How Structure, Ca Signals, and Cellular Communications Underlie Function in Precapillary Arterioles

L. Borisova, Susan Wray, D. Eisner, T. Burdyga

Rationale: Precapillary arterioles control blood flow to tissues and their correct function is vital. However, their small size has limited study and little is known concerning the calcium signals in their endothelial and muscle cells and how these relate to function.

Objective: We aimed to investigate whether these small vessels are specialized in terms of structure and calcium signaling.

Methods and Results: Using in situ confocal imaging we have studied the ultrastructure, Ca signaling and coordination of contraction in precapillary arterioles in ureter and vas deferens. We have compared the data to that from a small mesenteric artery. In the precapillary arteriole, 1 myocyte covers a \( \approx 10-\mu \text{m} \) length, and contraction of this single cell can decrease the diameter of this segment. In the mesenteric artery, more than 20 myocytes are required for this. In the precapillary arteriole, Ca signals arise solely from Ca release from the sarcoplasmic reticulum through phosphoinositol-3,4,5-inositol--induced Ca release and not via ryanodine receptors. Agonist-induced Ca signals do not require Ca entry into the cell, do not spread or synchronize with neighboring cells, and are unaffected by endothelial stimulation, thereby allowing local control. This contrasts with the mesenteric artery, where Ca entry and ryanodine receptors are important and stimulation of the endothelium removes the Ca signal and contraction.

Conclusions: These data reveal the structural and signaling specializations underlying how blood flow is locally regulated provide new insight into control of microcirculation, and provide a framework to explain its vulnerability to disease. (Circ Res. 2009;105:00-00.)

Key Words: calcium ■ vascular smooth muscle ■ endothelium

Precapillary arterioles are the final generation of terminal arterioles. They have diameters <30 \( \mu \text{m} \) and determine local blood flow to tissues via capillary beds, as opposed to the gross distribution of blood and control of pressure, performed by the larger conducting vessels and resistance arteries. Little direct investigation of Ca signaling, structure, and contractility has been possible in live, intact precapillary arterioles, because their small size virtually precludes dissection. Although vital microscopy and related techniques have produced useful information,\(^1\text{-}^3\) they are not well suited to investigations of cellular function, signaling, or morphology.\(^4\) A decreased role for the internal Ca store in small vessels has been suggested,\(^3,^6\) but generally the paucity of information in precapillary arterioles has led to the processes found in larger vessels: integration of neuronal and endothelial signals,\(^3,^7,^9\) being extrapolated to precapillary arterioles. However, consideration of the differences in their function, innervation, and amount of tone would suggest that the mechanisms and signaling controlling precapillary arterioles may differ from those present in other vessels.\(^10,^11\)

In addition to neurohormonal stimulation of myocytes, the endothelium via Ca signals and NO- and/or EDHF-mediated effects, can also affect vascular tone.\(^12\text{-}^15\) Little is known concerning endothelial Ca signals in precapillary arterioles or their contribution to relaxation; indeed, it is unclear how relevant flow-, pressure-, or shear-mediated relaxing mechanisms are to these smaller vessels.\(^1,^11\)

We now report unique mechanistic signaling data from in situ precapillary arterioles in 2 host tissues, obtained with confocal imaging of intracellular Ca, and directly compare the results with a well-characterized larger vessel, the mesenteric artery (\( \approx 230 \mu \text{m} \) in diameter).\(^16\text{-}^19\) We show, for the first time, that Ca signals induced by agonists in precapillary arteriolar myocytes rely exclusively on Ca release from the sarcoplasmic reticulum (SR) mediated by phosphoinositol-3,4,5-inositol (IP\(_3\)) receptors (IP\(_3\)Rs). As the signals do not pass and coordinate between cells, exquisite local control in segments of precapillary arterioles is achieved. The endothelium has no effect on agonist-induced tone but abolishes Ca spikes and contraction produced by L-type Ca channel entry. Thus, uniquely within the arterial system, in precapillary arterioles a single myocyte influences a significant part of the vessel and blood flow to the surrounding region.

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Methods

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org.

Calcium and Diameter Measurements

Following euthanasia, strips of rat ureter or vas deferens were dissected and arterioles within the tissue were studied as described previously.20 The in situ precapillary arterioles remain patent and their structure determined in the data obtained in ureteric tissue. Differences were taken as significant at P<0.05 in a Student t test.

Results

Structure

Figure 1A shows low magnification images of in situ precapillary arterioles in the ureter. Individual myocytes (Figure 1B) and endothelial cells (Figure 1C) can be resolved more clearly at higher magnification and their structure determined (Online Movies 1 and 2). Erythrocytes and fluid moving within the precapillary arteriole could also be seen (Online Movie 4). The myocytes of the precapillary arterioles were 100.4±4.2 μm long and 6.7±0.2 μm wide (n = 17) and made 2 or occasionally 3 turns around the endothelium, as could be seen by tracking a myocyte through the arteriole and highlighted in color in Figure 1B (i and iii). Thus, a single myocyte covered ∼10 μm (8.5±1.4 μm) of the length of a precapillary arteriole segment (Figure 1B).

In the small mesenteric arteries, the same (10-μm) length of a vessel segment contained >20 myocytes of length 92±3.5 μm and width 5.7±0.2 μm (n = 11). The myocytes formed a triple layer of cells in the wall with each cell contacting around 6 others (Figure 1D, i).

Effect of Single-Cell Activation

The structure described above suggested that activation of a single myocyte might affect a segment of the precapillary arteriole. Figure 2A shows examples of Ca rises in single myocytes as a result of nerve stimulation of the precapillary arteriole. The transmitted light Online Movie 4 also demonstrates the effects of single-cell rhythmic contraction on the diameter of the arteriole and blood flow in motion. An increase of Ca in a single myocyte causes contraction and a marked local decrease in the diameter of the precapillary arteriole, as shown in Figure 2B (i and ii; red circles and Ca traces, respectively). The luminal diameter of the arterioles in the area of a contracting cell was reduced to 53.3±6.2% of control, which brought the endothelial cells into close apposition (n = 10 cells, 5 vessels). Stimulation by phenylephrine (PhE) or ET-1 produced rhythmic (phasic) changes in the precapillary arteriole diameter and affected blood flow (Online Movies 3 and 4). In contrast, in mesenteric arteries contraction of one smooth muscle cell had no effect on the vessel diameter (Figure 2C, i, and 2B, ii; Online Movie 5).

How Are Myocyte Ca Signals Generated and Are They Coordinated in Precapillary Arterioles?

Because a single myocyte in precapillary arterioles can influence the same length of vessel that requires >20 cells in

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<tr>
<td>2-APB</td>
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<td>8-pCPT-cGMP</td>
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<tr>
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<td>EDHF</td>
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Solutions

Physiological saline of the following composition was used (mmol/L): NaCl 120.4, KCl 5.9, MgSO_4_ 1.2, CaCl_2_ 2, glucose 8, and HEPES 11. 2-Aminoethoxydiphenyl borate (2-APB), ryanodine, and U-73122, were from Calbiochem (Nottingham, UK), all other chemicals were from Sigma. Phenylephrine, endothelin-1 (ET-1), ryanodine, Na nitroprusside, and para-chlorophenylthioureas-3', 5'-cyclic monophosphate (8-pCPT-cGMP) were dissolved in water; 2-APB, cyclopiazonic acid, S-nitroso-N-acetyl-DL-penicillamine (SNAP), U-73122, and cytochalasin D in DMSO; and nifedipine in ethanol.

Statistics

Values are means±SEM, and n is number of vessels or cells. Experiments were performed on rat: mesenteric small arteries (n=5 to 15) and precapillary arterioles in ureter (n=5 to 15), with findings also being verified in vas deferens precapillary arterioles (n=4 to 7).
small diameter arteries, we hypothesized that: (1) the coordination of agonist-induced Ca signals produced by Ca entry, which is required to control arteries, is redundant in the precapillary arterioles; and (2) the mechanisms generating their Ca signals would differ. We therefore compared the role of the SR to that of Ca entry in generating and coordinating Ca signals, along with functional effects, in both vessels.

In preliminary experiments, we established that stimulation of the nerve endings produced asynchronous Ca oscillations, which were resistant to suramin but inhibited by prazosin or phentolamine. These data suggested that in precapillary arterioles, noradrenaline is the major neurotransmitter and that it produces global Ca oscillations. We therefore applied the long-lasting analog PhE to study the effect of local neurotransmitter release under well-controlled conditions and ET-1 to investigate the effects of a circulating hormonal agonist. The effects of both agonists were compared for both terminal arterioles and mesenteric arteries.

Figure 3A and 3B shows Ca measurements from mesenteric arteries stimulated by PhE (10 μmol/L) and ET-1 (20 nmol/L). Large, synchronized global oscillations of Ca were associated with rhythmic activity (vasomotion) and vasoconstriction (Figure 3A, ii; Online Movie 6). Small asynchronous Ca waves also occurred (* in Figure 3A, i). At low concentrations of PhE (≤1 μmol/L), only asynchronous Ca waves were produced and little or no change in artery diameter occurred (Online Movie 5). If external Ca was removed or nifedipine (1 to 10 μmol/L) applied, synchronous Ca oscillations rapidly disappeared (3.2±0.2 minutes), along with rhythmic activity and only single Ca waves were produced in response to agonists (Figure 3B, i; Online Movie 7). These nifedipine-resistant Ca waves were inhibited by both ryanodine, a blocker of ryanodine receptors (Figure 3B, ii), and the nonspecific IP3R blocker 2-APB (Figure 3B, iii), confirming reports that both release channels are involved in their generation.18 Similar effects of Ca removal and applications of nifedipine, ryanodine and 2-APB were also seen on the response to ET-1 (20 nmol/L).

In precapillary arterioles, a very different pattern of Ca signals was found. At both low and high concentrations, agonists (PhE, 1 to 100 μmol/L; ET-1, 1 to 20 nmol/L) only produced asynchronous Ca waves (see lack of synchronization between the 2 cells in Figure 3C, i and ii; Online Movie 9), and no role for Ca entry was found (Figure 3D). There was no transmission of waves to neighboring cells (Figure 3C; Online Movies 8 and 9). These waves produced rhythmic activity and local vasoconstriction (Figure 3C, iii). Thus, unlike mesenteric arteries, irrespective of agonist concentration, Ca waves never synchronized. The Ca waves were resistant to removal of external Ca (Figure 3D, i) and nifedipine (not shown) and continued with little decrement even after 30 to 90 minutes in Ca-free solution. In contrast to the mesenteric artery, in precapillary arterioles, ryanodine (which abolished responses to caffeine) did not affect agonist-induced Ca waves (Figure 3D, ii). However, as was the case in the mesenteric artery, 2-APB abolished them (Figure 3D, iii).

Calcium Signals in Endothelium

Endothelial cells could be seen clearly in precapillary arterioles (Figures 1C and 4A; Online Movies 1 and 2). They had nuclei that protruded into the lumen, an average cell length of...
61.8 ± 8.7 μm, and their greatest width was 3.9 ± 0.1 μm and height (thickness) 5.2 ± 0.9 μm (n = 21, 10 vessels). The endothelial cells of mesenteric artery were longer (80.6 ± 5.2 μm) and wider (10.9 ± 0.5 μm) (n = 15 cells, 5 vessels; Figure 1D, ii).

In agreement with previous studies, the endothelium of the mesenteric arteries showed no spontaneous activity. The endothelial cells of precapillary arterioles, however, were spontaneously active producing Ca puffs: small localized Ca waves occur randomly in cells. This precapillary arteriole was treated with 20 μmol/L cytochalasin to reduce tissue movement. Images showing changes in Ca signaling and diameter of a segment of the precapillary arteriole. The lower image shows an increase in [Ca²⁺] and contraction of 1 cell (identified by red circle), produced in response to a low concentration of PhE, so that only asynchronous nonrepetitive Ca waves occur randomly in cells. B, Time course of changes of [Ca²⁺] (top) and vessel diameter (bottom) from the region of the identified cell. C shows data for a mesenteric artery.

Effect of Endothelial Ca Signals on Myocyte Function

In mesenteric artery, myocytes were stimulated by ET-1 (20 nmol/L) or PhE (10 μmol/L) to produce Ca signals and mechanical responses (rhythmic activity and vasoconstriction) (Figure 5A). Maintaining this stimulation, the endothelium was activated by 1 μmol/L CCh: activation of Ca signaling in the endothelial cells produced inhibition of Ca signaling in the myocytes and vasodilation (Figure 5A; Online Movie 12), as reported by others. In contrast, in precapillary arterioles, prestimulation with CCh did not prevent normal stimulation of myocytes by PhE or ET-1, ie, Ca waves and tone were unaffected (Figure 5D; Online Movie 13). In the precapillary myocytes, the frequency of global Ca waves/oscillations evoked by PhE was 0.035 ± 0.003 Hz in the presence of CCh compared to 0.036 ± 0.002 Hz before CCh (n = 6). When waves were evoked by ET-1, their frequency was 0.046 ± 0.004 Hz in the presence and 0.044 ± 0.002 Hz in the absence of CCh (n = 8). CCh also had no effect on the tone produced by stimulation with ET-1. With CCh, the mean diameter decreased to 61.4 ± 3.4% compared to 64.1 ± 3.2% in its absence.

The above data suggested lack of NO- or EDHF-mediated effects on Ca signaling and changes in vascular tone induced by agonists in precapillary arterioles. We tested this by applying the NO donors Na nitroprusside (20 μmol/L) or SNAP (20 μmol/L); these also had no significant effect on the Ca signals or mechanical activity in precapillary arterioles (Figure 5F; n = 9). Frequency of oscillations in the presence of ET-1 was 0.046 ± 0.004 and 0.048 ± 0.004 Hz in the absence and presence of SNAP, respectively. SNAP or Na nitroprusside (not shown) abolished synchronized Ca oscillations and rhythmic activity in the mesenteric preparations (n = 7), mimicking the effects of CCh (Figure 5E). Consistent with the effects of NO donors, the membrane permeable analog of cGMP, 8-pCPT-cGMP (100 μmol/L), abolished Ca oscillations and rhythmic activity in mesenteric arteries but had no effect in precapillary arterioles (n = 4, 2 animals, data not shown). The only situation in which the endothelial Ca signals produced by CCh inhibited precapillary arteriolar myocyte Ca signaling and tone was when the vessel had been pre-
treated with a potassium channel blocker (tetraethylammonium, 5 mmol/L) in the presence of the L-type Ca channel agonist Bay K-8644 (1 μmol/L), so that fast propagating Ca spikes evoked by direct electric field stimulation were produced (Figure 5G). These electrically evoked Ca spikes, which depended only on Ca entry in both types of vessels (Figure 5G, ii)3 and produced transient vasoconstriction, were reversibly inhibited during activation of endothelial Ca signaling by CCh (Figure 4G, iii; Online Movie 16) but were resistant to NO donors, Na nitroprusside or SNAP (Figure 5H).

Discussion

Combining imaging with Ca signaling in intact, in situ precapillary arterioles has provided unique insight into the structure and mechanisms that enable them to perform their specialized function within the circulatory system. The data show that precapillary myocytes wrap at least twice around the arterioles, and the activation by Ca signaling of a single myocyte is sufficient to produce functional effects. The situation is clearly different in the small arteries, where tens of myocytes need to be simultaneously activated to bring about functional change. The control of tone by individual myocytes enables a very precise response of the microcirculation to local and systemic stimuli.

The use of in situ confocal Ca imaging brings many advantages to the study of the microcirculation, especially the maintenance of normal cell architecture, contacts and the ability to make detailed spatial and temporal measurements, but it is also the case that the arterioles are not pressurized. However, (1) the precapillary arterioles we studied maintained their cylindrical shape, were patent and red blood cells could be seen moving along the lumen (Online Movie 4); (2) in vivo, these small vessels function at relatively low pressures (<20 mm Hg)4; and (3) the precapillary arterioles were studied with vasoconstriction produced by Bay K-8644 (10 μmol/L). Traces show: Ca-free solution (i), with a 3 minute gap between the traces; ryanodine (Ry) (50 μmol/L) and nifedipine (Nif) (1 to 10 μmol/L) (ii); 30 minutes gap between the traces; and 2-APB (50 μmol/L) (iii). C, Asynchronous Ca oscillations in 2 sites in a cell and diameter (bottom trace) in a precapillary arteriole. D, Effects of Ca-free solution: nifedipine (i), ryanodine (ii), and 2-APB (iii) on 10 μmol/L PhE-induced Ca signaling in terminal arteriole; 30 minutes gap between the 2 parts.

Our results show that contraction of a single myocyte is sufficient to nearly occlude a precapillary arteriole. The question then arises whether this can also occur when the vessel is pressurized? Using LaPlace’s law and taking force generation in single vascular myocytes to be 0.5×10^5 N • m•^{-2.25} and the wall thickness (diameter of the myocyte) to be 6 μm, then in a precapillary arteriole with a radius of 15 μm, the pressure generated by a single myocyte is around 15 kPa (112 mm Hg). This will clearly exceed that of the pressure in these vessels and therefore contraction of 1 myocyte would be expected to be able to stop blood flow. For the mesenteric artery, using the same values for force generation and cell thickness but a diameter of 120 μm, we calculate a pressure of just 2 kPa (15 mm Hg) is generated by an individual myocyte, which is clearly insufficient to occlude the artery. Although these calculations are only approximate, they emphasize that activity in a single myocyte in a precapillary arteriole can have much more functional effect than is the case in a larger vessel.3
We found that a distinct pattern of Ca signals occurs in precapillary arterioles, with agonists producing only IP₃-mediated Ca waves. Unlike a previous suggestion,26 these signals do not require Ca entry, do not synchronize, and produce only local changes in precapillary arteriolar diameter. Our data reveal that precapillary arterioles do not possess the coordinated and propagated Ca signals necessary for recruiting a group of myocytes and synchronizing their responses to generate vasomotion in larger vessels or the mechanisms responsible for this. Consistent with this lack of synchronization, and in agreement with in vivo studies on some terminal arterioles,27 we did not see the propagated vasoconstriction responsible for communication with upstream arteries in larger vessels with neurogenic stimulation.

These differences in Ca signaling pathways between the precapillary arterioles and small arteries, which underlie their different roles within the circulatory system, presumably reflect differences in expression of ion channels and pumps. A gradient in responses and expression of channels and receptors with vessel diameter has previously been suggested.28 Although poorly defined, earlier studies comparing conduit and resistance vessels5,6 had led to the suggestion that the SR plays a less important role in small vessels.29 Our Ca signaling data have directly tested this in precapillary arterioles and do not support such conclusions. Using Ca-free solutions, we have shown that in precapillary arterioles, Ca entry is not required for agonists to produce constriction but rather IP₃-induced SR Ca release is paramount, and this feature can be regarded as a specialization of these vessels. Indeed, the continued functioning for over an hour in Ca-free solution is unique for smooth muscle. This suggests that in precapillary arterioles, there are either differences in plasma membrane Ca extrusion mechanisms or differences in the SR such as a particularly avid and tightly controlled Ca reuptake mechanism by the SERCA and its accessory proteins.

A surprising finding of the present study was that neither CCh nor NO donors (Na nitroprusside, SNAP) nor a membrane permeable analog of cGMP affected myocyte Ca signaling induced by agonists in precapillary arterioles. That NO donors dilate larger vessels more potently than terminal arterioles has been reported by others,30,31 and has led to the suggestion that guanylate cyclase or other intermediaries are absent from some small vessels,32,33 and that EDHF rather than NO is dominant in small vessels34 (although effects attributed to NO were found in cremaster microcirculation1). Our data are consistent with these findings. They also suggest that the effects of endothelial Ca signaling on arterial myocyte Ca are mediated not via SR Ca release but Ca entry, because CCh stimulation had no effect on SR Ca release but inhibited Ca spikes, which were dependent on Ca entry. We also found the endothelial cells of the arterioles in situ to be
and D. The effects in precapillary arterioles of adding ET-1 (10 nmol/L) and then CCh (1 μmol/L) alone or following CCh. B and D. The effects in precapillary arterioles of adding ET-1 (10 nmol/L) and then CCh (1 μmol/L) or PH (10 μmol/L) alone or following CCh. E and F. Effect of the NO donor SNAP (20 μmol/L) on Ca oscillations induced by PH in mesenteric (E) and vas deferens precapillary arterioles (F). G. The effect of direct muscle stimulation in the presence of Bay K 8644 (1 μmol/L) and tetraethylammonium (TEA) (5 μmol/L) (i). The resulting Ca spikes in myocytes are abolished by Ca-free solution (ii) and by CCh-induced (iii) endothelial Ca signal (iv). H. The lack of effect of SNAP on Ca spikes.

In summary, precapillary arterioles have been studied in more mechanistic detail than previously by using confocal imaging and Ca measurements. We have shown that both their structure and Ca signaling contribute to the functional specialization required in these vessels. The precapillary arterioles will respond functionally to changes in metabolites, paracrine factors, or nervous activity with single-cell control, and this may also explain why the role of the endothelium appears to be unimportant for controlling tone. It remains to be elucidated which factors are most important in different vascular beds and the mechanisms by which they influence myocyte Ca signaling. Changes in the structure and functions of the microcirculation underlie the morbidity associated with conditions such as diabetes, infection, and hypertension.35–38

By revealing differences in the control of function in precapillary arterioles from larger vessels, our work highlights the need for therapeutic interventions to be targeted to these newly described mechanisms.

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**Disclosures**

None.

**References**

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Supplementary Videos

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Movie 1. **Z-stepping through a Fluo-4 loaded precapillary arteriole.** A series of optical sections using spatial module was taken from a Fluo-4 loaded descending branch of the ureteric precapillary arteriole showing full perimeter of the blood vessel.

Movie 2. **Z-stepping through a Fluo-4 loaded precapillary arteriole, using colour to reveal details of myocyte morphology.** Movie 2 is a replica of Movie 1 in which four smooth muscle cells were artificially coloured using Jasc Software Animation Shop Movie maker to reveal details of their morphology.
Movie 3  Calcium signalling and mechanical response induced by activation of a single smooth muscle cell, in precapillary arteriole. Precapillary myocytes were stimulated by 1 μM of phenylephrine. Vasomotion induced by contraction of individual smooth muscle cells facilitates blood flow in precapillary arterioles.

Video 4. Vasomotion produced by mechanical oscillations of individual smooth muscle cell in precapillary arteriole in situ. This movie demonstrates vasomotion produced by mechanical oscillations of a single smooth muscle cell induced by 1nM ET-1 in ureteric precapillary arteriole in transmitted light.

Movie 5. Calcium signalling and mechanical response induced by activation of a single smooth muscle cell, in mesenteric artery. Smooth muscle cells were stimulated by 1 μM of phenylephrine. Vasomotion induced by contraction of individual smooth muscle cells in mesenteric artery had no effect on the diameter of the blood vessel.
Movie 6. Asynchronous intracellular and synchronized intercellular Ca oscillations associated with vasoconstriction/vasomotion evoked by phenylephrine in mesenteric artery myocytes. Mesenteric artery was stimulated by 10 µM phenylephrine.

Movie 7. Inhibition of synchronized Ca oscillations and vasoconstriction/vasomotion in mesenteric artery evoked by phenylephrine after exposure to Ca²⁺-free solution. Mesenteric artery was stimulated by 10 µM phenylephrine after 10 minutes exposure to Ca²⁺-free solution with 2 mM EGTA.
Movie 8. Asynchronous intracellular Ca waves evoked by phenylephrine in smooth muscle cells of precapillary arterioles. Tangential section of ureteric precapillary arterioles stimulated by 1 μM phenylephrine.

Movie 9. Asynchronous intracellular Ca oscillations evoked by endothelin-1 associated with a non-propagating vasoconstriction in a segment of precapillary arteriole. Radial section of precapillary arteriole stimulated by 10nM endothelin -1.
Movie 10. Spontaneous Ca signalling in endothelial cell of ureteric precapillary arteriole. Radial section of precapillary arteriole showing spontaneous activity in individual endothelial cell.

Movie 11. Calcium oscillations induced by carbachol in endothelial cells of precapillary arterioles exposed to Ca-free solution. Precapillary arteriole was pre-treated with Ca free solution with 2mM EGTA for 60 minutes.
Movie 12. Simultaneous recording of intracellular Ca signalling in endothelial cells and mesenteric myocytes: effects of carbachol after phenylephrine. This movie demonstrates that in the rat mesenteric artery activation of endothelial cell Ca signalling blocks Ca oscillations in smooth muscle cells leading to vasodilation.

Movie 13. Simultaneous recording of intracellular Ca signalling in endothelial cells and mesenteric myocytes: effects of phenylephrine after carbachol. This movie demonstrates that in the rat mesenteric artery activation of endothelial cell Ca signalling prior to agonist stimulation of smooth muscle cells selectively blocks synchronized Ca oscillations and vasomotion in rat mesenteric artery.
Movie 14. Lack of effect of endothelial cell Ca signalling on myocyte activity in precapillary arterioles: carbachol following endothelin-1. This movie demonstrates that activation of endothelial cell Ca signalling by carbachol on smooth muscle cells pre-stimulated by agonists did not terminate agonist – induced Ca oscillations in arteriolar myocytes.

Movie 15. Lack of effect of endothelial cell Ca signalling on myocyte Ca signalling in precapillary arterioles: endothelin-1 applied after carbachol. This movie demonstrates that activation of endothelial cell Ca signalling prior to agonist stimulation of arterioles had no effects on agonists – induced Ca oscillations in arteriolar myocytes.

Movie 16. Inhibitory effects of carbachol induced Ca signalling in endothelial cells on Ca spikes evoked by electrical field stimulation in the presence of TEA and Bay-K-8644 in the arteriolar myocytes. This movie demonstrates that activation of Ca signalling in endothelial cells can inhibit Ca signalling in arteriolar myocytes dependent on Ca entry via voltage gated Ca channels.