Propagation of calcium waves along endothelium of hamster feed arteries

Torben R. Uhrenholt,1,2* Timothy L. Domeier,1,2* and Steven S. Segal1,2,3,4,5,6
1The John B. Pierce Laboratory, 2Department of Cellular and Molecular Physiology, 3Department of Biomedical Engineering, and 4Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut; 5Department of Medical Pharmacology and Physiology, University of Missouri-Columbia; and 6Dalton Cardiovascular Research Center, Columbia, Missouri

Submitted 8 June 2006; accepted in final form 7 November 2006

Propagated calcium (Ca2+) waves along endothelium of hamster feed arteries. Am J Physiol Heart Circ Physiol 292: H1634–H1640, 2007. First published November 10, 2006; doi:10.1152/ajpheart.00605.2006.—An increase in tissue blood flow requires relaxation of smooth muscle cells along entire branches of the resistance vasculature. Whereas the spread of hyperpolarization along the endothelium can coordinate smooth muscle cell relaxation, complementary signaling events have been implicated in the conduction of vasodilation. We tested the hypothesis that Ca2+ waves propagate from cell to cell along the endothelium of feed arteries exhibiting spontaneous vasomotor tone. Feeder arteries of the hamster retractor muscle were isolated, pressurized to 75 mmHg at 37°C, and developed myogenic tone spontaneously. Smooth muscle cells and endothelial cells were loaded with the Ca2+ indicator Fluo-4. An acetylcholine stimulus was delivered locally using microiontophoresis (1-μm pipette tip, 1 μA, 1 s), and Ca2+ signaling within and along respective cell layers was determined using laser-scanning confocal microscopy. Acetylcholine triggered an increase in intracellular Ca2+ concentration ([Ca2+]i) of endothelial cells at the site of stimulation that preceded two distinct events: I) a rapid synchronous decrease in smooth muscle [Ca2+]i; and 2) an ensuing Ca2+ wave that propagated bidirectionally along the endothelium at ~111 μm/s for distances exceeding 1 mm. Maximal dilation of vessels with either nifedipine (1 μM) or sodium nitroprusside (SNP, 100 μM) reduced the distance that Ca2+ waves traveled to ~300 μm (P < 0.05). Thus Ca2+ waves propagate along the endothelium of resistance vessels with vasomotor tone, and this signaling pathway is compromised during maximal dilation with nifedipine or SNP.

blood flow; calcium imaging; conducted vasodilation; micrcirculation; myogenic tone

FEED ARTERIES AND ARTERIOLES of the resistance vasculature undergo dynamic coordinated changes in diameter during the control of tissue blood flow. The coordination of smooth muscle cell relaxation is readily effected via signals that travel along the endothelium; however, the nature of signaling among endothelial cells is controversial, particularly in regards to Ca2+ dynamics. The endothelium-dependent vasodilator acetylcholine (ACh) increases intracellular Ca2+ concentration ([Ca2+]i) of endothelial cells by release from internal stores (4, 25), thereby opening Ca2+-activated potassium (KCa) channels (4, 23, 25) to produce hyperpolarization. In feed arteries of hamster skeletal muscle, the focal application of ACh triggers hyperpolarization, which travels rapidly (several mm/s) along the endothelium and into smooth muscle cells through myoendothelial gap junctions (14, 15), resulting in closure of L-type Ca2+ channels and relaxation (23, 25). Thus the coordination of smooth muscle relaxation during conducted vasodilation can be explained through electromechanical coupling initiated by local increases in [Ca2+]i.

The generation of vasodilator autacoids within endothelial cells is also Ca2+ dependent (4, 23, 25), and evidence suggests that alternative signaling pathways may contribute to conducted vasodilator responses. For example, nitric oxide-mediated components of conducted vasodilation have been described both in vitro (12) and in vivo (3, 32), implying that [Ca2+]i increases at remote sites along the endothelium during conducted vasodilation. However, the autacoid-mediated signaling pathways effecting conducted vasodilation remain poorly defined, and the propagation of Ca2+ signals (e.g., Ca2+ waves) along the endothelium remains controversial (16), particularly as previous investigations have indicated that endothelial cell [Ca2+]i does not increase at remote sites (e.g., >250 μm from the stimulus) during conducted responses to ACh (11, 28, 35).

Feed arteries and arterioles have a circumferential layer of smooth muscle surrounding a longitudinally oriented monolayer of endothelial cells (15, 20, 29). This anatomical arrangement enables the observation of [Ca2+]i, simultaneously in both cell layers using confocal fluorescence microscopy, particularly when imaging along the curvature of the vessel surface. However, as confocal analyses are prone to movement artifact (particularly with single-wavelength Ca2+ indicators), the influence of vasomotor tone (and thereby the ability to undergo vasodilation) was eliminated in studies focused on [Ca2+]i, signaling associated with endothelium-dependent hyperpolarization (28, 35). In the present study, we tested the hypothesis that Ca2+ waves propagate from cell to cell along the endothelium of feed arteries exhibiting spontaneous vasomotor tone. Endothelial cells and smooth muscle cells of pressurized feeder arteries were loaded with the Ca2+ indicator Fluo-4 and imaged with confocal microscopy to analyze the [Ca2+]i dynamics along the vessel wall that precede and accompany conducted vasodilation. Our findings are the first to reveal that Ca2+ waves can propagate along the endothelium of microvessels for distances exceeding 1 mm.

* T. R. Uhrenholt and T. L. Domeier contributed equally to this work.

Address for reprint requests and other correspondence: S. S. Segal, Dept. of Medical Pharmacology and Physiology, School of Medicine, One Hospital Drive, MA 615 Medical Science Bldg., Univ. of Missouri-Columbia, Columbia, MO 65212 (e-mail: segalss@health.missouri.edu).
MATERIALS AND METHODS

Feed artery preparation. Procedures for preparing isolated feed arteries (14, 15) were approved by the John B. Pierce Laboratory Animal Care and Use Committee in accordance with Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). Briefly, male Syrian golden hamsters (n = 42, 80–120 g; Charles River Breeding Laboratories, Kingston, NY) were anesthetized (pentobarbital sodium, 65 mg/kg ip), and feed arteries of the cheek pouch retractor muscle were excised, cannulated, and pressurized to 75 mmHg in physiological saline solution (PSS; pH 7.4, 37°C) containing (in mM) 148.0 NaCl, 4.7 KCl, 2.0 CaCl₂, 1.17 MgSO₄, 0.026 EDTA, 2.0 MOPS, 5.0 glucose, and 2.0 pyruvate. Reagents were obtained from Sigma-Aldrich (St. Louis, MO) unless stated. The preparation was superfused continuously (3 ml/min) while visualized using an upright confocal microscope system (Olympus Fluoview 1000; Center Valley, PA). These resistance microvessels typically develop robust spontaneous myogenic tone with a resting internal diameter (50–70 μm) that is 50–60% of maximal diameter (13–15).

Laser-scanning confocal microscopy with Fluo-4. For imaging 
[Ca²⁺] dynamics, 1 μM Fluo-4-AM (Molecular Probes/Invitrogen; Carlsbad, CA) in PSS with 0.1% BSA + 2.5% dimethylsulfoxide was perfused through the vessel lumen for 30 min to load endothelial cells and smooth muscle cells. Excess dye was washed, and each vessel was equilibrated for 20 min to allow for deesterification of dye. Confocal laser-scanning fluorescence microscopy was performed using a ×20 (numerical aperture, 0.5) Olympus water immersion objective. The scanning area was set to 512 × 128 pixels without internal zoom, corresponding to 873 μm × 218 μm. Full-frame imaging was performed at 3 Hz using excitation from an argon laser at 488 nm with fluorescence monitored through a 505 nm long-pass filter. The focal plane of the microscope was positioned along the

---

Fig. 1. Analysis of calcium dynamics along the vessel wall. The confocal image plane was positioned near the vessel surface to simultaneously visualize endothelial cells (EC, parallel to vessel axis) and smooth muscle cells (SMC, perpendicular to vessel axis). A: line analysis. Endothelial cell-specific fluorescence changes were obtained by placing a line (1 pixel × 5 pixels) for averaging across endothelial cells imaged between smooth muscle cells (inset, vertical line); smooth muscle cell-specific fluorescence changes were obtained by placing a line (5 pixels × 1 pixel) for averaging across smooth muscle cells imaged between endothelial cells (inset, horizontal line). B: fluorescence (F/F₀) from endothelial cells (left) and smooth muscle cells (right) shown in A in response to an ACh stimulus (location of stimulus marked by asterisks in A and C, given at time marked by arrows in B and D). C: window analysis. A window (20 × 80 pixel region; white rectangle) was positioned to obtain an integrated fluorescence signal from both endothelial cells and smooth muscle cells. D: fluorescence (F/F₀) from averaging window shown in C following ACh stimulus. Scale bars in A and C, 50 μm.
surface of the vessel to visualize endothelial cell and smooth muscle cell fluorescence simultaneously. Laser power was adjusted (transmissivity 30.6%; photomultiplier tube voltage 450–550 V) to obtain images with a mean intensity of 1,500 arbitrary intensity units (range 200–4,095). This ensured fluorescence acquisition over the full dynamic range of Fluo-4 without pixel saturation or excessive photobleaching. Data are presented as the relative fluorescence intensity change (F/Fo), where Fo equals the average fluorescence intensity before stimulation. Whereas imaging along the vessel surface precluded accurate observation of vessel diameter, conducted vasodilation in feed arteries supplying the hamster retractor muscle has been well defined and shown to be similar between isolated preparations (13–15) and the intact system (30, 32).

Vessel stimulation and data acquisition. ACh was delivered using microiontophoresis (1 M; 1 µA for 200–1,000 ms, 1-µm micropipette tip internal diameter) onto a discrete site at the downstream end of the vessel located at one edge of the imaging window. The resulting fluorescence signals were recorded to computer for subsequent analysis using Olympus Fluoview software (version 1.3c). Prior to vessel stimulation, vasomotor tone was determined to be consistent along the length of each vessel segment by imaging diameter along the vessel edges. To reduce the probability of erroneous data interpretation due to motion artifact, only recordings in which both endothelial cells and smooth muscle cells along the vessel remained in focus were used for analysis, which occurred in ~25% of the present experiments. In a subset of experiments, vessels were dilated maximally with either nifedipine (1 µM) or sodium nitroprusside (100 µM) prior to ACh stimuli. During maximal dilation, we ensured that the endothelium was responsive and within the image plane by stimulating with ACh and confirming local fluorescence responses at sites along the entire vessel segment.

Data analysis and statistics. Loading endothelial cells and smooth muscle cells with the same calcium indicator was ideal for visualizing dynamic fluorescence responses in real time (see supplemental videos; the online version of this article contains supplemental data) as respective cell types are readily distinguished by their axial vs. circumferential orientation along the vessel (14, 20, 26, 38). However, analysis of observed fluorescence changes using this approach was problematic once a vessel shifted within the imaging plane upon the onset of movement. To circumvent these difficulties, we used two complementary approaches to quantify fluorescence signals: “line” analysis and “window” analysis (Fig. 1). Line analysis obtained fluorescence signal selectively from endothelial cells or from smooth muscle cells (Fig. 1, A–B) prior to movement onset. In this procedure, an averaging line (1 × 5 pixels) placed vertically (between smooth muscle cells) monitored endothelial cell-specific fluorescence, whereas a perpendicular line (5 × 1 pixels) placed horizontally (between endothelial cells) monitored smooth muscle cell-specific fluorescence. In contrast, the window analysis monitored fluorescence from all cells (endothelial cells and smooth muscle cells) lying within 20 × 80 pixel (34 × 136 µm) averaging windows positioned along the vessel wall (Fig. 1, C–D). Since window analysis was less sensitive to vessel movement, it was used to characterize fluorescence responses both during and preceding the onset of vasodilation. Low-intensity background pixels within these windows were excluded from averaging by setting a secondary threshold of ~50 arbitrary intensity units.

Under control conditions, Ca2+ waves propagated beyond the field of view (i.e., >750 µm; see RESULTS AND DISCUSSION). In maximally dilated vessels, the distance that Ca2+ waves traveled within the field of view was determined initially by visual inspection and confirmed by using window analysis along the vessel segment. Thus, using the caliper function of the Fluoview software, we calculated the distance between the site of stimulation and the furthest distance at which F/Fo increased in response to ACh. The velocity of Ca2+ wave propagation was estimated on the basis of the time elapsed for the leading edge of the Ca2+ wave to reach defined distances along the vessel. Statistical comparisons were performed using analysis of variance with Bonferroni correction for post hoc comparisons (SPSS 11.0; Chicago, IL). Differences were considered statistically significant with P < 0.05. Summary data are reported as means ± SE.

RESULTS AND DISCUSSION

The present experiments were designed to test the hypothesis that Ca2+ waves propagate along the endothelium of feed arteries exhibiting spontaneous vasomotor tone. Observations focused on simultaneous imaging of intra- and intercellular Ca2+ dynamics of endothelial cells and smooth muscle cells along the surface of pressurized feed arteries during conducted vasodilation. Delivery of ACh triggered an increase in F/Fo of endothelial cells in the region of stimulation (Fig. 2A), indicating the activation of local signaling events that release Ca2+ from internal stores (4, 25). This transient increase in endothelial cell [Ca2+]i preceded two distinct events. First, [Ca2+]i decreased simultaneously along the entire smooth muscle layer, observed as the synchronous reduction in fluorescence along the vessel wall (see supplementary videos 1 and 2). This coordinated response is illustrated graphically by the simultaneous decrease in F/Fo at the 100- and 500-µm remote sites (Fig. 2B) and reflects the initial local rise in endothelial cell

![Fig. 2. Endothelial cell- and smooth muscle cell-specific calcium dynamics.](http://ajpheart.physiology.org/)

*Fig. 2.* Endothelial cell- and smooth muscle cell-specific calcium dynamics. Line analysis of endothelial cells (EC; A) and smooth muscle cells (SMC; B) at 100 µm (○) and 500 µm (●) upstream from the ACh stimulus preceding the onset of vasodilation. Delivery of ACh and onset of vessel movement are indicated by vertical hatched lines. Traces are mean ± SE (n = 6). Prior to movement, note simultaneous fall in smooth muscle cell F/Fo at both sites during rise of endothelial cell F/Fo at 100 µm with no change in endothelial cell intracellular Ca2+ concentration at 500 µm.
[Ca\textsuperscript{2+}]_i activating KCa channels (4), leading to the initiation of hyperpolarization (5, 15, 25) and its rapid conduction along the endothelium into consecutive smooth muscle cells (14). In turn, hyperpolarization of smooth muscle cells closes L-type (i.e., voltage-gated) Ca\textsuperscript{2+} channels in the plasma membrane, thereby lowering [Ca\textsuperscript{2+}]_i to produce relaxation and vasodilation (2, 23, 25).

Second, the ACh stimulus triggered a Ca\textsuperscript{2+} wave that spread from cell to cell bidirectionally along the endothelium (see supplementary videos 1 and 2). To characterize these Ca\textsuperscript{2+} waves, averaging windows were positioned every 100 \( \mu \)m increments along the vessel upstream from the stimulus micropipette to monitor fluorescence signal arising from both endothelial cells and smooth muscle cells (Fig. 3). At the site of stimulation,
nifedipine (10–60 μM; n = 6; closed circles) or sodium nitroprusside (SNP, 100 μM; n = 6; open bars) eliminated vasomotor tone and reduced the distance that Ca²⁺ waves spread to ~300 μm with the ACh stimulus maintained at 1,000 ms. Values are means ± SE. *Significantly different from control with 1,000-ms ACh stimulus; P < 0.05.

F/F₀ increased immediately as a result of the endothelial cell [Ca²⁺], transient (Fig. 3A, closed circles). In contrast, F/F₀ initially fell at all remote sites due to the simultaneous decrease in smooth muscle cell [Ca²⁺], as explained above. The decreases in F/F₀ at remote sites were followed by sequential increases in F/F₀ as the Ca²⁺ wave propagating along the endothelium entered successive averaging windows (Fig. 3). Line analysis of endothelial cells at 500 μm upstream (Fig. 2A) demonstrated that endothelial cell [Ca²⁺] remained unchanged prior to the Ca²⁺ wave front. Although the greatest fluorescence intensity was observed around endothelial cell nuclei, fluorescence increased throughout the cytoplasm of endothelial cells during propagation of Ca²⁺ waves (see supplementary Fig. 1) with no delay between neighboring cells.

The distance traveled by Ca²⁺ waves increased with stimulus duration (Fig. 4). In response to a 1,000-ms stimulus, Ca²⁺ waves traveled the entire distance of the observed vessel segment. Although the total length of vessel within the scanning area was 873 μm (see MATERIALS AND METHODS), the maximum distance for observing propagation of Ca²⁺ waves was typically ~750 μm due to the ACh micropipette being positioned near the downstream end of the segment within the field of view (Fig. 4). Nevertheless, in three experiments in which we were able to maintain the appropriate image plane during vessel movement, it was verified that Ca²⁺ waves propagated for at least 1 mm by repositioning the stimulus pipette further downstream, beyond the scanning area. Across experiments, the velocity of Ca²⁺ wave propagation along the endothelium averaged 111 μm/s (Fig. 5), which is considerably faster than that observed for endothelial cells in primary culture (10–60 μm/s) (8, 9), in pulmonary capillaries (5 μm/s) (43), or in mesenteric arteries treated with nifedipine (~30 μm/s) (28).

**Pharmacological vasodilators attenuate propagation of Ca²⁺ waves.** Pharmacological vasodilators have been applied routinely during imaging studies to prevent vessel movement associated with endothelium-dependent hyperpolarization (28, 35). In the present study, maximal dilation of feed arteries with sodium nitroprusside or nifedipine prior to ACh stimulation decreased the distance that Ca²⁺ waves spread along the endothelium to ≤300 μm (Fig. 4; see also supplementary videos 2-4). Sodium nitroprusside (a nitric oxide donor) relaxes vascular smooth muscle primarily via Ca²⁺ desensitization (34). In contrast, nifedipine exerts its vasodilator effects by blocking L-type (voltage-gated) Ca²⁺ channels in the smooth muscle cell membrane, thereby lowering [Ca²⁺], (23, 25). In addition to these effects on smooth muscle, both sodium nitroprusside and nifedipine may have direct actions on the endothelium. For example, nitric oxide has been found to depress [Ca²⁺], responses to bradykinin and serotonin in primary cultures of aortic and cerebral microvascular endothelial cells (36, 41). Additionally, the expression of L- and T-type voltage-gated Ca²⁺ channels has been detected in endothelial cells isolated from the adrenal medulla and microvessels of the lung (37, 39). A technical limitation of confocal microscopy with single-wavelength Ca²⁺ indicators is the inability to accurately determine [Ca²⁺], particularly as cell thickness changes during vasodilation. Thus, while the respective effects of pharmacological vasodilators on [Ca²⁺], of endothelial cells and smooth muscle cells comprising intact hamster feed arteries remains to be established, the present findings illustrate the importance of maintaining vasomotor tone when investigating the propagation of Ca²⁺ signaling events along resistance microvessels.

As discussed above, smooth muscle cell relaxation during the conduction of hyperpolarization is attributable to voltage-
dependent closure of L-type Ca\textsuperscript{2+} channels. The question arises as to why the inhibition of L-type Ca\textsuperscript{2+} channels with nifedipine restricts Ca\textsuperscript{2+} wave travel (Fig. 4), whereas the closure of L-type Ca\textsuperscript{2+} channels during conducted hyperpolarization and vasodilation does not. This difference can be explained by the transient nature of both hyperpolarization and dilation during the conducted response to ACh (15) when compared with sustained maximal dilation during nifedipine treatment. Thus the sustained inhibition of L-type Ca\textsuperscript{2+} channels with nifedipine is likely to exert more profound effects on [Ca\textsuperscript{2+}]i dynamics in affected cells. As developed below, the actions of vasodilators on smooth muscle cell [Ca\textsuperscript{2+}]i may further affect the intracellular environment of neighboring endothelial cells.

**Development of a hypothesis: smooth muscle tone sensitizes endothelium for Ca\textsuperscript{2+} wave propagation.** The phenomenon of the Ca\textsuperscript{2+} wave was first described as a “tsunami” traversing the cytoplasm of medaka fish eggs and attributed to calcium-induced calcium release from internal stores (17). Calcium waves have since been observed in pulmonary capillary endothelium (43), in cultured endothelium (8, 9), and in endothelium of arteries dilated maximally with nifedipine (28, 35), albeit over considerably shorter distances (and slower propagation velocities) than reported here. Within cells, free Ca\textsuperscript{2+} diffuses <1 µm before being buffered, whereas diffusion of the Ca\textsuperscript{2+}-mobilizing molecule inositol 1,4,5-trisphosphate (IP\textsubscript{3}) is restricted to ~24 µm (1). Thus our finding that Ca\textsuperscript{2+} waves can propagate along feed artery endothelium for at least a millimeter implies a mechanism that involves regenerative Ca\textsuperscript{2+} signaling over distances far greater than can be explained by the diffusion of Ca\textsuperscript{2+} or IP\textsubscript{3} from the site of ACh stimulation. For example, Ca\textsuperscript{2+} release from endoplasmic reticulum receptors (IP\textsubscript{3} or ryanodine) may activate neighboring receptors to release Ca\textsuperscript{2+}, thereby providing regenerative signal propagation (6). Moreover, these receptors appear to be sensitized for Ca\textsuperscript{2+} release by increases in basal [Ca\textsuperscript{2+}]i (18).

The development of vasomotor tone reflects an increase in smooth muscle [Ca\textsuperscript{2+}]i via integrin-mediated Ca\textsuperscript{2+} influx (7). Pressurizing vessels results in depolarization and the opening of voltage-gated Ca\textsuperscript{2+} channels to promote Ca\textsuperscript{2+} entry (21, 24). As illustrated in arterioles from hamster cheek pouch and cremaster muscle, an elevation in smooth muscle cell [Ca\textsuperscript{2+}]i may be transmitted into endothelial cells through myoendothelial gap junctions (10, 42). The mechanical deformation of endothelial cells as smooth muscle cells develop tone (19) may also produce an elevation in the basal level of endothelial cell [Ca\textsuperscript{2+}]i (8, 33). Although endothelial cell [Ca\textsuperscript{2+}]i was not quantified here, we hypothesize that endothelial cells in vessels with vasomotor tone have higher basal [Ca\textsuperscript{2+}]i, and are thereby “sensitized” to propagate Ca\textsuperscript{2+} waves in response to the local [Ca\textsuperscript{2+}]i increase triggered by ACh (5).

**Summary and conclusion.** Conduction along resistance microvessels was first described as electrical in nature (22), an interpretation that has been supported consistently in subsequent studies (14, 31, 38, 40). In contrast, the present experiments uniquely illustrate the existence of a second signaling pathway triggered by ACh that involves the propagation of Ca\textsuperscript{2+} waves along the vessel wall and appears to be independent of electrical signaling. This conclusion is supported by the difference in velocity of Ca\textsuperscript{2+} waves estimated from the present data (~111 µm/s; Fig. 5) vs. that estimated for the conduction of electromechanical vasodilation using the same vessel preparation (~45 mm/s (13)). The difference in propagation velocity of respective signaling pathways becomes increasingly apparent as distance increases from the site of stimulation. For example, at 700 µm upstream, the onset of vasodilation subsequent to the fall in smooth muscle cell [Ca\textsuperscript{2+}]i precedes the arrival of the Ca\textsuperscript{2+} wave (see supplementary videos 1 and 2).

Previous studies have implicated hyperpolarization as a mechanism for elevating endothelial cell [Ca\textsuperscript{2+}]i (27) by increasing the electrochemical gradient for Ca\textsuperscript{2+} entry. This effect was proposed to underlie the generation of nitric oxide during conducted vasodilation (3). In contrast, the present findings reveal that cell-to-cell propagation of a Ca\textsuperscript{2+} wave represents the mechanism by which Ca\textsuperscript{2+} increases along the endothelium during conducted vasodilation and that this signaling pathway is temporally dissociated from the more rapid conduction of hyperpolarization. Whereas the precise mechanism of regenerative Ca\textsuperscript{2+} signaling along the endothelium remains to be established, our data uniquely demonstrate that, in the presence of vasomotor tone, the endothelium can propagate Ca\textsuperscript{2+} waves over distances that are sufficient to coordinate the activity of entire segments of the resistance vasculature.

**ACKNOWLEDGMENTS**

This work was included in a dissertation submitted to fulfill in part the requirements for the degree of Doctor of Philosophy in Yale University (T. L. Domeier).

**GRANTS**

This research was supported by the National Institutes of Health (NIH) Grants RO1-HL-56786 and RO1-HL-41026. T. L. Domeier was supported by NIH Grants T32-GM-07223, T32-GM-07527, and F31-NR-053186. T. R. Uhrenholt was supported by Danish Medical Research Grant 22-04-0544.

**REFERENCES**


CALCICUM WAVE PROPAGATION ALONG FEED ARTERY ENDOTHELIUM