Electrical Coupling Between Endothelial Cells and Smooth Muscle Cells in Hamster Feed Arteries

Role in Vasomotor Control

Geoffrey G. Emerson, Steven S. Segal

Abstract—Endothelial cells (ECs) govern smooth muscle cell (SMC) tone via the release of paracrine factors (e.g., NO and metabolites of arachidonic acid). We tested the hypothesis that ECs can promote SMC relaxation or contraction via direct electrical coupling. Vessels (resting diameter, 57 ± 3 μm; length, 4 mm) were isolated, cannulated, and pressurized (75 mm Hg; 37°C). Two microelectrodes were used to simultaneously impale 2 cells (ECs or SMCs) in the vessel wall separated by 500 μm. Impalements of one EC and one SMC (n = 26) displayed equivalent membrane potentials at rest, during spontaneous oscillations, and during hyperpolarization and vasodilation to acetylcholine. Injection of −0.8 nA into an EC caused hyperpolarization (≈5 mV) and relaxation of SMCs (dilation, ≈5 μm) along the vessel segment. In a reciprocal manner, +0.8 nA caused depolarization (≈2 mV) of SMCs with constriction (≈2 μm). Current injection into SMCs while recording from ECs produced similar results. We conclude that ECs and SMCs are electrically coupled to each other in these vessels, such that electrical signals conducted along the endothelium can be directly transmitted to the surrounding smooth muscle to evoke vasomotor responses. (Circ Res. 2000;87:474-479.)

Key Words: endothelium ■ smooth muscle ■ electrical coupling ■ resistance artery ■ conduction

Endothelial cells (ECs) promote smooth muscle relaxation through the release of NO, prostaglandins, and endothelium-derived hyperpolarizing factors (EDHFs). These paracrine factors promote K⁺ efflux from smooth muscle cells (SMCs), with ensuing relaxation. Recent evidence suggests that ECs may also effect SMC relaxation via direct myoendothelial coupling.

Electron microscopic studies demonstrate regions of close apposition between endothelium and smooth muscle. These regions are believed to contain myoendothelial gap junctions that promote the direct transfer of electrical and chemical signals between ECs and SMCs. In previous studies, membrane potential has typically been measured in a single cell, with electrical coupling inferred between respective cell types. A definitive measure of electrical coupling entails simultaneous recording from endothelium and smooth muscle, once demonstrated in strips of coronary artery, albeit in the absence of vasomotor responses. Dual simultaneous recordings have not been obtained from intact vessels that control oxygen delivery to target tissues. Therefore, it is not known whether electrical coupling between ECs and SMCs is of functional significance with respect to blood flow control.

In this study, we tested the hypothesis that ECs and SMCs are electrically coupled in resistance microvessels. Experiments were performed using feed arteries from the hamster retractor muscle, because these vessels are known to control blood flow to downstream tissue and have wall morphology (a single layer of ECs surrounded by a single layer of SMCs) that is conducive to intracellular recording. Electrophysiological responses were induced and recorded simultaneously from defined ECs and SMCs during vasodilation and vasoconstriction. Our data demonstrate that endothelium and smooth muscle are electrically coupled to each other and that such myoendothelial coupling plays a key role in coordinating vasomotor activity.

Materials and Methods

General

Procedures were approved by the Animal Care and Use Committee of The John B. Pierce Laboratory. Male Golden hamsters (n = 28; 80 to 90 g) were anesthetized with sodium pentobarbital (65 mg/kg IP), and feed arteries supplying the retractor muscle were isolated. In a chamber (1 mL) containing physiological saline, the vessel was cannulated and in vivo pressure (75 mm Hg) was restored. Spontaneous tone developed during equilibration for 30 minutes at 37°C. EC and SMC viability was tested with microiontophoresis of norepinephrine (a single layer of ECs surrounded by a single layer of SMCs) that is conducive to intracellular recording. Electrophysiological responses were induced and recorded simultaneously from defined ECs and SMCs during vasodilation and vasoconstriction. Our data demonstrate that endothelium and smooth muscle are electrically coupled to each other and that such myoendothelial coupling plays a key role in coordinating vasomotor activity.

Electrophysiology

For mechanical stability, the vessel was positioned on a Sylgard block submerged at the bottom of the vessel chamber. Two glass microelectrodes containing 1% propidium iodide in 2 mol/L KCl (resistance, 100 to 150 MΩ), each connected to an intracellular...
electrometer, were aligned with the vessel axis at a penetration angle of 60°. In 5 recordings, one microelectrode contained 4% Lucifer yellow in 135 mmol/L LiCl (resistance, 400 to 600 MΩ) to determine whether recordings were affected by the type of intracellular dye used. Both dyes are membrane-impermeant; however, dye can diffuse from an EC to its neighbors through gap junctions.14,24–26 To impale a cell, each microelectrode was advanced slowly into the vessel wall, and the base of the micromanipulator was gently tapped.24 When tip potential rapidly dropped to ~−30 mV24,25 and remained stable for >1 minute, the other microelectrode was advanced similarly into another cell at a separation distance of 500 μm. When both microelectrodes were lodged in cells simultaneously, the vessel was stimulated (see below). At the end of each recording, it was confirmed that tip potential returned rapidly to 0±2 mV on withdrawal from the cell. Vessels were then viewed using fluorescence microscopy to identify the cell type recorded from on the basis of the pattern of dye staining.24

Vessel Stimulation
During dual-cell impalements, current (≤±1.6 nA; 1.5 seconds) was injected into one electrode, or ACh was microiontophoresed onto the distal end of the vessel. The vasomotor response was measured using video calipers positioned at the recording electrode farthest from the stimulus. Electrical and vasomotor responses (resolutions, 1 mV and 1 μm, respectively) were acquired at 400 Hz.

Pharmacology
To inhibit the effects of NO, cytochrome P-450 metabolites, or metabolites of arachidonic acid, vessels were incubated with N-nitro-l-arginine (L-NNA; 100 μmol/L, 30 minutes), 17-octadecynoic acid (ODYA; 10 μmol/L, 30 minutes), or indomethacin (10 μmol/L, 15 minutes).27

Electron Microscopy
Feed arteries were fixed in situ with Karnovsky’s fixative for 10 minutes. The tissue was excised, postfixed for 20 minutes, and stored in 0.1 mol/L sodium cacodylate buffer with 7% sucrose for 4 days. Vessels were osmicated, stained en bloc with 3% uranyl acetate, and embedded in epoxy resin. Tissue was sectioned at ~50 nm thickness, stained with uranyl acetate and lead citrate, and examined on a Zeiss 109T transmission electron microscope.

Data Analysis
Representative tracings were selected to illustrate typical responses. Summary data are presented as mean±SEM. Statistical analyses are given in context.

Results
On pressurization, feed arteries (n=28) initially dilated to their maximal diameter (82±3 μm). Spontaneous tone developed during equilibration (resting diameter, 57±3 μm) and persisted throughout the experiment (3 to 5 hours). All vessels dilated to ACh and constricted to phenylephrine.

Recorded cells were identified by dye (propidium iodide or Lucifer yellow) that diffused from the microelectrode during intracellular recording.24 Because there is typically one SMC layer in these vessels, intracellular microelectrodes readily penetrated to ECs (∼50% of impalements) with no apparent damage to (or labeling of) surrounding SMCs. During EC impalement, multiple ECs (oriented parallel to vessel axis, Figure 1A) were labeled, regardless of the dye used. In contrast, smooth muscle labeling was confined to the impaled cell (oriented perpendicular to the vessel axis, Figure 1B). Moreover, dye did not spread from ECs to SMCs or vice versa. Propidium iodide labeled cell nuclei, whereas Lucifer yellow labeled the entire cell (Figure 1C). Electrophysiological responses were similar whether electrodes contained propidium iodide or Lucifer yellow. Resting membrane potential and electrophysiological responses were unchanged throughout a given impalement (up to 1.5 hours); vasomotor responses were unchanged in the impaled region throughout the experiment (up to 5 hours).

Two to five simultaneous dual-cell recordings were obtained from each vessel. During recordings from one EC and one SMC (n=26), membrane potential in both cells was identical (∼−28±1 mV), and oscillations in membrane poten-
tial were simultaneous and equivalent (Figure 2). Injection of negative (hyperpolarizing) current into the EC resulted in simultaneous hyperpolarization of the SMC, with ensuing vasodilation (lag time between onset of hyperpolarization and onset of vasodilation, 1.7 ± 0.1 seconds). Conversely, injection of negative current into the SMC caused simultaneous hyperpolarization of the EC, with ensuing vasodilation (lag time, 1.6 ± 0.1 seconds). When ACh was microiontophoresed onto the downstream end of the vessel, simultaneous hyperpolarization of both cell types ensued (Figure 2B), and vasodilation conducted along the entire vessel (n = 8). Addition of L-NNA decreased resting diameter (n = 7; 75 ± 4 versus 67 ± 3 μm; P < 0.05, paired t test; reversed with 1 mmol/L l-arginine) but did not alter the vasodilatory response to ACh. Further, indomethacin (n = 4), 17-ODYA (n = 3), or L-NNA and 17-ODYA in combination (n = 3) had no effect on the vasodilatory response to ACh. These findings argue against a role for NO or metabolites of arachidonic acid in mediating responses to ACh. Electron microscopy revealed regions of electron-dense apposition between ECs and SMCs (Figure 2C), indicating the presence of myoendothelial gap junctions.11

To test whether electrical coupling within the endothelium is greater than electrical coupling between ECs and SMCs, current was injected into one cell while the electrical response in the other cell was recorded.20 Each combination of cell-cell coupling was evaluated, as follows: (1) endothelial-endothelial, (2) endothelial-smooth muscle, (3) smooth muscle-endothelial, and (4) smooth muscle-smooth muscle (Figures 3 and 4). For all combinations, injection of −0.8 nA evoked simultaneous hyperpolarization of the second cell and vasodilation that conducted along the entire feed artery. In a complementary manner, injection of +0.8 nA evoked depolarization and constriction that conducted along the vessel. In each case, the electrical response of the noninjected cell was instantaneous in onset and offset for both hyperpolarization and depolarization. The magnitudes of electrical and vaso-motor responses were similar for all cell-cell combinations (Figures 3 and 4), as were the delays between the onset of hyperpolarization and the onset of vasodilation (1.7 ± 0.1 seconds) and between depolarization and vasoconstriction (1.3 ± 0.1 seconds).

In all cell-cell combinations, responses to −0.8 nA were consistently greater than responses to +0.8 nA (Figures 3 and 4). To further explore this relationship, the current intensity was varied between −1.6 and +1.6 nA (Figure 5). Throughout this range, pooled for all cells that compose the conduction pathway, the “transfer resistance” (Figure 4, legend) for positive current was ≈25% of that for negative current (1.3 ± 0.5 versus 6.0 ± 0.5 MΩ, respectively; P < 0.01, paired t

Figure 2. Simultaneous recording of membrane potential (Em) from an EC and an SMC. A, Illustration of an isolated vessel with microelectrodes impaled in an EC and an SMC. A micropipette filled with ACh is positioned at the downstream end of the vessel (with respect to superfusion of physiological saline; Flow) to trigger hyperpolarization and vasodilation, which conduct along the entire vessel. Scale bar = 250 μm. B, Simultaneous recording of diameter, membrane potential of EC, and membrane potential of SMC. During the recording, 3 stimuli were applied, as follows: −0.8 nA was injected into EC (solid bar), −0.8 nA was injected into SMC (open bar), and ACh was delivered by microiontophoresis (↑; 500 nA, 500 ms). Each stimulus evoked dilation along the entire vessel; diameter was recorded at site of SMC impalement. Note simultaneous oscillations in membrane potential (magnified inset). This record is representative of n = 26 dual-cell recordings from ECs and SMCs. Scale bar = 5 seconds. C, Electron micrograph of feed artery wall in cross section. EC (with prominent nucleus, adjacent to the vessel lumen, L) and SMC (underlying the adventitia, A) are separated by internal elastic lamina except in the region of EC:SMC contact, where electron-dense regions characteristic of myoendothelial gap junctions11 are present (arrowhead). Scale bar = 0.2 μm.
Nevertheless, for ECs as well as SMCs, the relationship between electrophysiological and vasomotor responses was approximately linear (\(1 \text{ mV/m} \text{m} \)); Figure 5C). This proportionality between electrical and mechanical responses to current injection is consistent with electromechanical coupling.\(^{25,28}\) For rapid (\(1\text{-Hz}\)) oscillations in membrane potential (eg, Figures 2B and 3D), electrical events were presumably too brief to be accompanied by corresponding mechanical events.

Controls were performed for current injection as follows. (1) Throughout experiments, stimuli were delivered with microelectrodes positioned in the bath to confirm that the bridge of the electrometer was balanced similarly for positive and negative current injections. (2) Current injection into the bath adjacent to the vessel failed to evoke vasomotor or electrophysiological responses (\(n=6\)). (3) Injection of current into a cell failed to evoke a response from a recording electrode positioned in the bath adjacent to the vessel (\(n=7\)). (4) During simultaneous impalements, increasing stimulus duration (eg, to 15 seconds) correspondingly prolonged electrical responses and enhanced changes in vessel diameter (\(n=5\); data not shown).

Discussion

We present the first dual simultaneous electrophysiological recordings obtained concomitant with vasomotor responses. Our findings demonstrate that ECs and SMCs are electrically coupled to each other in resistance microvessels that control blood flow to the hamster retractor muscle. Instantaneous, bidirectional transmission of electrical signals between endothelium and smooth muscle ensures that both cell types consistently display equivalent membrane potential. Thus, myoendothelial coupling enables electrical responses originating in either the endothelium or the smooth muscle to evoke robust vasomotor responses along the entire vessel segment.

Endothelium-dependent agonists (eg, ACh) can hyperpolarize and relax SMCs by a mechanism independent of NO, EDHFs, and prostaglandins.\(^{15,16,29}\) Conversely, SMC-specific agonists (eg, phenylephrine) can alter EC calcium\(^{13}\) and membrane potential.\(^{17,18,28,30}\) Electron microscopy has revealed electron-dense regions of membrane apposition between SMCs and ECs in conduit vessels,\(^{8,9}\) resistance arteries,\(^{11}\) and arterioles.\(^{6,7}\) Such regions of membranous contact (eg, Figure 2C) are believed to contain gap junctions.

Whereas putative gap junction antagonists can inhibit responses that depend on cell coupling,\(^{15-19}\) there is a paucity of direct evidence\(^{21}\) that electrical signals spread freely between ECs and SMCs. Moreover, the role of myoendothelial elec-
Myoendothelial coupling promotes synchronization of cells in the vessel wall. In arterioles in vivo, myoendothelial coupling ensures that SMCs are electrically coupled to each other (albeit indirectly) via the endothelium and vasoconstriction, respectively, through electromechanical coupling in governing vascular resistance has not been defined.

Hyperpolarization and depolarization evoke vasodilation and vasoconstriction, respectively, through electromechanical coupling (Figure 5C). This behavior is consistent with voltage-operated calcium channels in SMCs. However, the present findings demonstrate that injection of current into an EC evokes vasodilation or vasoconstriction (according to polarity) and that EC membrane potential itself is a powerful determinant of electromechanical coupling within the smooth muscle layer (Figure 5C). Both electrical and mechanical responses to negative current were consistently larger than those to equivalent positive current (Figures 3 and 4), and depolarizations were frequently followed by a slight, transient hyperpolarization (eg, Figure 3). These features of the response to positive current are consistent with the presence of voltage-activated potassium channels.

Myoendothelial coupling promotes synchronization of cells in the vessel wall. In arterioles and feed arteries, cells are organized such that each EC spans ~20 SMCs and each SMC spans ~20 ECs. This wall morphology promotes heterologous cell-to-cell contact. Further, homologous coupling within the endothelium promotes electrical conduction along the vessel axis. The integrity of the EC layer is required for conducting hyperpolarization and vasodilation along hamster feed arteries, indicating relatively poor coupling between SMCs. Here we demonstrate in the same vessels that myoendothelial coupling is of similar electrical resistance to that within the endothelium (Figure 4). In turn, myoendothelial coupling ensures that SMCs are electrically coupled to each other (albeit indirectly) via the endothelium (Figures 3 and 4).

The importance of NO, EDHFs, and prostaglandins during endothelium-dependent vasodilation is well demonstrated. Although we cannot rule out a contribution of these paracrine agents to current-induced vasodilation, their involvement is unlikely given the instantaneous and bidirectional nature of electrical transmission and the failure of established pharmacological interventions to block responses to ACh.

In summary, we present the first simultaneous measurements of EC membrane potential, SMC membrane potential, and diameter responses. Our data establish that electrical signals travel freely and bidirectionally between ECs and SMCs to evoke robust vasomotor responses. We conclude that myoendothelial coupling can serve as a rapid, integral component of blood flow control.

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References
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