Electrical activation of endothelium evokes vasodilation and hyperpolarization along hamster feed arteries

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Electrical activation of endothelium evokes vasodilation and hyperpolarization along hamster feed arteries. Am J Physiol Heart Circ Physiol 280: H160–H167, 2001.—Endothelial cells are considered electrically unexcitable. However, endothelium-dependent vasodilators (e.g., acetylcholine) often evoke hyperpolarization. We hypothesized that electrical stimulation of endothelial cells could evoke hyperpolarization and vasodilation. Feed artery segments (resting diameter: 63 ± 1 μm; length 3–4 mm) of the hamster retractor muscle were isolated and pressurized to 75 mmHg, and focal stimulation was performed via microelectrodes positioned across one end of the vessel. Stimulation at 16 Hz (30–50 V, 1-ms pulses, 5 s) evoked constriction (−20 ± 2 μm) that spread along the entire vessel via perivascular sympathetic nerves, as shown by inhibition with tetrodotoxin, ω-conotoxin, or phenolamine. In contrast, stimulation with direct current (30 V, 5 s) evoked vasodilation (16 ± 2 μm) and hyperpolarization (11 ± 1 mV) of endothelial and smooth muscle cells that conducted along the entire vessel. Conducted responses were insensitive to preceding treatments, atropine, or Nω-nitro-l-arginine, yet were abolished by endothelial cell damage (with air). Injection of negative current (≤1.6 nA) into a single endothelial cell reproduced vasodilator responses along the entire vessel. We conclude that, independent of ligand-receptor interactions, endothelial cell hyperpolarization evokes vasodilation that is readily conducted along the vessel wall. Moreover, electrical events originating within a single endothelial cell can drive the relaxation of smooth muscle cells throughout the entire vessel.

THE FUNCTIONAL ELEMENTS of resistance arteries include perivascular nerves, smooth muscle cells, and endothelial cells. Perivascular nerves and smooth muscle cells are electrically excitable, such that depolarization promotes calcium entry into the cytoplasm, leading to neurotransmitter release and vasoconstriction, respectively. Endothelial cells are generally thought to be electrically unexcitable because they typically do not exhibit voltage (i.e., depolarization)-activated currents (6, 15), though exceptions have been reported (2, 3). Nevertheless, endothelial cells exhibit voltage (i.e., depolarization)-activated currents (6, 15), though exceptions have been reported (2, 3). Furthermore, endothelial cells exhibit voltage-activated currents (6, 15), though exceptions have been reported (2, 3).

METHODS

Animal care and use. Procedures were approved by the Animal Care and Use Committee of The John B. Pierce Laboratory and performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council. Male Golden hamsters (40–50 days old; 80–100 g) were obtained from Charles River Breeding Laboratories (Kingston, NY). Hamsters were anesthetized with pentobarbital sodium (65 mg/kg ip); supplemental doses (20 mg/kg ip) were given as needed. After vessels were harvested (see Surgery), the hamster was given an overdose of pentobarbital intraperitoneally.

Solutions and drugs. Three physiological (pH 7.4) salt solutions were prepared before each experiment. Solution 1 (for superfusion of vessels during experiments) contained (in mmol/l) 148 NaCl, 4.7 KCl, 2.0 CaCl2, 1.17 MgSO4, 0.026
EDTA, 2.0 3-(N-morpholino)propanesulfonic acid (MOPS), 5.0 glucose, and 2.0 pyruvate. These reagents were obtained from Sigma (St. Louis, MO) or J. T. Baker (Phillipsburg, NJ). Solution 2 contained all of the above plus 1% albumin (no. 10856, Amersham; Cleveland, OH) and was used for perfusing the vessel lumen. Solution 3 contained 1% albumin but excluded CaCl₂ and was used during dissection and cannulation of the vessel.

Surgery. While we viewed through a stereo microscope (SV8, Zeiss; Thornwood, NY), a 2-cm incision was made over the left or right scapula, and the skin was retracted to expose the underlying retractor muscle of the cheek pouch (25). Superficial connective tissue was removed using microdissection, and the muscle was gently reflected to expose feed arteries extending from the thoracodorsal artery. Connective tissue around a feed artery and its adjacent collecting vein was trimmed away, and the vessel pair was then severed at proximal and distal ends and placed in a chilled (4°C) dissecting dish containing solution 3. The vein was pinned onto a Sylgard (Dow Corning; Midland, MI) surface to secure the vessel pair. The feed artery was dissected away with great care and transferred into a vessel chamber containing solution 3 (4°C).

Vessel cannulation. A water-jacketed vessel chamber (1 ml volume; Technical Services, Pierce Laboratory; New Haven, CT) contained the feed artery. A pair of cannulation pipettes (heat polished outer diameter, 30–60 μm) were pulled (P-97, Sutter Instruments; Novato, CA) from borosilicate glass capillary tubes (GC150T-10, Warner Instruments; New Haven, CT) and positioned in the vessel chamber using micromanipulators (MT-XYZ, Newport; Irvine, CA). Each pipette was filled with solution 2 and connected to a hydrostatic column mounted on a vertical pulley to control pressure. While we observed through the stereo microscope and used fine forceps, one end of the vessel was pulled onto a pipette and secured with 11-0 monofilament nylon suture (7715, Ethicon; Somerville, NJ). Pressure was raised to 10 cmH₂O to expel residual blood, and then the free end was secured onto the other pipette in a similar manner.

The vessel chamber was transferred to a fixed stage above an inverted microscope (Diaphot, Nikon; Garden City, NY) on a vibration isolation table (Technical Manufacturing Corporation; Peabody, MA). Temperature and transmural pressure were raised gradually (over 30 min) to 37°C and 75 mmHg (in vivo) and held constant for 30 min. Superfusion of solution 1 through the chamber was continuous (4 ml/min) and laminar along the vessel as determined by movement of toluidine blue dye released from micropipettes near the vessel wall. Endothelial and smooth muscle cell viability were tested with microinjection of fluorescein diacetate (500 nA, 500 ms) and acridine orange (1.0 mol/l) and phenylephrine (PE, 0.5 mol/l), respectively, using stimulus micropipettes (borosilicate glass; GC120F-10, Warner; tip diameter, 2 μm) positioned adjacent to the vessel wall (9, 24).

Electrical stimulation. Focal electrical field stimulation (EFS) was performed using two stimulus micropipettes filled with 0.9% saline. Each micropipette was connected via Ag/AgCl wires to the respective poles of a stimulation isolation unit (SIU5, Grass; Quincy, MA) driven by a square-wave stimulator (S48, Grass). At the downstream (with respect to superfusate flow) end of the vessel, the cathode was positioned in the adventitia, and the anode was positioned directly across the vessel (tip separation: ~150 μm; Fig. 1A).

Stimulus pulses (1 ms at 16 Hz for 5 s) were delivered at “low” (30–50 V), “medium” (50–80 V), and “high” (80–110 V) intensity to directly activate perivascular nerves (14, 22), smooth muscle cells (21), and endothelial cells (see RESULTS). Direct current EFS (DCEFS; 30 V, 5 s) enabled stimulation of smooth muscle cells and endothelial cells without activating perivascular nerves (see RESULTS). When the stimulus micropipettes were elevated ~100 μm (so that the vessel was no longer directly between them), EFS produced no vasomotor response (n = 4), confirming that the electrical field was highly localized between the microelectrodes.

Video microscopy. Images were acquired in brightfield using a Leitz L25 objective (numerical aperture = 0.35); illumination was provided by a 100-W halogen lamp (Nikon.
LWD condenser; numerical aperature \( = 0.52 \). The optical image was projected onto a video camera (KP-D50, Hitachi; Japan) and displayed on a video monitor (PVM-1343MD, Sony; Japan) at a total magnification of \( \times 1.000 \). Internal diameter was measured (resolution, \( \sim 1 \mu m \)) using video calipers (Microcirculation Research Institute; College Station, TX) calibrated with a stage micrometer. The output of the calipers was directed to a data acquisition system (PowerLab 400, CB Sciences; Dover, NH).

**Vasomotor responses.** Vasomotor responses were recorded as changes in diameter; the magnitude of each response was calculated by subtracting the resting diameter from the peak response diameter. Responses were evaluated at the site of stimulation and at defined distances (determined with a calibrated eyepiece reticule) along the vessel upstream of the stimulus. The microscope rested on a motorized stage (MT-150MD, Warner), which enabled repositioning of the field of view without disturbing the vessel preparation or micropipettes secured to the fixed stage. Thus a given stimulus was repeated up to four times while sites were monitored at defined distances along the vessel. The order in which respective sites were studied was randomized between experiments; individual stimuli were separated by \( \sim 2 \) min to allow complete recovery. Preliminary experiments established that tachyphylaxis to these stimulus protocols was negligible for the duration of an experiment (2–4 h). All observations were made in the central 2 mm of vessel segments; this precaution minimized potential effects from cannulated ends.

**Electrophysiology and cell labeling.** Membrane potential was recorded with an electrometer (IE-210, Warner) using microelectrodes pulled (P-97, Sutter) from glass capillary tubing (GC100F-10, Warner). Electrode tips were filled with 1% propidium iodide (P-1304, Molecular Probes; Eugene, OR) in 2 mol/l KCl and backfilled with 2 mol/l KCl; tip resistance was 100–150 MΩ. An Ag/AgCl pellet positioned in the chamber effluent served as the reference electrode. The output of the electrometer was connected to the data acquisition system and an audible baseline monitor (ABM-D, World Precision Instruments; Sarasota, FL). Data were acquired at 100 or 400 Hz.

Our criteria for successful intracellular recording were 1) a sharp negative deflection of potential upon entering the cell, 2) a stable potential for \( > 1 \) min before the applied stimulus, 3) continuous recording during the stimulus and response, 4) clear exit from the cell, and 5) characteristic cell labeling (9, 24) with propidium iodide. During some impalements, negative and/or positive current (\( \pm 1.6 \) nA) was injected into the impaled cell through the recording electrode. After each recording, the vessel was illuminated with a 100-W mercury lamp through a rhodamine filter set (G-2A, Chroma; Battleboro, VT). Images of cell labeling were acquired with a cooled CCD camera (SPOT, Diagnostic Instruments; Sterling Heights, MI) and arranged for presentation using Adobe Photoshop (Adobe Systems; San Jose, CA).

**Air bubble treatment.** To disrupt the endothelium without damaging the smooth muscle cell layer (9), an air bubble (0.05 ml) was introduced into one of the cannulating pipettes, and pressure in the opposite cannula was reduced to 20 mmHg; the pressure gradient (55 mmHg) pushed the air bubble through the vessel lumen. After the bubble had

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**Fig. 2. Vasodilation to focal DCEFS encompasses the entire feed artery.**

**A:** drawing (not to scale) of the vessel wall before (dotted line) and during (solid line) focal DCEFS with vasodilation (arrows). **B:** representative diameter responses at distances indicated along vessel during DCEFS. Horizontal bar indicates 5 s DCEFS. **C:** summary data showing the magnitude (peak – resting value) of diameter responses from 26 vessels (resting diameter = 70 \( \pm \) 4 \( \mu m \); maximal diameter = 105 \( \pm \) 4 \( \mu m \)). Note that site of stimulation remained constricted relative to the rest of vessel \( (*) P < 0.05; \) one-way ANOVA with Tukey’s post hoc comparisons.)
passed, the lumen was reperfused with solution 2 reequilibrated for 30 min, and responses to ACh and PE microiontophoresis were then reevaluated (see RESULTS). DCEFS was then reapplied at the downstream end of the vessel while responses were reevaluated along the vessel.

**Pharmacology.** Tetrodotoxin (TTX, 10⁻⁶ mol/l; Calbiochem; La Jolla, CA) or α-conotoxin GVIA (CON, 1.5 × 10⁻⁷ mol/l; Auspep; Louisville, KY) was used to inhibit perivascular nerves. Atropine (10⁻⁶ mol/l; Sigma), phentolamine (Phe, 10⁻⁶ mol/l; Research Biochemicals International; Natick, MA), nifedipine (10⁻⁶ mol/l; Sigma), and N⁵-nitro-L-arginine (L-NNA; 10⁻⁴ mol/l; Sigma) were used to inhibit muscarinic receptors, α-adrenoceptors, L-type calcium channels, and synthesis of nitric oxide, respectively. At the conclusion of each experiment, sodium nitroprusside (10⁻⁵ mol/l; Sigma) was added to the vessel chamber to evaluate maximal diameter and intrinsic vasomotor tone.

**Statistics.** Summary data are presented as means ± SE. Statistical analyses were performed on a personal computer using SigmaStat (version 2.03, SPSS; San Rafael, CA) as detailed in results and the figures. Differences were considered statistically significant with *P* < 0.05.

**RESULTS**

**Vasomotor responses.** Upon pressurization, isolated vessels (*n* = 55) first dilated to their maximal diameter (95 ± 2 μm) and then constricted to a stable resting diameter (63 ± 1 μm). At the site of microiontophoresis, vessels dilated (by 26 ± 2 μm; *n* = 26) to ACh and constricted (by 28 ± 3 μm; *n* = 24) to PE, confirming the integrity of endothelial cells and smooth muscle cells, respectively (9, 24). Electron microscopy confirmed that the smooth muscle layer was typically one or two cells thick (B. Doran and S. Segal, data not shown).

Low EFS (30–50 V) caused vasoconstriction (−20 ± 2 μm; *n* = 12) that propagated along the entire vessel segment (Fig. 1A). In the presence of CON (*n* = 8), TTX (*n* = 4), or Phe (*n* = 4), constriction in response to EFS was limited to the region immediately beneath the stimulus microelectrode (Fig. 1B), as indicated by a one-sided dimpling of the vessel (constriction, −10 ± 1 μm; *n* = 16). Nifedipine inhibited this constriction (*n* = 4).

![Fig. 3. Electrophysiological responses during DCEFS. Vessels (*n* = 19; resting diameter = 56 ± 2 μm; maximal diameter = 85 ± 4 μm) were stimulated for 5 s with focal DCEFS (horizontal bars) while diameter (*D*) and membrane potential (*Eₜₚₚ*) were measured 1,000 μm upstream from the stimulus site.](http://ajpheart.physiology.org/\Downloadedfrom\Http://ajpheart.physiology.org/\by\10.2203.2106.2016)
6), indicating the direct activation of L-type calcium channels in this response. Medium EFS (50–80 V) evoked circumferential constriction (−15 ± 2 μm) in the vicinity of the electrode (Fig. 1B) that was also inhibited by nifedipine (n = 12). High EFS (80–110 V) evoked vasodilation (10 ± 2 μm) along the entire vessel (Fig. 1B) with the persistence of local circumferential vasoconstriction (−15 ± 2 μm).

In response to DCEFS, vasodilation was similar to that observed in response to high-intensity EFS (Fig. 1C), though the local vasoconstriction was less pronounced. Responses to DCEFS were sustained for the duration of the stimulus (up to 30 s) and vessels recovered within 10–15 s. Neither CON nor TTX altered the response to DCEFS (data not shown), indicating perivascular nerves were not involved in vasodilation.

Vasodilation to DCEFS encompassed the entire vessel segment (Fig. 2). Responses began ~2 s after stimulus onset and peaked near the end of the 5-s pulse. Although atropine abolished vasodilation to ACh (24 ± 6 vs. 0 ± 0 μm; n = 4; P < 0.05, paired Student’s t-test) and L-NNA decreased resting diameter (from 75 ± 5 to 66 ± 4 μm; n = 5; P < 0.05, paired Student’s t-test; reversed with 1 mmol/l L-arginine; Sigma), neither agent altered vasodilation to DCEFS (data not shown). Thus neither ACh nor nitric oxide (7) mediated vasodilator responses to DCEFS.

**Electrophysiological responses.** DCEFS produced hyperpolarization of endothelial cells and smooth muscle cells that conducted along the length of the vessel segment (Fig. 3). In both cell types, the hyperpolarization was similar in magnitude and lasted for the entire 5-s stimulus. The magnitude of vasodilation increased significantly with the magnitude of hyperpolarization (Fig. 4). To confirm that electrophysiological responses to DCEFS were true cellular events rather than stimulus artifacts, DCEFS was performed during intracellular recording with the stimulus micropipettes moved above the vessel by 100 μm (n = 4) or with the recording electrode placed in the adventitia rather than in a cell (n = 4). In both circumstances, only capacitance spikes were recorded coincident with the beginning and end of the 5-s stimuli.

**Current injection.** Injection of −0.8 to −1.6 nA into an endothelial cell caused hyperpolarization and reproduced vasodilator responses to DCEFS (Fig. 5), con-

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**Fig. 4.** Intensity dependence of vasomotor and electrophysiological responses to DCEFS. A: representative tracings of diameter and $E_m$ recorded from an endothelial cell 1,000 μm proximal to stimulus microelectrodes during stimuli of increasing intensity (indicated below respective 5-s scale bars). B: summary data (n = 12; resting diameter = 55 ± 3 μm; maximal diameter = 80 ± 4 μm) for peak diameter and $E_m$ responses to DCEFS. Electrophysiological responses were similar between endothelial cells (n = 10) and smooth muscle cells (n = 2) and therefore pooled (*main effect of stimulus intensity on ΔDiameter and $ΔE_m$; P < 0.05, one-way ANOVA).
firming that endothelial cell hyperpolarization in and of itself produces vasodilation. Injection of negative current into smooth muscle cells caused similar vasodilation, whereas injection of positive current (0.8 to 1.6 nA) into either cell type resulted in vasoconstriction (8; present data not shown).

Endothelial cell damage. Perfusion of a vessel with an air bubble caused visible rounding of endothelial cells and, in some regions, complete removal of the endothelial cell layer. Cell debris was then present in the vessel lumen and cannulating micropipettes. Strikingly, after air treatment, DCEFS no longer caused vasodilation along the vessel, and local vasoconstriction was enhanced relative to control ($P < 0.05$; Fig. 6, A and B). Air treatment abolished the dilator response to ACh (Fig. 6C) but did not significantly change resting diameter, the constrictor response to PE (Fig. 6D), or the dilator response to sodium nitroprusside (25 ± 5 vs. 16 ± 7 μm; $n = 5$).

DISCUSSION

The present experiments demonstrate selective electrical activation of perivascular nerves, smooth muscle cells, or endothelial cells of intact pressurized resistance arteries. Each stimulus was characterized by a particular vasomotor response that was selectively inhibited with established interventions. Whereas the activation of perivascular nerves and smooth muscle cells with electrical stimuli is well documented, we present novel evidence that endothelial cells can also be electrically “activated.” In practice, direct hyperpolarization of even a single endothelial cell had the powerful effect of relaxing smooth muscle cells along the entire vessel.

Electrical stimulation of perivascular nerves. As expected (14, 21, 22), focal activation of perivascular nerves with repetitive (16 Hz) stimuli produced vasoconstriction along the entire vessel. In contrast, DCEFS failed to activate perivascular nerves, confirming that intermittent stimuli are required to maintain repetitive action potentials and sustain neurotransmitter release. Because the propagation of vasoconstriction was antagonized by $\alpha$-adrenergic blockade, our data indicate that norepinephrine was the primary vasoactive neurotransmitter released in these experiments. However, as stimulation parameters are changed, other neurotransmitters may be released (13). To eliminate this possibility with higher voltage or DC stimulation, vessels were treated with CON (27). In the presence of CON, responses were not different from those during exposure to TTX or Phe. Because neither atropine nor l-NNA altered responses to DCEFS, our findings argue further that neither ACh nor nitric oxide was released from perivascular nerves (20) or from endothelial cells (18).

Electrical activation of smooth muscle cells. In the presence of TTX, Phe, or CON, EFS directly activated smooth muscle cells in the vicinity of the microelectrode, whether using 16 Hz or DC stimuli. This vasomotor response was mediated by the opening of volt-
maximal diameter 105

6 to acetylcholine (ACh; C)(PE; D)

dulating and recording microelectrodes were measured because of the electrical artifact when stim-
response; however, local depolarization could not be cell depolarization near the cathode gave rise to this sensitivity to nifedipine. Presumably, smooth muscle 
age-operated calcium channels as indicated by its sensitivity to nifedipine. Presumably, smooth muscle cell depolarization near the cathode gave rise to this response; however, local depolarization could not be measured because of the electrical artifact when stimulating and recording microelectrodes were <100 μm apart. Local vasoconstriction was graded (Fig. 1B), with low intensity EFS causing partial constriction (one-sided “dimpling”) and medium intensity EFS causing pronounced circumferential constriction. In turn, this behavior suggests that low intensity EFS activated only those voltage-gated calcium channels closest to the tip of the cathode, whereas higher intensities activated smooth muscle cells circumferentially within the electrical field.

Individual smooth muscle cells can encircle feed arteries of the hamster retractor muscle (9). The dim-

plung observed during low intensity EFS therefore indicates that regions of individual smooth muscle cells can be activated in the vicinity of the electrical stimulus (where current density is highest) while the rest of the cell (and adjacent cells) remains inactivated. These observations further suggest that, with focal activation, neither depolarization nor the elevation of intracellular calcium is uniform throughout individual smooth muscle cells. Their long, tapered morphology (9, 12), together with electrotonic decay of a low-intensity stimulus, may underlie this behavior.

It is of interest to note that the intense, circumfer-

tional vasoconstriction evoked by intermediate or high-
voltage electrical stimulation (Fig. 1) or by PE micro-

iontophoresis (data not shown) did not spread along the vessel wall; i.e., there was no conducted vasoconstric-
tor response, as reported previously for these ves-
sels (9, 19). In contrast, arterioles supplying the epi-

thelium of the hamster cheek pouch can conduct vasoconstriction in response to PE for several millime-
ters along the smooth muscle layer, independent of the endothelium (24). Our findings collectively indicate that smooth muscle contraction does not conduct from cell to cell as readily along feed arteries of the retractor muscle as it does along arterioles of the adjacent cheek pouch, despite similarities in vessel size and wall morphology (9, 12, 22). Nevertheless, perivascular nerves [which are absent from the epithelial region of the cheek pouch (11)], effectively constrict smooth muscle cells in unison along feed arteries and arterioles of the hamster retractor muscle (19, 22) and present data.

**Electrical activation of endothelial cells.** High inten-
sity EFS or DCEFS resulted in hyperpolarization and vasodilation that conducted along the entire feed ar-
tery; responses were similar to those evoked by ACh (9). Whereas little is known of ionic currents of endo-
thelial cells in vivo, voltage-operated (i.e., depolariza-
tion activated) ion channels have typically not been found in culture (6, 15). Nevertheless, endothelial cells abound with inward rectifying potassium channels (15). From the present data, we hypothesize that electrical stimulation activates these channels, giving rise to the conduction of hyperpolarization and vasodila-
tion.

Endothelial cell “activation” by an agonist [e.g., ACh (9) or bradykinin (6)] or through shear stress (16) characteristically elicits hyperpolarization. With DCEFS or injection of negative current, endothelial cells were hyperpolarized directly; ligand- and mech-

anoreceptor events (which activate multiple signaling pathways) were bypassed. From our recent demonstra-
tion that the endothelium is a highly effective conduc-
tion pathway (9), we conclude that current injected into a single endothelial cell can encompass the entire ves-

el. Whether hyperpolarization travels along the endo-
thelium electrotonically or through activating a “neg-
ative-going action potential” (4) remains to be determined. For feed arteries of the hamster retractor muscle, endothelial cell hyperpolarization evokes smooth muscle cell hyperpolarization and relaxation via direct myoendothelial coupling (8).
In summary, we demonstrate selective activation of the functional cellular elements of resistance arteries (e.g., perivascular nerves, smooth muscle, and endothelium) using defined electrical stimuli in conjunction with specific interventions. Whereas focal activation of perivascular nerves or smooth muscle caused vasoconstriction, electrical activation of even one endothelial cell was accompanied by hyperpolarization and vasodilation that conducted along the entire vessel. We suggest that electrical field stimulation offers a powerful method for evoking endothelium-dependent vasodilation, independent of ligand-receptor or mechanoreceptor interactions.

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