants by endothelium, however, is important for their localization by adhesion to the endothelial surface. In addition to the release of monocyte chemoattractants by endothelium, other endothelial products that influence leukocytes and vascular smooth muscle include prostacyclin, the most potent antagonist of platelet aggregation yet discovered, also influences vascular tone, stimulating cyclic adenosine 3',5'-monophosphate (AMP) formation in smooth muscle cells and resulting in vasodilation. Endothelium-derived relaxing factors (EDRFs), originally described by Furchgott and Zawadzki, are the subject of intensive study. Nitric oxide has recently been shown to be released from endothelial cells and to mimic the biological activity of EDRF, suggesting that at least one form of EDRF is nitric oxide. It appears likely that other EDRFs will be identified and, as will be discussed, direct gap junctional communication between endothelium and smooth muscle cells is also likely to play a role in vasorelaxation.

In Table 1, endothelial cell communication is classified as either humoral or contact-mediated. Humoral communication involves the release of substances from endothelial cells that bind to receptors on other cells. Contact-mediated communication involves direct physical contact between cells. The table lists various endothelial cell communication mechanisms and their functions and references.

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EC = endothelial cell; SMC = smooth muscle cell; LDL = low density lipoprotein; EDGF or MDGF = endothelium-derived and macrophage-derived growth factors, respectively; EDRF = endothelium-derived relaxing factors.
thelial cells in vitro,
smooth muscle cells and tissue macrophages can also produce factors that are chemotactic for monocytes. PDGF, a potent mitogen secreted by endothelial cells, is a well-defined molecule shown to be chemotactic for monocytes in vitro. Reciprocally, a variety of agents secreted by monocyte-derived macrophages can in turn influence endothelial metabolism. Recent examples of such interactions include endothelial cell proliferation induced by monocyte-derived growth factors, induction of neovascularization in corneal implants, and the induction of prostacyclin synthesis. Other ultrastructurally defined junctions between endothelium and smooth muscle cells, myoendothelial junctions, have been demonstrated in cultured arterial cells. The importance of communication, structures resembling gap junctions, in the microcirculation. With the use of various tracers, dyes, and electrophysiological techniques, functional communication has been demonstrated in cultured arterial cells. Myoendothelial junctions in large arteries such as the aorta and coronary vessels have largely been overlooked. Frequent fenestrations of the internal elastic lamina allow for the rapid diffusion of hydrophilic molecules in the microcirculation and in larger arteries, leading to the hypothesis that endothelium and smooth muscle cells may act as a coupled system allowing smooth muscle responses to secondary signals generated in the endothelium and efficiently transferred through such junctions.

In addition to evidence supporting the existence of myoendothelial gap junctions in large vessels, the implications of gap junctional communication pathways for the rapid transmission of changes of membrane potential mediated by endothelial ion channel activity will be considered in relation to our recent experiments with whole cell patch clamping of endothelial cells.

**Endothelial Cell–Smooth Muscle Cell Gap Junctional Communication in Vitro**

Following the pioneering studies by Pitts and Simms as applied to endothelial cell cultures, the establishment of gap junctional communication between endothelial cells and smooth muscle cells in vitro using a reversible microcarrier-mediated technique was demonstrated. As outlined in Figure 2, [H]nucleotide readily diffuses through gap junctions between the cells. The technique of microcarrier-mediated attachment allows for the rapid separation of the two cell populations without the use of proteolytic enzymes, thereby conserving surface proteins. To extend this approach to communication in vascular tissue, short-term organ cultures of bovine aorta were prepared. The endothelium was removed by brief collagenase digestion, after which a stream of culture fluid was directed over the luminal surface to remove any residual endothelial cells. Cultured bovine aortic endothelial cells prelabeled with [H]uridine (a nucleoside that is rapidly phosphorylated to a nucleotide in the cytoplasm, where it remains trapped) were then plated onto deendothelialized aortic explant. After overnight incubation, endothelial cells formed a monolayer on the surface of the aortic tissue. Transfer of labeled nucleotide from endothelial cells to a population of intiminal cells was demonstrated by autoradiography (Figure 3). Grains were localized over the body of the intiminal cells with very low background counts in the interstitial space. There was little, if any, further transfer to medial smooth muscle cells. These studies suggest that endothelial cells and intiminal cells (probably smooth muscle in origin) are in metabolic communication through gap junctions in aortic tissue.

**Morphological Evidence for Endothelial Gap Junctional Communication in Human Coronary Artery**

Coronary arterial segments were obtained from the heart of a 14-year-old boy undergoing cardiac trans-
plantation for idiopathic congestive cardiomyopathy. They were dissected from the heart within 10 minutes of removal and fixed in 2.5% glutaraldehyde, 2% paraformaldehyde in cacodylate buffer, pH 7.4. Gross and conventional histological examination of the coronary tree revealed mild diffuse intimal thickening without atherosclerosis. Cells in the subendothelial intimal space were frequently in contact with the abluminal surface of the endothelium, as shown in Figure 4A and B by transmission electron microscopy. In some cases, the cell bodies were closely apposed, whereas in other sections the cell processes formed a “tongue and groove” relationship with the endothelium (see Figure 4B) similar to that described for some forms of myoendothelial junctions. Sequential ultrathin sectioning of regions of cell-to-cell contact combined with manipulation of the viewing angle revealed regions of gap junctional communication between the endothelium and subendothelial intimal cells (Figure 4C), representing communication channels between the cytoplasm of the respective cells. The overall thickness of the double membrane was reduced to 150 Å in this region where ordered domains of connecting channels (connexons) could be seen. The channels were linked to each other in the extracellular space between the membranes, creating a gap of about 30 Å, indi-
provide evidence for gap junctional communication

The intimal cells involved in such endothelial communication in coronary artery were poorly differentiated and have not yet been clearly identified by monoclonal antibody immunocytochemistry. They are probably of smooth muscle origin; intimal smooth muscle cells are often poorly differentiated compared with those of the media.

The three experimental approaches just outlined provide evidence for gap junctional communication between endothelium and intimal cells in larger arterioles, analogous to the well-defined myoendothelial communication exhibited in the microcirculation and in arterioles and arteries of intermediate size. A number of studies have documented the presence of gap junctions between medial smooth muscle cells and endothelium in mouse coronary artery, glomerular arterioles and renal interlobular arteries of rat and mouse, neonatal and young swine pulmonary arteries, and carotid artery of young rabbits. The pattern that emerges from these studies is a progressive decrease with age in the frequency of myoendothelial cell contact as the internal elastic lamina is laid down. Contact becomes limited to fenestrations in the elastic tissue that allow heterocellular processes to meet, creating cytoplasmic bridges including gap junctions. Conversely, as the amounts of elastic tissue decrease in smaller arteries and arterioles, the incidence of myoendothelial contacts increases dramatically, especially in the microcirculation. In mature animals, particularly humans, diffuse intimal thickening is extensively developed in all major arteries. There is some debate as to whether it represents a nonpathological or pathological milieu that precedes focal atherogenesis. Our demonstration that intimal cells of probable smooth muscle origin frequently share regions of membrane contact with the endothelium, including gap junctions, shows that endothelial cells are not restricted to homocellular communication in their monolayer but are also electrically and biochemically linked to other intimal and medial cells.

What is the function of these gap junctions? Since the endothelium is the interface between blood and vascular tissue, (1) endothelial cells may act as a receptor system for subendothelial cells, transferring small second messenger molecules through gap junctions, or (2) myoendothelial junctions may represent an electrically coupled system that influences smooth muscle tone. Our recent studies of chemically activated and flow-activated endothelial ion channel activity, of relevance to this latter possibility, are outlined in the next paragraph.

Acetylcholine-Activated K⁺ Current in Bovine Aortic Endothelial Cells

The presence of endothelial cells is essential for acetylcholine (ACH)-induced relaxation of precontracted blood vessels, and endothelial cells possess cholinergic ACh receptors, as shown functionally and by receptor binding studies. The initial steps in activation of endothelial cells by ACh, however, are not known. Using a whole cell patch clamp technique, we recently described the activation by ACh of a muscarinic K⁺ channel in cultured aortic endothelial cells and suggested that the resulting hyperpolarization of the endothelial cell represents an initial cellular response linked to ACh-induced vasoreactivity mediated by direct junctional conductors to smooth muscle cells. This mechanism, which locally would be highly efficient, may work in conjunction with or independently of EDRF mechanisms.

The average resting membrane potential of endothe-
Endothelial cell-intimal cell gap junctions in human coronary artery. Human coronary artery intima (A–C) is shown in transverse thin section. Regions of contact between the abluminal surface of endothelial cells and poorly differentiated cells of a diffusely thickened intima were common (A, B). The intimal cells, probably of smooth muscle origin, were sometimes observed to be in an invagination of the endothelial abluminal surface (B). Sequential thin sections at various angles through regions of contact revealed structures resembling intercellular gap junctions (C). A classic gap junction in rat liver is shown (D). (A, x 2925; B, x 3450; C, x 24,750; D, x 22,500).

Endothelial cells was measured as $-77 \text{ mV}$. When the cells were hyperpolarized to membrane potentials negative to $-90 \text{ mV}$, as shown in Figure 5, a large inward current was identified (control). This inward rectifier probably sets the resting membrane potential because it is the dominating current in these cells. More important, however, was the finding that administration of ACh to the bath activated a second current that added to the inward rectifier. The current-voltage relationship was obtained by clamping the membrane potentials at different values and recording the current in the whole cell membrane (see Figure 5B). The ACh-induced current, $I_{\text{K,ACh}}$, is the difference between the control current and that in the presence of ACh, the pure ACh-mediated current represented by triangles in Figure 5. The inward current increased in a dose-dependent fashion with ACh; the dose-response curve was approximated by single saturation kinetics with a half-maximal effect at $51 \pm 26 (SD) \text{ nM ACh} (n = 6)$. The current induced by $1 \mu\text{M ACh}$ was fully inhibited.
by 1 μM atropine, indicating that the response is mediated by a muscarinic receptor. Muscarinic receptors are often coupled to cellular responses by guanosine 5'-triphosphate (GTP) binding proteins (G-proteins), which can be irreversibly inhibited by pertussis toxin. Endothelial cell pretreatment with pertussis toxin resulted in no significant change in the ACh-dependent current. Furthermore, dialysis of the cell interior with GTP-free solutions did not reverse the muscarinic current, although such a treatment does so in cardiac atrial cells. Nor did dialysis of the cell interior with 20 μM GTPS (a nonhydrolyzable GTP analogue) for several minutes before the ACh challenge affect the ACh-induced current. Therefore, we have concluded that it seems unlikely that the muscarinic receptor is coupled by G-proteins to the ion channel. With respect to endothelium-smooth muscle interactions, ion channel activity may play a role in cell communication by influencing the membrane potential of underlying smooth muscle cells by open gap junctions. 

In more recent studies, whole cell patch clamp recordings of single arterial endothelial cells exposed to controlled levels of laminar shear stress in capillary flow tubes have revealed a K⁺-selective, shear stress-activated ionic current that is unlike previously described stretch-activated currents. The current (designated $I_{k.s}$) varied in magnitude as a function of shear stress (half-maximal effect at 0.70 dyn/cm²), desensitized slowly, and recovered rapidly and fully on cessation of flow. The effects of $I_{k.s}$ and $I_{k.ACh}$ were additive. $I_{k.s}$ activity represents the earliest and fastest stimulus-response coupling of hemodynamic forces to endothelial cells found to date, and we suggest that its activity, which results in hyperpolarization of the endothelium, is likely to be relevant to the regulation of vascular tone by way of gap junctions or EDRF-related mechanisms (or both). The following list summarizes the ion channel activity reported to date (October 1987) in vascular endothelial cells:

- $I_{k.1}$: Activated primarily by hyperpolarization; inward rectifier; K⁺ selective; single-channel conductance ~ 30 pS; sets the resting membrane potential.
- $I_{k.ACh}$: Muscarinic-gated; inward rectifier; K⁺ selective; dose-dependent activation; half-maximal activity is at 50 nM ACh; no desensitization; independent of GTP binding protein; repolarizes or hyperpolarizes the cell; may be related to vasodilation.
- $I_{sShe}$: Stretch (suction)-activated; cation selective; single-channel conductance ~ 40 pS.
- $I_{k.s}$: Shear stress (flow)-activated; K⁺ selective; shear stress amplitude-dependent; saturates at 15 dyn/cm²; slow desensitization; full recovery in no flow; hemodynamic force regulation of tone.

**Implications of Endothelial Communication for Vessel Wall Biology**

While many of the effects of endothelium-derived mitogens, chemoattractants, and relaxing factors are known, much less is understood concerning the function of endothelial gap junctional communication.
Changes of endothelial and smooth muscle cell ion concentrations are communicated very efficiently by gap junctions, which are very likely to play a role in vasoregulation, perhaps in association with EDRF. Segal and Duling recently provided evidence for ACh-mediated vasodilator activity in arterioles that is not accountable by simple diffusion of a humoral mediator such as EDRF and is independent of blood flow. Furthermore, other circumstantial evidence favors the involvement of gap junctions. There appear to be three plausible interpretations of such rapid bidirectional transmission of ACh-mediated vasodilatation. First, ACh induces local EDRF release resulting in repolarization of the underlying smooth muscle cells lying immediately beneath the stimulation point; gap junctional communication between the smooth muscle cells then allows bidirectional transmission of the effect. Second, gap junctional communication between endothelial cells may allow hyperpolarization in response to ACh to be transmitted throughout the endothelial monolayer. If ionic changes are linked to EDRF release by the endothelium, then electrical transmission (by gap junctions) in the endothelial monolayer may result in release of EDRF at a distance from the original stimulus. Neither mechanism depends on endothelial cell–smooth muscle cell gap junctional communication. A third scenario, however, requires endothelial and smooth muscle cells to be in contact, and ACh-induced ionic changes of the endothelium (hyperpolarization) are then communicated directly to underlying smooth muscle cells; the ionic changes are then transmitted upstream and downstream by low resistance communication channels. The role of endothelial ion channel activity is important in each of these mechanisms, providing an efficient link between ACh receptor affinity and smooth muscle repolarization.

Recently, there has been renewed interest in gap junctions as regulators of cell proliferation and transformation. In large arteries, where intimal hyperplasia is an integral part of atherogenesis, the modification of normal growth regulatory mechanisms between vascular cells is of some importance. Breakdown of gap junctional communication was proposed by Sheridan and Atkinson as a potential mechanism for vascular smooth muscle cell proliferation, but direct experimental evidence is lacking. In the microcirculation, the role of pericytes in the regulation of neovascularization may also require contact between these cells and capillary endothelial cells. Orlidge and D'Amore have demonstrated the inhibition of endothelial growth in culture by the presence of pericytes, which were required to be in direct contact with the endothelial cells, although it is not yet clear whether pericyte–endothelial cell gap junctions are necessary.

On the apical side of the endothelium, an important contact-mediated relationship occurs with circulating leukocytes. Endothelial cell–leukocyte adhesion molecules have been identified on the surface of endothelial cells, and the factors regulating their expression are under intense investigation. The adhesion and emigration of lymphocytes from the blood follow a similar pattern to that of monocytes and are markedly increased during inflammatory episodes. Recent work (E. Guinan, B. Smith, P. Davies, J. Pober, unpublished data, 1987) has demonstrated that bidirectional gap junctional communication between adherent lymphocytes and endothelial cells can occur in vitro; it is not clear, however, whether such mechanisms occur in vivo and whether, once in the vessel wall, lymphocytes and monocyte-derived macrophages can interact by contact-mediated mechanisms with endothelium or with other vascular cells.

The vessel wall, whether it be an artery or of simpler structure in the microcirculation, presents a complex picture of intercellular and intermolecular communications. In addition to the regulation of coagulation and permeability, communication with other cells of the vessel wall is essential for normal physiological function, and its disturbance can initiate pathological changes in blood vessels. Subtle alterations of endothelial function over long periods rather than overt breakdown of endothelial structure are likely to be the prevalent cause of diseases of the large arteries. To investigate mechanisms of endothelial communication, it is often necessary to use in vitro techniques, particularly isolation of pure populations of cells and the reconstitution of vessel components in coculture. Verification of similar mechanisms in vivo is harder to obtain. However, in addition to the use of bioassays, explosive developments in molecular biology and immunocytochemistry are providing the tools to interpret a variety of cellular changes in intact tissue, approaches that can be expected to provide major advances in our understanding of cell communication processes.

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