Intracellular Calcium, Currents, and Stimulus–Response Coupling in Endothelial Cells

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Vascular endothelium appears to be a unique organ. It not only responds to numerous hormonal and chemical signals but also senses changes in physical parameters such as shear stress, producing mediators that modulate the responses of numerous cells, including vascular smooth muscle, platelets, and leukocytes. In many cases, the initial response of endothelial cells to these diverse signals involves elevation of cytosolic Ca\(^{2+}\) and activation of Ca\(^{2+}\)-dependent enzymes, including nitric oxide synthase and phospholipase A\(_2\). Both the release of Ca\(^{2+}\) from intracellular stores, most likely the endoplasmic reticulum, and the influx of Ca\(^{2+}\) from the extracellular space contribute to the [Ca\(^{2+}\)] increase. The most important trigger for Ca\(^{2+}\) release is inositol 1,4,5-trisphosphate, which is generated by the action of phospholipase C, a plasmalemmal enzyme activated in many cases by the receptor–G protein cascade. Ca\(^{2+}\) influx appears to be related to the activity of receptor–G protein–enzyme complex and to the degree of fullness of the endoplasmic reticulum but does not involve voltage-gated Ca\(^{2+}\) channels. The magnitude of the Ca\(^{2+}\) influx depends on the electrochemical gradient, which is modulated by the membrane potential, \(V_m\). Under basal conditions, \(V_m\) is dominated by a large inward rectifier K\(^+\) current. Some stimuli, e.g., acetylcholine, have been shown to hyperpolarize \(V_m\), thus increasing the electrochemical gradient for Ca\(^{2+}\), which appears to be modulated by activation of Ca\(^{2+}\)-dependent K\(^+\) and Cl\(^-\) currents. However, the lack of potent and specific blockers for many of the described or postulated channels (e.g., nonselective cation channel, Ca\(^{2+}\)-activated Cl\(^-\) channel) makes an estimation of their effect on endothelial cell function rather difficult. Possible future directions of research and clinical implications are discussed. (*Hypertension* 1993;21:112–127)

**Key Words** • calcium • endothelium • ion channels

**D** uring the last decade, it has become increasingly clear that the endothelial cells lining blood vessels are more than a barrier between blood and surrounding tissues. In fact, the endothelium is a highly sophisticated, regionally differentiated organ that regulates vascular tone in both conduit and smaller resistance vessels\(^1-3\) and plays a role in hemostasis, cellular proliferation, inflammation, and immunity.\(^4-11\) Endothelial cells communicate humorally with their neighboring smooth muscle cells by synthesis and release of vasoactive compounds such as endothelin,\(^12\) prostaglandins,\(^13\) and endothelium-derived relaxing factor (EDRF),\(^14\) which is composed of nitric oxide\(^15\) and related nitrosothiols.\(^16\) In addition, direct electrotonic interaction between endothelial and vascular smooth muscle cells (VSMCs) has also been proposed.\(^17,18\) The intracellular signal that links systemic or local external stimuli to the synthesis and release of EDRF and prostaglandins appears to be the level of free cytosolic calcium, [Ca\(^{2+}\)]. Furthermore, [Ca\(^{2+}\)], also seems to be involved in the contraction of endothelial cells and the increased permeability of microvessels in response to inflammatory agents (for review, see Reference 19). Thus, a detailed knowledge of intracellular Ca\(^{2+}\) homeostasis is essential for our understanding of the physiology, pathophysiology, and pharmacology of endothelial cells. The following sections review the importance of the endothelium in various disease states and the mechanisms underlying cytoplasmic Ca\(^{2+}\) regulation under basal and stimulated conditions.

**Importance of the Endothelium in Various Disease States**

Increased systemic vascular tone, which is thought to result from enhanced circulating hormones (e.g., noradrenaline, angiotensin II), is among the most common hemodynamic findings in patients with heart failure.\(^20\) However, heightened vasoconstriction correlates poorly with the plasma levels of these substances.\(^20\) The poor correlation could possibly be explained by the involvement of local, endothelium-dependent factors, such as an imbalance of endothelium-derived relaxing and contracting factors.\(^21-23\) This view is supported by numerous studies conducted in isolated vascular segments as well as in intact laboratory animals and human subjects in a variety of disease states, including systemic and coronary atherosclerosis, pulmonary and systemic hypertension, and heart failure (Table 1).
**Isolated Vascular Segments**

In ring preparations of coronary arteries of pigs with hypercholesterolemia and atherosclerosis, endothelium-dependent relaxation in response to bradykinin, serotonin, and ADP was impaired. Similar observations have been made in small coronary resistance arteries of atherosclerotic monkeys, in which endothelium-dependent vasodilation was also found to be depressed. The impairment of EDRF-mediated relaxation (in response to substance P, bradykinin, and calcium ionophore A23187) has been confirmed in isolated human epicardial coronary arteries as well.

**Intact Laboratory Animals and Human Subjects**

In a canine model of pacing-induced heart failure, the acetylcholine-mediated relaxation of the femoral artery was depressed, whereas vasomotor responses to nitroglycerin and norepinephrine were unchanged. As predicted from the experiments on isolated vascular segments, patients with both mild and severe coronary atherosclerosis reacted to acetylcholine, an endothelium-dependent vasodilator, with vasoconstriction or decreased vasodilation, whereas their response to nitroglycerin was normal. Endothelial dysfunction was evident in coronary resistance arteries in patients suffering from angina pectoris who had normal coronary angiograms. The forearm vasculature of patients with essential hypertension also showed reduced response to acetylcholine, whereas the response to nitroprusside was indistinguishable from that of healthy control subjects. Several studies in patients with heart failure caused by ischemic or idiopathic cardiomyopathy report attenuated endothelium-dependent vasodilation for both forearm and coronary blood flow.

The endothelium may also contribute to the increased vasomotor tone associated with certain forms of hypertension. For example, the plasma concentrations of immunoreactive endothelin, the most potent endothelium-derived vasoconstrictor peptide, were elevated in patients with severe systemic essential hypertension. The endothelium-derived contracting factors in the pathophysiology of hypertension.

**Signal Transduction Mechanisms in Endothelial Cells**

The endothelium-dependent relaxation of vascular tone is mediated by many compounds such as vasoactive...
peptides (bradykinin, substance P) and autacoids (acetylcholine, histamine, ATP, serotonin), which depend on receptors on the endothelial cell surface. However, other stimuli, such as shear stress, hypoxia, and calcium ionophores, are receptor independent. The effects of the vasoactive constrictor peptide endothelin have been reviewed recently.33

Some of the compounds that produce vasodilation are also neurotransmitters or cotransmitters. These substances cause vasoconstriction when acting directly on VSMCs, as would be expected after release from perivascular nerves. For example, ATP released from perivascular nerves leads to P₂X-purinoceptor-mediated contraction of vascular smooth muscle, whereas luminal ATP acts on P₂Y-purinoceptors located on endothelial cells, resulting in EDRF-mediated vasodilation.54–55 It is therefore essential to identify the sources of vasoactive substances that induce endothelium-dependent responses by binding to endothelial cell receptors. One readily available and well-known source is cellular blood constituents such as platelets and erythrocytes, which release serotonin, ATP, and ADP.52,55 Another potential source is the endothelium itself, as choline acetyltransferase, substance P, serotonin, vasopressin, angiotensinogen, angiotensin II, histamine, ATP, and endothelin have been localized in endothelial cells from various blood vessels with the use of biochemical techniques and immunocytochemical staining in combination with electron microscopy.54–59 In fact, human umbilical vein and rabbit aortic endothelial cells have been shown to release ATP, substance P, acetylcholine, and bradykinin into the culture medium.60–62 However, there is no conclusive evidence as to the physiological significance of this release mechanism in vivo.

One ubiquitous transduction pathway for receptor-generated signals is through GTP-binding regulatory proteins (G proteins).64–66 although growth factors67–69 and protein kinase C agonists70,71 may act independently of G proteins (Figure 1). The subsequent activation of effectors (e.g., phospholipase C, phospholipase A₂, adenyl cyclase) produces intracellular second messengers such as inositol 1,4,5-trisphosphate (IP₃), diacylglycerol, arachidonic acid, and cyclic AMP72–74 and activation of ion channels,75 resulting in Ca²⁺ influx into the cytoplasm (Figure 1). This chain of events results in an increase in [Ca²⁺], (see below), which activates Ca²⁺-dependent enzymes such as nitric oxide synthase76,77 or phospholipase A₂. Finally, vasoactive compounds such as EDRF, putative endothelium-derived hyperpolarizing factor (EDHF), and prostaglandin I₃ are formed and released.78,79 However, documentation of a particular signal transduction pathway under defined conditions (species, regional vascular origin, conditions for cell isolation, culture, and experiment) is sometimes incomplete. Although a reasonable amount of information is available with respect to muscarinic, histaminergic, and purinergic stimuli, bradykinin, and thrombin (see Table 2), little is known about shear stress, an important physiological stimulus.

Although most of the stimuli listed in Table 2 need G proteins for signal transduction, their role in muscarinic and purinergic transmission has yet to be confirmed. Although a few of these agonists transduce their signal via pertussis toxin-sensitive G proteins, as shown in functional studies for ß₂-adrenergic, serotoninergic, and histaminergic transduction,80,114 functional as well as biochemical experiments have failed to demonstrate an effect of pertussis toxin for the majority of agonists (Table 2). Experiments using AlF₃123 or nonhydrolyzable GTP analogues point to the presence of pertussis toxin-insensitive G proteins in the signal transduction mechanism for bradykinin receptors probably involves pertussis toxin-sensitive as well as pertussis toxin-insensitive G proteins.83,84

Despite the diversity in receptors and G proteins, receptor activation appears to be followed by the increase in [Ca²⁺], and by the release of EDRF. The Ca²⁺ mobilization step can be modulated by both diacylglycerol-activated protein kinase C and cyclic AMP–dependent protein kinase. For example, phorbol myristate acetate has been shown to inhibit bradykinin- and ATP-induced Ca²⁺ release in bovine aortic endothelial cells, whereas cyclic AMP had the opposite effect and cyclic GMP had no effect.85 Although endothelial cells have been shown to release arachidonic acid metabolites (e.g., prostaglandins I₂ and E₂ and thromboxane...

**FIGURE 1.** Schematic shows endothelial cell model of mechanisms involved in signal transduction and ion transport. R, receptor; G, guanine nucleotide binding regulatory protein; PLC, phospholipase C; PLA₂, phospholipase A₂; PG, prostaglandins; IP₃, inositol 1,4,5-trisphosphate; IP₄, inositol 1,3,4,5-tetrakisphosphate; DG, diacylglycerol; PKC, protein kinase C; ER, endoplasmic reticulum; Iₛ, K⁺ inward rectifier; Iₑ[ACh], transient A-type K⁺ current; Iₑ[Ca²⁺], calcium-activated K⁺ current; Iₑ[nonselective cation current]; Iₑ[stretch-activated cation current]; Iₑ[Ca²⁺], stretch-activated K⁺ current.
TABLE 2. Signal Transduction Pathways for Some Endothelium-Dependent Modulators of Vascular Tone

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>G</th>
<th>PTX</th>
<th>Effector enzyme</th>
<th>IP3†</th>
<th>Vasoactive compound</th>
<th>Endothelial cell type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>B2</td>
<td>+</td>
<td>- (+)</td>
<td>PLA₂, PLC</td>
<td>+</td>
<td>EDRF/NO</td>
<td>BPA, BA, PA, PCA</td>
<td>80–82, 83–89</td>
</tr>
<tr>
<td>Thrombin</td>
<td>T-</td>
<td>- (+)</td>
<td>- (+)</td>
<td>PLA₂, PLC</td>
<td>+</td>
<td>EDRF/NO</td>
<td>HUV, PA</td>
<td>90–100</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>M₁, M₂</td>
<td>?</td>
<td>?</td>
<td>PLC</td>
<td>+</td>
<td>EDRF/NO</td>
<td>Rat aorta, BA, BBMV</td>
<td>80, 101–105</td>
</tr>
<tr>
<td>Carbachol</td>
<td>M₁</td>
<td>?</td>
<td>?</td>
<td>PLA₂</td>
<td>-</td>
<td>EDHF?</td>
<td>Rat PA + MA</td>
<td>106–111</td>
</tr>
<tr>
<td>Histamine</td>
<td>H₁</td>
<td>+</td>
<td>+</td>
<td>PLC</td>
<td>+ (−)</td>
<td>EDRF/NO</td>
<td>BA, RA, GPCA</td>
<td>17, 112, 113</td>
</tr>
<tr>
<td>ATP/ADP</td>
<td>P₂</td>
<td>+</td>
<td>−</td>
<td>PLC</td>
<td>+</td>
<td>EDRF/NO</td>
<td>BA, HUV, BBMV</td>
<td>80, 119–122</td>
</tr>
</tbody>
</table>

R, receptor; G, guanine nucleotide binding protein; PTX, pertussis toxin sensitivity; IP3, inositol 1,4,5-trisphosphate; +, yes; −, no; PLC, phospholipase C; EDRF/NO, endothelium-derived relaxing factor/nitric oxide; BPA, bovine pulmonary artery; BA, bovine aorta; PA, porcine aorta; GPCA, guinea pig coronary artery; GPPA, guinea pig pulmonary artery; AC, adenylate cyclase; DBA, dog basilar artery.

A₂, which requires the activation of phospholipase A₂, it is often not clear whether this release of metabolites is solely due to an increase in [Ca²⁺].

Although muscarinic receptor–induced, EDRF-mediated vasodilation and the concomitant hyperpolarization of VSMCs are well known, there has been some confusion until recently with respect to the receptor subtypes and the signal transduction pathways in endothelial cells. Binding and functional data but not autoradiographic findings suggest that endothelial cells possess muscarinic receptors, which are heterogeneous with respect to subtype, vascular region, and species. However, interpretation of these data requires some caution, because muscarinic receptors can also be found on smooth muscle cells and neurons. A recent study comparing cultured aortic endothelial cells and VSMCs showed that cholinergic stimuli enhanced prostacyclin and cyclic GMP production in endothelial cells but not in VSMCs. These effects were mediated by the activation of M₁- and M₃-receptors but not the M₂-receptor subtype, which is in apparent conflict with a radioligand binding study that reported the presence of M₁-receptors only in isolated bovine aortic endothelial cells. These apparent discrepancies could be explained by altered expression of muscarinic receptors in cultured cells. Over time, shear stress also affects the cytoskeleton, 7 which may be due to a flow-activated calcium gradient and various sites, the steps linking shear stress to cellular responses have to be further elucidated.
Agonist-Evoked Ca$^{2+}$ Transient

Our understanding of the basis of Ca$^{2+}$ mobilization in endothelial cells after exposure to extracellular ligands has evolved through the use of different techniques, including intracellular fluorescent Ca$^{2+}$ indicator dyes. Many groups have shown that several ligands, including bradykinin, ATP, histamine, and thrombin, can elevate [Ca$^{2+}$], in endothelial cells in monolayer culture from a basal level of 50–100 to 400–800 nM. The Ca$^{2+}$ spike is reached within 20–30 seconds, followed by a plateau phase that persists for 5–8 minutes (Figure 2). A variety of experiments have shown that the initial component of the Ca$^{2+}$ transient results from release from intracellular stores, whereas the delayed component contributing to the plateau phase is dependent on Ca$^{2+}$ influx from the extracellular space.

In general, agonists that increase [Ca$^{2+}$], in endothelial cells are coupled by receptors and G proteins to phospholipase C. Generation of IP$_3$ is thought to activate IP$_3$ receptors in internal stores, resulting in Ca$^{2+}$ mobilization. The plasmalemmal Ca$^{2+}$ influx pathway is incompletely characterized; however, both Ca$^{2+}$ depletion of intracellular stores and inositol 1,3,4,5-tetrakisphosphate (IP$_4$) have been shown to activate Ca$^{2+}$ influx through the plasmalemma (see below).

Other mediators such as diacylglycerol and Ca$^{2+}$ may act as second messengers in regulating both the Ca$^{2+}$ and subsequent cellular response. In addition, arachidonic acid and sphingosine derivatives, which have been shown recently to mediate Ca$^{2+}$ release and the inhibition of several key enzymes, such as protein kinase C and Ca$^{2+}$/calmodulin-dependent protein kinase, may also be involved; although, in the case of the latter, the mechanism of action is not understood.

Studies in brain cells, hepatocytes, pancreatic acinar cells, and VSMCs provide evidence, with limited confirmation from endothelial cells, that IP$_3$ binds to a receptor on the endoplasmic reticulum (ER) (recently reviewed; see References 148–153). On average, IP$_3$ releases 50% of the Ca$^{2+}$ stored in the ER pool. The remaining Ca$^{2+}$ can be released by calcium ionophores or by agents such as thapsigargin or 2,5-di(tert-butyl)-1,4-benzohydroquinone (BHQ), which inhibit the Ca$^{2+}$-ATPase in the ER. Thus, it seems that these cells contain several distinct Ca$^{2+}$ storage compartments, which may be involved in agonist-induced Ca$^{2+}$ release. In fact, it has been suggested that different Ca$^{2+}$ pools may exist in a number of cells and participate in a coordinated fashion in IP$_3$-mediated Ca$^{2+}$ release and subsequent Ca$^{2+}$-activated Ca$^{2+}$ release, leading to such phenomena as Ca$^{2+}$ waves and oscillations. Although these pools may be con-
tained within the ER, it has been proposed that the IP₂-sensitive Ca²⁺ pool is localized in a unique membrane compartment termed calciosome, which contains the Ca²⁺-binding protein calsequestrin. ¹⁰⁶ The IP₂-binding site, which can be blocked by heparin and other compounds, ¹⁰⁹–¹¹¹ and the Ca²⁺ release channel that it controls are probably separate domains within the same protein.¹⁴⁸,¹₅₃ IP₂-sensitive Ca²⁺ release channels isolated from aortic smooth muscle (not yet confirmed in endothelial cells) have a single-channel conductance of approximately 10 pS and are blocked by cinnarizine and flunarizine but not by the classic calcium channel blockers.¹⁴⁸,¹₅₃ Furthermore, the function of the channel protein can be modulated by phosphorylation (see Reference 153). However, the molecular mechanisms involved in Ca²⁺ release from the ER are still unclear. On the one hand, there is evidence to support the presence of a Ca²⁺ leak channel, an IP₃-gated Ca²⁺ channel, and an ATP-driven Ca²⁺ pump in the ER membrane (Figure 1), which are responsible for basal as well as stimulus-induced Ca²⁺ release and Ca²⁺ reuptake. On the other hand, a Ca²⁺ leak channel is not required to account for the continuous cycling of Ca²⁺ across the ER membrane, because the passive efflux ("leak") of Ca²⁺ from the ER could occur through the same channel that mediates the IP₃-stimulated release.

The influx of extracellular Ca²⁺, which constitutes the delayed component of the agonist-stimulated increase in [Ca²⁺]ᵢ, is still poorly understood with respect to both its pathway and control mechanisms. The influx pathway is probably a receptor-mediated or second-messenger-operated, calcium-permeable, nonselective cation channel.¹⁵²,¹⁵⁷ A recent study has implicated both intracellular Ca²⁺ and IP₃ in controlling the slow influx of extracellular calcium, ¹⁷⁸ which is in contrast to an earlier report¹¹⁰ in which IP₃ and IP₇ did not regulate Ca²⁺ influx. Other studies, however, indicate that the Ca²⁺ content of the ER controls the Ca²⁺ permeability of the plasmalemma.¹⁴⁵,¹⁴⁶,¹⁷³ The data on which this model is based were gathered using at least two different experimental approaches that use the measurement of [Ca²⁺]ᵢ fluxes or [Ca²⁺]ᵢ by means of fluorescent dyes. One approach in endothelial cells uses repeated agonist stimulation of IP₃-sensitive Ca²⁺ release under conditions of normal extracellular Ca²⁺, nominally Ca²⁺-free solutions, or solutions in which Mn²⁺ replaces Ca²⁺.¹⁴⁶,¹⁷₆ The other approach uses compounds such as thapsigargin or BHQ for IP₃-independent emptying of the intracellular Ca²⁺ pool and activation of the Ca²⁺ entry pathway in endothelial cells.¹⁴⁵ As has been pointed out recently,¹⁷⁷,¹⁷₈ Ca²⁺ and Mn²⁺ influx after depletion of intracellular Ca²⁺ stores could also occur through two different pathways that are controlled by different mechanisms. The inability to discern whether cellular responses to external agonists reflect a variety of pathways thus may reflect limitations of the current experimental techniques, including the degree of expression of a particular pathway in a given cell.

Mn²⁺ has been a useful tool in addressing this question, because Mn²⁺ influx is not detectable under basal conditions, and Mn²⁺ influx after exposure to an agonist should therefore reflect influx through the plasmalemma alone. Using histamine as an agonist, Jacob ¹⁴⁶ demonstrated that when internal Ca²⁺ stores were discharged by transiently exposing cells in a Ca²⁺-free solution to histamine and then to extracellular Mn²⁺, Mn²⁺ entry was seen. By exposing the cells to extracellular Ca²⁺, Jacob was able to show that Mn²⁺ influx was dependent on the duration of exposure to extracellular Ca²⁺. This was interpreted to mean that 1) the rate of Mn²⁺ influx depends on the degree of repletion of internal stores by Ca²⁺, and 2) the mechanism for activation of the Ca²⁺ influx in the plasmalemma is IP₃ independent.¹⁴⁶ Similar observations have been made using BHQ or thapsigargin to deplete the intracellular Ca²⁺ stores.¹⁴₃ This mechanism has been postulated by Putney ¹²⁹ to be the capacitively coupled mechanism for Ca²⁺ influx into the cytosol. According to these data, extracellular Ca²⁺ could enter a restricted cytosolic compartment and be taken up into the ER by a Ca²⁺-ATPase. The internal Ca²⁺ store would thereby control the plasmalemmal channel, which would then be progressively closed as the [Ca²⁺]ᵢ in the internal stores increased. A leak pathway between the restricted compartment and the major part of the cytosol would account for the refilling transient seen in many cells. A corollary of this capacitative model is the close apposition of ER and plasmalemma. Although the link between these two structures is not known, one could speculate about a gap junction–like pore for Ca²⁺ transport or other connections involving parts of the cytoskeleton.¹⁷³

Electrophysiological Properties

Exposure to a variety of stimuli has been shown to transiently increase [Ca²⁺]ᵢ in endothelial cells. Within a few seconds of exposure to various ligands, [Ca²⁺]ᵢ increases from basal levels to a peak, which is followed by a maintained plateau phase. The initial peak is unaffected by removal of extracellular Ca²⁺; however, the plateau phase depends on the presence of extracellular Ca²⁺ and, in fact, has been shown to depend on the calcium electrochemical gradient.¹⁸¹–¹₈₅ Although these cells have been demonstrated to be nonexcitable,¹₈₄–¹₉₃ i.e., they do not have voltage-activated Ca²⁺ channels (for the only exception, see References 194 and 195), the dependence of the delayed component of the Ca²⁺ transient on the calcium electrochemical gradient indicates that knowledge of the electrical pathways is essential to our understanding of both the regulation of membrane potential in endothelial cells and calcium homeostasis within these cells (also compare earlier publications: References 178 and 196–199). The different current pathways are summarized in Table 3.

Basal Conditions

Membrane potential. Numerous investigators have measured resting potential in endothelial cells from various preparations using the "tight seal" technique, and a wide range of values has been reported, from approximately –80 up to nearly 0 mV (4.0–5.4 mM [K⁺]),¹₇₂,¹₈₂–¹₈₆,¹₈₈–¹₉₀,²₀₂–²₀₄,²₁₁–²₁₃ When resting potential was measured after a high seal resistance had been obtained, the relatively hyperpolarized values and the response of the resting potential to changes in [K⁺] suggest (Mullins-Noda modified constant field equation) that the membrane is selectively permeable to K⁺ over Na⁺, with a Pₖ/Pₙ of 0.027 (assuming a Na-K pump coupling ratio of 3:2).¹⁸⁵ Accurate measurement of the resting potential of endothelial cells is a prereq-


<table>
<thead>
<tr>
<th>Current</th>
<th>Activation, agonist*</th>
<th>Blockade</th>
<th>Whole-cell current slope conductance</th>
<th>Single-channel conductance‡</th>
<th>Permeability ratio§</th>
<th>Endothelial cell type</th>
<th>Reference</th>
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<tbody>
<tr>
<td>( I_{k1} )</td>
<td>( \text{Ba}^{2+}, \text{Cs}^+ )</td>
<td>~266 pS/pF $</td>
<td>25 pS (150 K(^+))</td>
<td>...</td>
<td>...</td>
<td>BA</td>
<td>186</td>
</tr>
<tr>
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<td>( \text{Ba}^{2+}, \text{Cs}^+ )</td>
<td>~230 pS/pF $</td>
<td>36 pS (140 K(^+))</td>
<td>...</td>
<td>...</td>
<td>BPA</td>
<td>184</td>
</tr>
<tr>
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<td>( \text{Ba}^{2+} )</td>
<td>~104 pS/pF</td>
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<td>...</td>
<td>...</td>
<td>...</td>
<td>BPA</td>
</tr>
<tr>
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<td>( \text{Ba}^{2+}, \text{Cs}^+ )</td>
<td>~160 pS/pF</td>
<td>26 pS (145 K(^+))</td>
<td>...</td>
<td>...</td>
<td>BA</td>
<td>17</td>
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<td>( \text{Cs}^+ )</td>
<td>~150–160 pS/pF$</td>
<td>23 pS (140 K(^+))</td>
<td>...</td>
<td>...</td>
<td>BA, BPA</td>
<td>191</td>
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<td>( \text{Ba}^{2+}, \text{Cs}^+ )</td>
<td>~130–220 pS/pF</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>BPA</td>
<td>192</td>
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<td>35 pS (200 K(^+))</td>
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<td>...</td>
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<td>27 pS (140 K(^+))</td>
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<td>...</td>
<td>HUV</td>
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<td>( \text{Ba}^{2+} )</td>
<td>~250–360 pS/pF</td>
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<td>( \text{VD} )</td>
<td>4-AP</td>
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<td>( I_{k(A)} )</td>
<td>( \text{ACh} )</td>
<td>Atropine</td>
<td>~70 pS/pF</td>
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<td>...</td>
<td>...</td>
<td>BA</td>
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<td>( \text{ACh} )</td>
<td>Atropine</td>
<td>~86 pS/pF</td>
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<td>( \text{VD}, [\text{Ca}^{2+}]_i )</td>
<td>...</td>
<td>...</td>
<td>~150 pS (140 K(^+))</td>
<td>...</td>
<td>...</td>
<td>BA</td>
</tr>
<tr>
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<td>( [\text{Ca}^{2+}]_i \uparrow ) (BK)</td>
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<td>...</td>
<td>~80 pS/pF</td>
<td>...</td>
<td>...</td>
<td>BA</td>
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<td>( I_{k(A)} )</td>
<td>( [\text{Ca}^{2+}]_i \uparrow ) (BK)</td>
<td>...</td>
<td>...</td>
<td>Increase</td>
<td>...</td>
<td>...</td>
<td>BPA</td>
</tr>
<tr>
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<td>( [\text{Ca}^{2+}]_i \uparrow ) (ATP)</td>
<td>...</td>
<td>...</td>
<td>~71 pS/pF</td>
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</tr>
<tr>
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<td>( [\text{Ca}^{2+}]_i \uparrow ) (BK)</td>
<td>...</td>
<td>...</td>
<td>Increase</td>
<td>40 pS (140 K(^+))</td>
<td>...</td>
<td>BA</td>
</tr>
<tr>
<td>( I_{k(A)} )</td>
<td>( [\text{Ca}^{2+}]_i \uparrow ) (ACh)</td>
<td>Heparin</td>
<td>Increase</td>
<td>9 pS</td>
<td>...</td>
<td>...</td>
<td>RA</td>
</tr>
<tr>
<td>( I_{c(a)} )</td>
<td>( \text{V}, [\text{Ca}^{2+}]_i \uparrow ) (A23187)</td>
<td>...</td>
<td>...</td>
<td>360 pS</td>
<td>...</td>
<td>...</td>
<td>PA</td>
</tr>
<tr>
<td>( I_{c} )</td>
<td>( \text{V} )</td>
<td>...</td>
<td>382 pS (140 Cl(^-))</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>BA</td>
</tr>
<tr>
<td>( I_{n} )</td>
<td>( \text{VH} )</td>
<td>...</td>
<td>12 pS (140 K(^+))</td>
<td>... ( \text{K}^+ = \text{Na}^+ )</td>
<td>...</td>
<td>...</td>
<td>BA</td>
</tr>
<tr>
<td>( I_{n} )</td>
<td>( \text{Thrombin, BK} )</td>
<td>...</td>
<td>~50 pS/pF</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>( I_{n} )</td>
<td>( [\text{Ca}^{2+}]_i \uparrow ) (BK)</td>
<td>...</td>
<td>...</td>
<td>Increase</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>( I_{n} )</td>
<td>( [\text{Ca}^{2+}]_i \uparrow ) (ATP, BK)</td>
<td>...</td>
<td>...</td>
<td>~3.2 pA/pF</td>
<td>2.5 pS</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>( I_{n} )</td>
<td>( [\text{Ca}^{2+}]_i \uparrow ) (histamine, A23187)</td>
<td>...</td>
<td>...</td>
<td>Increase</td>
<td>20–25 pS (140 K(^+))</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>( I_{n} )</td>
<td>( \text{VH}, \text{histamine}, \text{thrombin} )</td>
<td>...</td>
<td>...</td>
<td>28 pS (140 K(^+))</td>
<td>K:Na:Ca=1:0.9:0.2</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>( I_{n} )</td>
<td>( \text{VH} )</td>
<td>...</td>
<td>~50 pS (140 K(^+))</td>
<td>K:Na:Ca=1:1:100</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>( I_{n} )</td>
<td>( \text{Histamine} )</td>
<td>...</td>
<td>26.4 pS (140 K(^+))</td>
<td>K:Na:Ca=1:1:15.7</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

| \( I_{k(n)} \) | Shear stress | \( \text{Ba}^{2+}, \text{Cs}^+ \) | 91 pS/pF | ... | ... | ... | BA | 187 |
| \( I_{k(n)} \) | Shear stress | ... | 39 pS | Ca\(^{2+}\):Na\(^+\)=6:1 | ... | ... | ... | PA | 210 |

\( I_{k1} \), inward rectifier; \( \text{Ba}^{2+}, \text{extracellular Ca}^{2+} \); \( \text{Cs}^+, \text{extracellular Cs}^+ \); BA, bovine aorta; BPA, bovine pulmonary artery; HUV, human umbilical vein; \( I_{k(A)} \), transient outward current; \( 4-\text{AP}, 4\)-aminopyridine; \( I_{k(C)} \), acetylcholine-activated K\(^+\) current; ACh, acetylcholine; RA, rabbit aorta; \( I_{k(O)} \), Ca\(^{2+}\)-activated K\(^+\) current; BK, bradykinin; ATP, adenosine triphosphate; \( I_{k(G)} \), Ca\(^{2+}\)-activated Cl\(^-\) current; PA, pig aorta; \( I_{c(a)} \), Cl\(^-\) current; \( I_{c(o)} \), agonist-activated nonselective cation current; IP\(_3\), inositol 1,3,4,5-tetrakisphosphate; RPA, rat pulmonary artery; \( I_{k(g)} \), stretch-activated K\(^+\) current; \( I_{k(n)} \), stretch-activated nonselective cation current.

*Voltage-activated (V) by hyperpolarization (H) or depolarization (D); \( [\text{Ca}^{2+}]_i \uparrow \) (BK), activated by increased cytosolic Ca\(^{2+}\) concentration due to effect of bradykinin.

†Conductance determined in symmetrical K\(^+\) solutions with concentrations of 150 mM.

‡\( I_{k(A)} \), \( I_{k(C)} \), \( I_{k(G)} \), \( I_{k(O)} \), \( I_{k(n)} \) are carried mainly by K\(^+\), as inferred from 1) ion substitution experiments, 2) reversal potential measurements, 3) change in reversal potential per 10-fold change in [K\(^+\)] ratio.

§Estimated from linear parts of current–voltage relation, assuming cell capacitance of 30 pF.

||Nonselectivity based on ion substitution experiments and shifts of reversal potential.

#X\(^+\) stands for Mn\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\).
Figure 3. Tracings show Ca\(^{2+}\) transient and whole-cell currents in a single, voltage-clamped, bovine aortic endothelial cell (H2218R08). Panel A: Time course of fura-2 fluorescence ratio. Fura-2 (pentapotassium salt, 50 \(\mu\)M) was loaded via a patch electrode; intracellular fura-2 was alternately excited at 340 and 380 nm; intensity of emitted light was measured at 510 nm. Horizontal bar denotes exposure of the cell to bradykinin (5 \(\mu\)M). Numbers (1–7) at various points along the Ca\(^{2+}\) transient correspond to times that respective current traces were recorded (depicted in panels B–D). Panels B–D: The cell was voltage clamped at a holding potential of \(-40\) mV and stimulated with ramp pulses (-120 to +60 mV; duration, 160 msec) at 2-second intervals. Before the beginning of each ramp, the cell was clamped to -120 mV for 5 msec to allow for settlement of the capacitance transient (blanked in panels B–D). Holding current is displayed at left of panels B–D. Note the different ordinate in panel C. During initial seconds of [Ca\(^{2+}\)]\(_t\) increase, there is no change in inward current (2) and only a slight increase in outward current (2). This is consistent with Ca\(^{2+}\) released from endoplasmic reticulum. While [Ca\(^{2+}\)]\(_t\) reaches its peak value (3–4–5), outward current increases dramatically, which could be interpreted as activation of a Ca\(^{2+}\)-dependent K\(^+\) current. The concomitant slight increase in inward current could be caused by the inward rectifier or the activation of a Cl\(^-\) current. The small shift of reversal potential to a more positive value, however, suggests that a nonselective cation current is probably activated as well. Note the considerable linearization of the I–V relation. Traces 6 and 7 (panel D) show return of the I–V relation to its normal N-shape, and [Ca\(^{2+}\)]\(_t\) approaches control levels again.


gsite to understanding the basic electrophysiology and roles of various ion channels in the plasmalemma. The wide variability in observed resting potentials may reflect differences among species, level of the vascular tree, and tissue culture conditions. However, accurate measurement of the resting potential can be problematic in small single cells with high input impedance, in which the magnitude of the leakage conductance due to seal resistance becomes critical. The size of the leakage conductance determines the degree of variance between the true and measured resting potential. On the other hand, measurement of resting potential in a confluent monolayer is less dependent on seal resistance. Mehrke et al.\(^{213}\) have demonstrated that the histogram of the resting potentials of bovine aortic endothelial cell monolayers is bimodal, with one peak around \(-25\) mV and another peak around \(-85\) mV. The two peak values and the transition between them can be explained by an N-shaped current–voltage relation of the endothelial cell membrane. In contrast, resting potential measurements in cultured guinea pig coronary endothelial cell monolayers showed a reasonable Gaussian distribution around \(-35\) mV. One interpretation of these differences in resting potential values is that the regional differences in electrophysiological properties exist between endothelial cells at different levels of the vascular tree.

Ionotropic currents. Numerous studies of voltage-clamped bovine aortic and pulmonary artery endothelial cells have shown that the basal current–voltage relation of these cells inwardly rectifies near the resting potential (Figure 3 and Table 3). In the range of potentials from -40 to +50 mV, endothelial cells display very small, time-independent outward currents, whereas hyperpolarization to voltages negative to the reversal potential results in a large inward current (Figure 3). Unitary current recordings obtained using the patch-clamp technique demonstrate a channel with permeation and gating properties comparable to those reported for the inward rectifier in other cell types. Experiments per-
formed by other investigators have shown that the single-channel conductance estimated from inward current was 25–27 pS, and no evidence of current reversal was observed (Table 3). As Campbell et al.\(^{183}\) have pointed out, this time-independent inward rectifying background current is the primary determinant of resting potential in these cells. It has been postulated that endothelial cells are electrically coupled not only to each other but also to VSMCs.\(^{17,18}\) In fact, in terminal arterioles, numerous gap junctions between endothelial cells and the underlying one or two layers of VSMCs have been found in a recent electron microscopic study on primate hearts. Myoendothelial gap junctions were found to be more abundant in coronary resistance vessels than in large coronary arteries.\(^{214–218}\) Thus, the maintenance of a relatively hyperpolarized resting potential has been postulated to be of importance to both endothelial cell function and VSMC relaxation.\(^{11,12}\)

Repeated studies have failed to demonstrate the presence of a steady-state inward current in endothelial cells. However, one investigator\(^{183}\) noted the presence of a fast-rising, rapidly inactivating outward current analogous to the transient outward or A current in approximately one third of the cells analyzed.

**Agonist-Evoked Conductance Changes**

Transmembrane potential measurements after exposure to a variety of agonists (e.g., bradykinin and ATP) have revealed a transient hyperpolarization that parallels the agonist-induced transient rise in cytosolic Ca\(^{2+}\) levels. The magnitude of the hyperpolarization depends on the resting potential recorded in the cell. In cells with low resting potential (−10 to −30 mV), agonists induced a large hyperpolarization, whereas in those with membrane potential more negative than −80 mV, the hyperpolarization was much smaller in amplitude.\(^{182,183,189}\) Although Ca\(^{2+}\) influx occurs during the delayed phase of the Ca\(^{2+}\) transient, the anticipated depolarization does not occur, because the magnitude of the inward current is too small to offset the outward current generated by the Ca\(^{2+}\)-activated K\(^+\) channel or the Ca\(^{2+}\)-activated Cl\(^−\) channel.

The absence of voltage-gated Ca\(^{2+}\) channels in endothelial cells obtained from conduit vessels suggests that exposure to an agonist such as bradykinin activates a receptor-mediated Ca\(^{2+}\) influx pathway,\(^{184}\) which contributes to the Ca\(^{2+}\) transient in these cells (a receptor-operated Ca\(^{2+}\) channel has also been described in other cells; see References 220–222). Johns et al.\(^{184}\) have reported that thrombin and bradykinin rapidly activate a large inward current in bovine pulmonary artery endothelial cells. Ionic substitution experiments suggest that the influx pathway does not discriminate between Na\(^+\) and Ca\(^{2+}\); i.e., the pathway is nonselective. On the other hand, other investigators have failed to demonstrate an inward current during the early stages (<30 seconds) after exposure to bradykinin\(^{192}\) (Figure 3). One can dismiss the explanation that those cells failing to demonstrate this inward current during the early stages were unresponsive to bradykinin, because an increase in outward current resulting from activation of calcium-activated K\(^+\) channels was reported in one such study (see below). One explanation for this apparent discrepancy is that in the experiments reported by Johns et al.\(^{184}\) shear stress was generated by the flow of agonist solution over the cell surface. However, it should also be noted that, although a variety of nonselective cation channels have been described in endothelial cells (Table 3), it is not known if endothelial cells from different species or different levels of the vascular tree have different types (isoforms) of the nonselective channels expressed in their plasmalemma.

In contrast to the controversy over the inward current changes early during exposure to the agonist, we and others\(^{75,190,193,208,223}\) (see Figure 3) have observed a delayed (>30 seconds) increase in inward current that developed after exposure to agonist. Bregestovski et al.\(^{190}\) and, more recently, Nilius and Riemann\(^{193}\) have described an inward, nonselective cation current evoked by histamine in human umbilical vein endothelial cells, which showed a latency of >60 seconds and was mimicked by application of A23187 (also compare Reference 209). The unitary conductance of 20–26 pS and the reversal potential measurements suggest that the channel is nonselective although, when internal K\(^+\) was replaced with Na\(^+\), only inward currents were observed. Histamine in the pipette solution was unable to elicit single-channel activity but clearly induced single-channel activity in cell-attached membrane patches if present in the bath solution. Although both extracellular and cytosolic Ca\(^{2+}\) were necessary for histamine to activate the channel, Bregestovski's experiments\(^{190}\) allow no unequivocal conclusion with respect to the activation mechanism. These unitary currents have also been observed in the absence of agonist, as has a higher conductance, nonselective cation channel.\(^{208}\) A more recent study has demonstrated a lower conductance, Ca\(^{2+}\)-permeable, IP\(_3\)- and Ca\(^{2+}\)-sensitive channel in endothelial cells.\(^{75}\) With the use of Mn\(^{3+}\) as the charge carrier and patch-clamp techniques (cell-attached, inside-out, and outside-out patches), single-channel activity was recorded with a slope conductance of 2.5 pS and a mean open time of 230 msec. IP\(_3\) was ineffective, and inositol 1,3,4,5,6-pentakisphosphate (IP\(_5\)) was much less effective than IP\(_3\) in enhancing channel activity. Finally, IP\(_3\) did not activate the channel at a Ca\(^{2+}\) concentration of 0.1 \(\mu M\), suggesting some cooperativity between Ca\(^{2+}\) and IP\(_3\) in activating the channel. Although this channel shares many of the attributes of the receptor-operated channel predicted from whole-cell recordings, the identification of more than one type of channel that is permeable to Ca\(^{2+}\) in the plasmalemma suggests that additional experiments will be needed to clarify the relative contributions of these pathways to Ca\(^{2+}\) influx in endothelial cells.

Recent studies have provided some insight into the ionic mechanism underlying the transient hyperpolarization of membrane potential that has been observed after exposure to a variety of agonists, such as bradykinin. Voltage-clamp studies have demonstrated that when intracellular Ca\(^{2+}\) concentration is not buffered, exposure to an agonist results in a transient increase in outward current\(^{197}\) (Figure 3).

Single-channel studies in cell-attached and inside-out patches using standard patch-clamp technique have demonstrated a large, calcium-activated K\(^+\) channel (150±10 pS) that is also voltage dependent. The physiological relevance of this channel is uncertain, as it was observed infrequently.\(^{209}\) Another calcium-activated K\(^+\) channel was observed when ATP was applied outside.
the pipette. Since the same channel was activated when the cell surface around the pipette was exposed to A23187, the authors concluded that exposure to ATP activated a Ca^{2+}-dependent channel. The unitary conductance at negative potentials was 40±2 pS ([K⁺], 212 mM), and the conductance became increasingly nonlinear near the zero current potential value. Open probability, which was analyzed in inside-out patches, was found to be negligible (P<0.05) at [Ca^{2+}] of 0.4 μM and increased substantially at [Ca^{2+}] > 1.0 μM. This P₀ versus [Ca^{2+}] relation implies that, during a typical Ca^{2+} transient, the open probability does not attain its maximal value. As can be seen in Figure 3, the increase in outward currents follows the increase in [Ca^{2+}], although the exact relation between I_{cj} or open probability, which was analyzed in inside-out patches, and single-channel currents are still needed to resolve channel types to the calcium-activated potassium current, its activity could be attenuated by a variety of compounds: It is increased by Ca^{2+}, bradykinin, vasoressin, and angiotensin II and decreased by acetylcholine, histamine, norepinephrine, and the cyclic nucleotides cyclic AMP and cyclic GMP.

Although the maintenance of resting [Ca^{2+}] in endothelial cells appears to be little affected by the voltage-dependent Na^{+}-Ca^{2+} exchanger, its activity could be modulated indirectly by compounds that affect the Na⁺,K⁺-ATPase or the Na⁺-K⁺-Cl⁻ cotransporter, thus changing electrochemical gradients. Yet another modulatory role in [Ca^{2+}]: homeostasis could be played by the Na⁺-H⁺ exchanger, because, among other processes, intracellular Ca^{2+} release, Ca^{2+}-ATPases, and Ca^{2+}-binding depend on the pH. Thus, pumps, exchangers, and cotransporters could influence the milieu in which the aforementioned mechanisms of [Ca^{2+}]: homeostasis are active.
Future Directions and Conclusions

As knowledge of endothelial cell function has evolved, our understanding of Ca\(^{2+}\) homeostasis has markedly increased. Ca\(^{2+}\) influx into the cytosol from both the ER and the extracellular space through the plasma membrane contribute to the Ca\(^{2+}\) transient. Although endothelial cells are inexcitable, Ca\(^{2+}\)-permeable channels (Table 3) are responsible for Ca\(^{2+}\) influx, whereas Ca\(^{2+}\)-activated K\(^{+}\) channels and inward rectifier K\(^{+}\) channels are responsible for maintaining the cell at a relatively hyperpolarized potential during the plateau phase of the Ca\(^{2+}\) transient to maximize the calcium electrochemical gradient. The redundancy in ion channel types points to the importance of these pathways in the function of endothelial cells as well as the need for identifying their relative contribution to Ca\(^{2+}\) and K\(^{+}\) fluxes. In addition, there is little information about the blocking action of a variety of compounds on the individual currents. This limits the extrapolation of the contribution of a particular current to the function of the endothelial cell.

Many clinical and laboratory studies suggest the involvement of the endothelium in disease states such as atherosclerosis, hypertension, and heart failure. However, the pathophysiological basis of endothelial cell dysfunction remains to be elucidated. More information is also needed with respect to signal transduction pathways and receptor subtypes mediating the actions of endothelium-dependent vasoactive compounds.

One major problem that has not yet been mentioned is the source or sources of the available evidence. A number of studies have dealt with the remarkable regional heterogeneity of the regulation of vascular tone and have shown differences not only between arterial and venous vasculature but also between large arteries, arterioles, and capillaries. Small arteries, arterioles, and capillaries are generally considered the most important with respect to systemic vascular resistance. However, the majority of endothelial cell data available and presented in this paper has been collected from large arteries of multiple animal species, as well as from human umbilical vein endothelial cells, which are probably not representative of the endothelial cell from the resistance vessels. Therefore, our present ideas should be reevaluated in microvascular endothelial cells.

Finally, the application of molecular biological techniques to study ion channels in endothelial cells will be of interest not only to investigators studying these cells but also to investigators studying ion channels in general, given the plethora of stretch-activated, receptor-operated, and nonselective channels in endothelial cells.

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126  Hypertension  Vol 21, No 1 January 1993


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