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Calcium dynamics in vascular smooth muscle

Gregory C. Amberg¹ and Manuel F. Navedo²

¹Vascular Physiology Research Group, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO 80523

²Department of Pharmacology, University of California, Davis, Davis, CA 95616

Abstract

Smooth muscle cells are ultimately responsible for determining vascular luminal diameter and blood flow. Dynamic changes in intracellular calcium are a critical mechanism regulating vascular smooth muscle contractility. Processes influencing intracellular calcium are therefore important regulators of vascular function with physiological and pathophysiological consequences. In this review we discuss the major dynamic calcium signals identified and characterized in vascular smooth muscle cells. These signals vary with respect to their mechanisms of generation, temporal properties, and spatial distributions. The calcium signals discussed include calcium waves, junctional calcium transients, calcium sparks, calcium puffs, and L-type calcium channel sparklets. For each calcium signal we address underlying mechanisms, general properties, physiological importance, and regulation.

Introduction

The otherwise unassuming inorganic divalent cation calcium (Ca^{2+}) is the most important signaling molecule in mammalian cells. Cellular responses to changes in intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) often underlie our definition of what a given cell “is” (22). For example, following an increase in $[\text{Ca}^{2+}]_i$, endocrine cells secrete hormones and neurons release neurotransmitters. Muscle cells, including vascular smooth muscle cells, generally contract when $[\text{Ca}^{2+}]_i$ rises. Ca^{2+} induces contraction in these cells by complexing with the ubiquitous Ca^{2+} binding protein calmodulin and subsequently increasing the activity of myosin light-chain kinase (MLCK; see Figure 1). Changes in the contractile state of vascular smooth muscle either increases or decreases vascular diameter, which in turn increases or decreases blood flow through the vessel and subsequently the vascularized tissue. Thus, events which influence $[\text{Ca}^{2+}]_i$ are critical regulators of vascular function with clear physiological (and pathophysiological) ramifications.

Due to the importance of Ca^{2+} , it is not unexpected that changes in vascular smooth muscle $[\text{Ca}^{2+}]_i$ (i.e., “ Ca^{2+} dynamics”) are governed by a complex array of cellular processes. Indeed, to arrive at the appropriate physiological response, vascular smooth muscle cells must integrate multiple signaling events that influence $[\text{Ca}^{2+}]_i$ either directly or indirectly (see Figure 1). In this paper we provide an overview of the major Ca^{2+} signals that contribute to changes in vascular smooth muscle $[\text{Ca}^{2+}]_i$. Note that many excellent and more extensive reviews on the individual topics discussed here are readily found in the published literature.

Calcium signals in vascular smooth muscle

Sources of Ca^{2+} available for cytoplasmic signaling in vascular smooth muscle cells include Ca^{2+} influx through ion-permeable channels located in the plasma membrane and Ca^{2+} release from intracellular Ca^{2+} stores (e.g., the sarcoplasmic reticulum). Ca^{2+} influx in vascular smooth muscle is mediated primarily by voltage-dependent L-type Ca^{2+} channels (39) with contributions from other channels including but not limited to voltage-dependent T-type Ca^{2+} channels, Ca^{2+} -permeable members of the transient receptor potential (TRP) superfamily of cation channels, and Ca^{2+} -permeable ligand-gated cation channels. Ca^{2+} release from intracellular stores is mediated by two types of Ca^{2+} -permeable ion channels located in sarcoplasmic reticular membranes: 1) Ryanodine receptors and 2) inositol 1,4,5-trisphosphate (IP_3) receptors.

Cytoplasmic signals produced by these Ca^{2+} fluxes can be loosely classified as either more or less localized with respect to total cell volume (see Figure 2). Here we have grouped the “less than global” subcellular Ca^{2+} signals observed in vascular smooth muscle into five categories: 1) Ca^{2+} waves, 2) junctional Ca^{2+} transients, 3) Ca^{2+} sparks, 4) Ca^{2+} puffs, and 5) L-type Ca^{2+} channel sparklets. Note that IP_3 -receptor-mediated Ca^{2+} puffs have not been definitively isolated and visualized in vascular smooth muscle cells to the best of our knowledge. This non-arbitrary categorization of subcellular Ca^{2+} signaling events is based on a wealth of experimental evidence obtained by numerous research groups over the past few decades. The general biophysical characteristics (when known) of each of these subcellular Ca^{2+} signals is presented in Table 1.

In contrast to increased global $[\text{Ca}^{2+}]_i$, which is invariably associated with contraction (29), the role of specific subcellular Ca^{2+} signals is not always apparent. For instance, while Ca^{2+} events such as junctional Ca^{2+} transients are inherently contractile (32, 63, 65), Ca^{2+} microdomains such as Ca^{2+} sparks may induce vasodilation (see below; (46)). This highlights the complexity of Ca^{2+} dynamics in vascular smooth muscle and the need for further investigation of subcellular Ca^{2+} signaling in these cells.

Calcium waves

As suggested by the moniker, Ca^{2+} waves are propagating $[\text{Ca}^{2+}]_i$ elevations produced by sequential series of Ca^{2+} release events from the sarcoplasmic reticulum that extend from one end of the cell to the other (23, 38, 66). Ca^{2+} waves have been shown to be produced by Ca^{2+} release via IP_3 and ryanodine receptors located in the sarcoplasmic reticulum (20, 26). By their nature Ca^{2+} waves inherently require a regenerative Ca^{2+} release mechanism that promotes propagation of the wavefront. This is thought to occur by a Ca^{2+} -induced Ca^{2+} -release (CICR) mechanism between adjacent IP_3 and/or ryanodine receptors (7, 9, 25, 26). A schematic representation of an IP_3 receptor-dependent Ca^{2+} wave is shown in Figure 3. In this scenario, the Ca^{2+} wave is initiated by localized SR Ca^{2+} release via IP_3 -dependent opening of IP_3 receptors. A self-perpetuating Ca^{2+} wave arises when the initiating Ca^{2+} induces Ca^{2+} -dependent opening of adjacent IP_3 receptors (i.e., Ca^{2+} -induced Ca^{2+} -release). Ca^{2+} release via ryanodine receptors has also been implicated in Ca^{2+} wave generation via frequent discharge sites in rat and rabbit portal vein (7, 20, 64). In addition, application of the alkaloid ryanodine eliminated Ca^{2+} waves in pressurized rat cerebral arteries (25, 62). This suggests that in some arterial smooth muscle cells Ca^{2+} waves involve SR Ca^{2+} release via ryanodine receptors.

The occurrence of Ca^{2+} waves in excised vessel preparations is variable to moderate under resting conditions. For example, smooth muscle cells in mouse cremaster feed arteries (62) and rat retinal arterioles exhibit moderate basal Ca^{2+} wave activity (56) while mouse and rat mesenteric arterial smooth muscle cells often possess minimal Ca^{2+} wave activity in the

absence of stimulation (38, 65). The occurrence of basal Ca^{2+} waves appears to be dependent on phospholipase C (PLC) activity (62). Consistent with a role of PLC in regulating Ca^{2+} waves, increased production of IP_3 by PLC following stimulation of α_1 adrenergic receptors by norepinephrine in mesenteric arteries (31) and ET_A receptors by endothelin-1 in retinal arterioles (56) increases Ca^{2+} wave frequency.

Ca^{2+} waves are postulated to contract vascular smooth muscle by at least two distinct mechanisms (21). Ca^{2+} released into the cytosol during the wave event can contribute to global Ca^{2+} thus increasing MLCK activity and promoting contraction (33). This is perhaps the most common interpretation of Ca^{2+} wave function. However, in contrast to this direct mechanism, Ca^{2+} waves can also influence contraction indirectly by interacting with and changing the activity of plasmalemmal Ca^{2+} -activated ion channels. Ca^{2+} waves in rat retinal arteriole smooth muscle cells elicit Ca^{2+} -activated chloride (Cl_{Ca}) currents (56). Stimulation of Ca^{2+} -activated chloride channels results in a depolarization of the plasma membrane via Cl^- efflux (an inward current by convention; see Figure 3), thus enhancing voltage-dependent L-type Ca^{2+} channel activity, and consequently, Ca^{2+} influx, global $[\text{Ca}^{2+}]_i$, and ultimately contraction.

As noted above, Ca^{2+} waves are associated with increased vascular smooth muscle contraction in excised vessels. For example, in rat mesenteric arteries, asynchronous Ca^{2+} waves (with respect to adjacent smooth muscle cells) following neurogenic activation of α_1 receptors by norepinephrine contribute to gradually-developing contractions (31). Contractile responses to bath applied α_1 agonists (e.g., phenylephrine) also include a rapid initial component due to synchronous Ca^{2+} wave activity. This is likely an experimental artifact resulting from strong uniform (i.e., non-physiological) activation of α_1 receptors on a large population of smooth muscle cells (31, 67). Despite a substantial body of experimental data, the physiological significance of Ca^{2+} waves is poorly understood. For example, Ca^{2+} waves in mouse mesenteric arteries appear to cease following the development of tone (65) and evidence suggests that Ca^{2+} waves, which are readily observed in numerous excised vessels (as described above), are not apparent in corresponding *in vivo* experiments (36). Thus, much work is required to understand the physiological role of Ca^{2+} waves in the vasculature.

Junctional Calcium Transients

Short-lived Ca^{2+} influx events evoked by neural (i.e., sympathetic) stimulation of vascular smooth muscle cells are defined as junctional Ca^{2+} transients (34, 63). In contrast to Ca^{2+} waves, junctional Ca^{2+} transients remain localized, as they do not possess a regenerating or propagating mechanism. Action potentials arriving at perivascular sympathetic nerve terminals culminate in release of neurotransmitters, including ATP and norepinephrine. ATP activates postjunctional vascular smooth muscle P2X_1 purinergic receptors and norepinephrine stimulates α_1 adrenergic receptors giving rise to Ca^{2+} waves as described above (31, 32, 35) (see Figure 4).

P2X_1 receptors are ligand-gated Ca^{2+} -permeable nonselective cation channels (16). In vascular beds innervated by the sympathetic nervous system (e.g., mesenteric arteries), stimulation of vascular smooth muscle P2X_1 receptors by ATP generates localized junctional Ca^{2+} transients on account of the relatively high Ca^{2+} permeability of these channels (32, 34). Temporally mirroring the kinetics of the junctional Ca^{2+} transients, the Ca^{2+} influx associated with these events induces a rapid but brief increase in contraction (31, 32). Non-neuronal stimulation of P2X_1 receptors has also been described. In mouse renal afferent arterioles, increasing intraluminal pressure promotes local release of ATP,

stimulation of smooth muscle P2X₁ receptors, and contraction (24). This paracrine signaling mechanism is thought to contribute to autoregulation of blood flow in the kidney.

In addition to evoking local junctional Ca²⁺ transients, activation of P2X₁ receptors by ATP induces depolarizing excitatory junctional potentials as a result of Na⁺ and (to a lesser extent) Ca²⁺ influx (16, 67). Depolarization increases the opening of L-type Ca²⁺ channels resulting in increased Ca²⁺ influx and elevated global [Ca²⁺]_i. Thus, stimulation of P2X₁ receptors with ATP increases vascular smooth muscle Ca²⁺ influx by two independent mechanisms: 1) Local junctional Ca²⁺ transients via Ca²⁺ entry through the P2X₁ receptors and 2) non-localized Ca²⁺ influx through L-type Ca²⁺ channels following depolarizing excitatory junction potentials. These two complimentary Ca²⁺ influx mechanisms summate to underlie the initial component of sympathetic-mediated contraction of rat mesenteric arteries (67).

Calcium Sparks

Ca²⁺ sparks are localized Ca²⁺ microdomains produced specifically by the opening of the ryanodine receptor class of intracellular Ca²⁺ release channels located in sarcoplasmic reticular membranes (12). In vascular smooth muscle Ca²⁺ sparks have minimal direct impact on global [Ca²⁺]_i and contraction as a consequence of their limited spatial spread (46). However, Ca²⁺ sparks can have substantial influence on global [Ca²⁺]_i and contraction through indirect mechanisms. Accordingly, regions of sarcoplasmic reticulum are often found in close juxtaposition to the plasma membrane of vascular smooth muscle cells (~10–20 nm; (14, 28, 57)). As noted above, Ca²⁺-activated plasmalemmal ion channels (e.g., K_{Ca} and Cl_{Ca}) are expressed in various vascular smooth muscle cells. Ca²⁺ sparks occurring in close proximity to these channels can increase local [Ca²⁺]_i to levels sufficient (i.e., μM; (48)) for channel activation (see Figure 5). Thus, the contractile response of vascular smooth muscle to Ca²⁺ sparks depends on the expression and coupling of the sparks to Ca²⁺-activated plasmalemmal ion channels.

In rat and mouse cerebral arteries, large-conductance, Ca²⁺-activated K⁺ (BK; maxi K) channels are a primary K_{Ca} target for Ca²⁺ sparks (27, 46). Ca²⁺ spark activation of BK channels results in a stereotypical temporal pattern of hyperpolarizing K⁺ currents called spontaneous transient outward currents (STOCs) (6, 46). Simultaneous recording of intracellular Ca²⁺ and membrane potential has provided definitive evidence that Ca²⁺ sparks give rise to STOCs as the two events correlate not only in time but also in magnitude (1, 10, 46, 48). In rat cerebral arteries elimination of Ca²⁺ sparks eliminates contractile responses to BK channel inhibition (30, 46) thus indicating that Ca²⁺ sparks are the physiological activators of BK channels in these vascular smooth muscle cells.

In rabbit portal vein, Ca²⁺ sparks are coupled to Cl_{Ca} channels (59). In contrast to Ca²⁺ spark activation of BK channels, which results in hyperpolarization, Ca²⁺ spark activation of Ca²⁺-activated Cl[−] channels causes plasma membrane depolarizing outward currents (via Cl[−] efflux; see Figure 5). Consequently, similar to Ca²⁺-activated K⁺ channels, activation of Ca²⁺-activated Cl[−] channels by Ca²⁺ sparks produces a signature pattern of inward current called spontaneous transient inward currents (STICs) that correlate in time and magnitude with each other. Depolarization induced by Ca²⁺-activated Cl[−] channel activity results in activation of voltage dependent L-type Ca²⁺ channels and increased contraction (see Figure 5) (51).

The dependence of Ca²⁺-activated ion channels on underlying Ca²⁺ sparks provides an opportunity for regulation of vascular smooth muscle function by vasodilators and vasoconstrictors. In rat cerebral arteries, vasodilators increase the hyperpolarizing influence of BK channels by increasing the sensitivity of the BK channels to Ca²⁺ and by increasing

Ca^{2+} spark (thus STOC) frequency (49, 54). These effects are mediated by activation of adenosine 3', 5'-cyclic monophosphate (cAMP)/protein kinase A (PKA) and guanosine 3', 5'-cyclic monophosphate (cGMP)/protein kinase G (PKG) signaling cascades. Thus, vasodilators can increase BK channel activity by increasing the coupling strength between the channel and the Ca^{2+} sparks (increasing STOC amplitude) and increasing the occurrence of Ca^{2+} sparks (increasing STOC frequency). Conversely, vasoconstrictors associated with protein kinase C (PKC) decrease the hyperpolarizing influence of BK channels by decreasing BK channel sensitivity to Ca^{2+} (reducing STOC amplitude) and by reducing the occurrence of Ca^{2+} sparks (reducing STOC frequency) (8, 54).

The coupling strength between Ca^{2+} sparks and BK channels is also associated with vascular dysfunction during diseases such as hypertension. The sensitivity of vascular smooth muscle BK channels to Ca^{2+} is greatly dependent on expression of BK channel $\beta 1$ subunits (10). Genetic ablation of the $\beta 1$ subunit decreases the sensitivity of cerebral arterial smooth muscle BK channels to Ca^{2+} . As a consequence, Ca^{2+} sparks in these cells do not efficiently generate corresponding STOCs (10). This results in increased contraction of excised cerebral arterial segments and increased mean arterial blood pressure. Similarly, in rat models of hypertension, the function and expression of BK channel $\beta 1$ subunits in cerebral arteries is decreased and contributes to vascular dysfunction (1, 4).

Calcium Puffs

Ca^{2+} puffs are localized Ca^{2+} microdomains produced by the opening of sarcoplasmic reticulum IP_3 receptors (58). Although Ca^{2+} puffs have been observed in colonic smooth muscle cells (5), they have not been visualized in vascular smooth muscle cells. However, indirect experimental evidence in rat cerebral arterial smooth muscle cells indicates that physiologically-relevant localized IP_3 receptor-dependent Ca^{2+} release does occur (17, 18). Indeed, in these smooth muscle cells, IP_3 -dependent Ca^{2+} release has been shown to promote the opening of transient receptor potential melastatin 4 (TRPM4) channels. Opening of these Ca^{2+} -activated, Na^+ -permeable cation channels causes arterial smooth muscle membrane depolarization, opening of voltage-dependent L-type Ca^{2+} channels, Ca^{2+} influx, and ultimately contraction (see Figure 6) (17–19). A more detailed explanation of these findings can be found in the accompany paper by Gonzales and Earley in this special issue of Microcirculation.

Calcium Sparklets

Ca^{2+} sparklets are distinct Ca^{2+} microdomains produced by plasmalemmal Ca^{2+} -permeable channels. Despite the name, Ca^{2+} sparklets are *not* small Ca^{2+} sparks. Ca^{2+} sparklets arise from Ca^{2+} influx through plasmalemmal ion channels (see below) and (as discussed above) Ca^{2+} sparks arise from the release of Ca^{2+} from the sarcoplasmic reticulum through ryanodine receptors (see Figure 7).

The original definition of a “ Ca^{2+} sparklet” was a visualized Ca^{2+} influx event produced by a voltage-dependent L-type Ca^{2+} channel (60). Since that time, Ca^{2+} influx events produced by other Ca^{2+} -permeable channels have been visualized and referred to as “ Ca^{2+} sparklets” (e.g., (55). To remove ambiguity we suggest that the term “ Ca^{2+} sparklet” be refined to specifically designate the visualization of a Ca^{2+} influx event through a Ca^{2+} -permeable plasmalemmal ion channel. The need for different terms denoting Ca^{2+} influx through different channels can then be eliminated by preceding “sparklets” with the name of the underlying channel (e.g., “L-type Ca^{2+} channel sparklets”, “TRPV4 sparklets”, etc...).

L-Type Calcium Channel Sparklets

Cav1.2 L-type Ca^{2+} channels are the main source of Ca^{2+} influx in vascular smooth muscle cells (39). Conventional electrophysiological recordings of steady-state L-type Ca^{2+} channel activity (50) provide no information with regard to potential spatial heterogeneity of L-type Ca^{2+} channel activity throughout the smooth muscle plasma membrane. However, when conventional electrophysiology and advanced Ca^{2+} imaging techniques (e.g., total internal reflection fluorescence (TIRF) microscopy) are used together this experimental limitation is overcome (41,60).

Using a combinatorial approach of electrophysiology and TIRF microscopy, Ca^{2+} influx through single L-type Ca^{2+} channels (i.e., L-type Ca^{2+} channel sparklets) is readily observed in smooth muscle cells isolated from rat and mouse cerebral and mesenteric arteries (2, 3, 40–42). Data from experiments using this approach have confirmed the importance of Cav1.2 channels in vascular smooth muscle and yielded unexpected information with regard to spatial organization and regulation of L-type Ca^{2+} channels (40, 41)). Previous conventional electrophysiological data suggested that, under steady-state conditions, Ca^{2+} influx in vascular smooth muscle cells was the end result of stochastic opening of L-type Ca^{2+} channels dispersed broadly throughout the plasma membrane (50). However, the exceptional temporal and spatial resolution provided by TIRF microscopy revealed that Ca^{2+} influx in vascular smooth muscle cells through L-type Ca^{2+} channels is not stochastic but rather segregates strikingly into sites of low and high activity (see Figure 7).

Consistent with conventional electrophysiological data, sites of low activity L-type Ca^{2+} channel influx arise from stochastic opening of randomly dispersed L-type Ca^{2+} channels. In contrast, sites of high activity L-type Ca^{2+} channel function result from non-stochastic, apparently coordinated opening of clustered channels (15, 43). High activity L-type Ca^{2+} channel sparklet sites require AKAP150-targeted kinase activity (i.e., PKC and PKA), account for approximately 50 % of the steady-state Ca^{2+} entry through L-type Ca^{2+} channels in isolated rat and mouse cerebral arterial smooth muscle cells. In addition, High activity L-type Ca^{2+} channel sparklets contribute to myogenic tone in mouse mesenteric arteries and are necessary for contractile responses of mouse mesenteric and rat cerebral arteries to angiotensin II (3, 11, 44, 45, 47). Microdomains of elevated Ca^{2+} generated by high-activity L-type Ca^{2+} channel sparklet sites also induce changes in gene expression via specific activation of the Ca^{2+} /calcineurin/NFAT signaling pathway (47, 52, 53). A more detailed explanation of these concepts can be found in the accompany paper by Navedo and Amberg in this special issue of Microcirculation.

Other Considerations and Future Directions

The Ca^{2+} signals described above are defined by the molecular mechanisms underlying their initiation and are classified as different “less than global” Ca^{2+} events with unique properties. While mechanisms underlying Ca^{2+} entry into vascular smooth muscle cytosol clearly distinguish one Ca^{2+} signal from another, termination of these events is also critical. Ca^{2+} signals in vascular smooth muscle are terminated by no less than five distinct mechanisms: 1) Diffusion into the surrounding cytosol, 2) extrusion via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, 3) extrusion via the plasma membrane Ca^{2+} -ATPase, 4) sequestration via the sarcoplasmic reticular Ca^{2+} -ATPase (SERCA), and 5) sequestration and redistribution via mitochondria. Detailed discussion of these important mechanisms in regulating Ca^{2+} signaling in vascular smooth muscle is beyond the scope of this review. However, these mechanisms clearly influence the vascular smooth muscle Ca^{2+} dynamics. For example, mitochondria are known to be important modulators of IP_3 -dependent Ca^{2+} signaling (37), the $\text{Na}^+/\text{Ca}^{2+}$ exchanger regulates L-type Ca^{2+} channel function (68), and sarcoplasmic

reticular Ca^{2+} -ATPase function influences ryanodine receptor-dependent Ca^{2+} sparks (13, 61).

Three major areas of future research are necessary to further our understanding of Ca^{2+} dynamics in vascular smooth muscle. First, cryptic Ca^{2+} signals such as Ca^{2+} puffs (see above) need to be visualized directly. Similarly, novel, discrete Ca^{2+} signals associated with store operated Ca^{2+} entry (SOCE) are in need of identification and characterization. Second, the relationship between local Ca^{2+} signals (e.g., Ca^{2+} waves and Ca^{2+} sparklets) and global Ca^{2+} needs to be clarified. Third and finally, future investigations should explore Ca^{2+} dynamics with respect to mechanisms regulating intracellular and intercellular signaling in the intact vascular syncytium. Experiments along these lines are necessary not only to assess the validity of experiments performed on isolated smooth muscle cells, but also to examine the influence of other cell types (i.e., endothelial cells) on Ca^{2+} signaling in vascular smooth muscle. Advances in Ca^{2+} imaging techniques such as high-speed confocal microscopy and availability of cell-specific Ca^{2+} indicators are already and will continue to be critical in advancing these areas of research.

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Figure 1. Overview of calcium dynamics in vascular smooth muscle

Ca²⁺ influences the state of vascular smooth muscle contraction directly and indirectly. Direct mechanisms include changes (Δ) in Ca²⁺ influx and release from intracellular Ca²⁺ stores leading to increased Ca²⁺ within the cell, binding of Ca²⁺ to calmodulin (CaM), and activation of myosin light chain kinase (MLCK). Indirect mechanisms include changes in Ca²⁺ influx via alterations in plasma membrane potential (Vm), Ca²⁺ release from intracellular stores, Ca²⁺ sequestration, and Ca²⁺ sensitization of the contractile apparatus.

Figure 2. Overview of calcium signaling events in vascular smooth muscle

Discrete subcellular Ca^{2+} signaling events (junctional Ca^{2+} transients, Ca^{2+} waves, Ca^{2+} sparks, Ca^{2+} puffs, and Ca^{2+} sparklets) can be classified as either more or less localized with respect to total cell volume. * Direct visualization of Ca^{2+} puffs in arterial smooth muscle is lacking; however, abundant indirect evidence supports their existence.

Figure 3. Vascular smooth muscle calcium waves

Norepinephrine (NE)-dependent stimulation of α_1 adrenergic receptors (α_1R) on the vascular smooth muscle cell plasma membrane (VSMC PM) leads to activation of phospholipase C (PLC) and the production of inositol 1,4,5-trisphosphate (IP3). IP3 in turn promotes the opening of IP3 receptors (IP3R) on the sarcoplasmic reticulum (SR) resulting in a propagating series of Ca^{2+} release events (i.e., a Ca^{2+} wave). Ca^{2+} waves increase contraction directly by elevating global intracellular Ca^{2+} ($[Ca^{2+}]_i$). Ca^{2+} waves also modulate contraction indirectly by altering plasma membrane potential (and thus voltage-dependent Ca^{2+} influx through Cav1.2 L-type Ca^{2+} channels) by stimulating plasmalemmal Ca^{2+} -activated chloride channels.

Figure 4. Vascular smooth muscle junctional calcium transients

Neuronal action potentials (AP) promote the release of ATP and norepinephrine (NE) from perivascular nerve terminals. Norepinephrine stimulates α_1 adrenergic receptors (α_1R) giving rise to Ca^{2+} waves as described in Figure 3 while ATP opens Ca^{2+} -permeable purinergic receptors (P2XR) on the vascular smooth muscle cell plasma membrane (VSMC PM) producing localized junctional Ca^{2+} transients. Purinergic receptor activation also leads to plasma membrane depolarization (+ Vm) and increased opening of voltage-dependent Cav1.2 L-type Ca^{2+} channels (L). Ca^{2+} influx from junctional Ca^{2+} transients and L-type Ca^{2+} channels summate to increase contraction.

Figure 5. Vascular smooth muscle calcium sparks

Ca^{2+} -permeable ryanodine receptors (RR) on the sarcoplasmic reticulum (SR) produce highly localized sites of elevated Ca^{2+} (i.e., Ca^{2+} sparks). Ca^{2+} sparks near the vascular smooth muscle cell plasma membrane (VSMC PM) increase the activity of plasmalemmal ion channels including Ca^{2+} activated K^+ (K) and Ca^{2+} -activated Cl^- (Cl) channels. Ca^{2+} spark-dependent activation of K^+ channels generates hyperpolarizing outward currents (via K^+ efflux) resulting in decreased opening of voltage-dependent Cav1.2 L-type Ca^{2+} channels (L) and decreased contraction. Conversely, Ca^{2+} spark-dependent activation of Cl^- channels generates depolarizing ($+V_m$) inward currents (via Cl^- efflux) resulting in increased opening of L-type Ca^{2+} channels and increased contraction.

Figure 6. Localized IP₃ receptor-dependent SR Ca²⁺ release in vascular smooth muscle
 Ca²⁺-permeable inositol 1,4,5-trisphosphate receptors (IP₃R) on the sarcoplasmic reticulum (SR) can produce non-propagating localized sites of elevated Ca²⁺. IP₃ receptor Ca²⁺ release events near the vascular smooth muscle cell plasma membrane (VSMC PM) increase the activity of plasmalemmal ion channels such as transient receptor potential melastatin 4 (TRPM4). Ca²⁺ puff-dependent activation of TRPM4 channels generates depolarizing (+ V_m) inward currents (via Na⁺ influx) resulting in increased opening of voltage-dependent Cav1.2 L-type Ca²⁺ channels (L) and increased contraction.

Figure 7. Vascular smooth muscle L-type calcium channel calcium sparklets

Cav1.2 L-type Ca^{2+} channels (L) in the vascular smooth muscle cell plasma membrane (VSMC PM) produce highly localized Ca^{2+} influx events (i.e., Ca^{2+} sparklets). Brief stochastic opening of single L-type Ca^{2+} channels produce low activity Ca^{2+} sparklet sites while prolonged opening of one or more clustered L-type Ca^{2+} channels produce high activity Ca^{2+} sparklet sites. Ca^{2+} influx from low and high activity Ca^{2+} sparklet sites contribute to global intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) resulting in contraction. Elevated Ca^{2+} concentrations at high activity Ca^{2+} sparklet sites (and perhaps global intracellular Ca^{2+} as well) contribute to changes in gene expression associated with increased contraction.

Table 1

Properties of vascular smooth muscle subcellular Ca^{2+} signals (approximate values). NA = not available.

	Ca^{2+} waves (31, 61)	Junctional Ca^{2+} transients (34)	Ca^{2+} sparks (8, 26)	Ca^{2+} puffs	Ca^{2+} sparklets (3, 41)
Area (μm^2)	$\approx 50 \mu\text{m}$ (full-width, half maximum)	≈ 25	≈ 15	NA	≈ 1
Duration ($t_{0.5}$; ms)	NA	≈ 150	≈ 50	NA	$\tau_1 \approx 35$ $\tau_2 \approx 150$
Amplitude (F/F_0)	≈ 2	≈ 3	$\approx$	NA	NA