Published in final edited form as:

Basic Clin Pharmacol Toxicol. 2012 January ; 110(1): 80–86. doi:10.1111/j.1742-7843.2011.00798.x.

Calcium and Electrical Signaling along Endothelium of the Resistance Vasculature

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Abstract

This MiniReview is focused on the nature of intercellular signaling along the endothelium that helps to coordinate blood flow control in vascular resistance networks. Vasodilation initiated by contracting skeletal muscle ascends from arterioles within the tissue to encompass resistance arteries upstream and thereby increase blood flow during exercise. In resistance vessels, acetylcholine microiontophoresis or intracellular current injection initiates hyperpolarization that conducts through gap junction channels (GJCs) along the vessel wall resulting in conducted vasodilation (CVD). Both ascending vasodilation and CVD are eliminated with endothelial cell (EC) disruption, pointing to common signaling events and mutual dependence upon EC integrity. As demonstrated by electrical coupling and dye transfer during intracellular recording, their longitudinal orientation and robust expression of GJCs enable ECs to play a predominant role in CVD. Once conduction is initiated, a major interest centers on whether CVD is purely passive or involves additional “active” signaling events. Here, we discuss components for Ca^{2+} and electrical signaling with an emphasis on intercellular coupling through endothelial GJCs. We stress the importance of understanding relationships between intracellular Ca^{2+} dynamics, EC hyperpolarization and CVD while integrating findings from isolated ECs into more complex interactions *in vivo*. Whereas endothelial dysfunction accompanies cardiovascular disease and the components of intra- and inter-cellular signaling are increasingly well defined, little is known of how Ca^{2+} signaling and electrical conduction along microvascular endothelium are altered in diseased states. Thus, greater insight into how these relationships are governed and interact is a key goal for continued research efforts.

Coordination of Blood Flow Control

Throughout the body, the magnitude and distribution of tissue blood flow are governed by resistance microvessels (internal diameter 20–150 μm) which are typically organized as branching arteriolar networks that arise from parent feed arteries (FAs) [1]. From a resting level of vasomotor tone, vasodilation within an arteriolar network increases the delivery of oxygen and nutrients to, and metabolite removal from, tissue regions of cellular activity. When vasodilation is localized to discrete vessel segments or individual branches, any increase in local blood flow is restricted. However, intercellular signal transmission through gap junction channels (GJCs) enables dilation initiated at discrete sites to spread from cell to cell along and between vessel branches and from daughter branches into proximal arterioles and FAs. This coordinated vasomotor response reduces total network resistance to blood flow, thereby augmenting tissue perfusion [2]. Contraction of skeletal muscle fibers or

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stimulation with acetylcholine (ACh) consistently evokes such coordinated responses from the resistance vasculature, giving rise to reproducible paradigms for studying what are now recognized as ascending vasodilation in response to exercise and conducted vasodilation (CVD) in response to ACh [2]. While inferred during exercise, key signaling events that have been shown to underlie CVD using ACh as a stimulus entail the initiation of hyperpolarization through intermediate (IK_{Ca} ; $K_{Ca3.1}$) and small (SK_{Ca} ; $K_{Ca2.3}$) Ca^{2+} -activated K^+ channels (referred to collectively as IK_{Ca}/SK_{Ca}) and the ensuing spread of hyperpolarization along the endothelium [3], with vasodilation occurring as hyperpolarization spreads into smooth muscle cells (SMCs) through myoendothelial GJCs [4]. The focus of this MiniReview is on understanding the mechanisms by which Ca^{2+} and electrical signals are initiated within and communicated among endothelial cells (ECs) of the resistance vasculature. Further, we identify areas for continued research efforts that are essential to providing insight needed to address how these processes are altered with vascular disease.

Calcium Signaling in Microvascular Endothelial Cells

Intracellular calcium release in response to agonist stimulation

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is a key regulator of EC function [5]. Elevations in EC $[Ca^{2+}]_i$ can occur as a result of receptor activation, mechanical stress or impairment of Ca^{2+} reuptake by the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA). Receptor activation via G protein coupled receptor (GPCR) agonists such as bradykinin, angiotensin II, serotonin and ACh is a well-characterized signaling event that increases EC $[Ca^{2+}]_i$. Since the discovery that ACh induces smooth muscle cell (SMC) relaxation via an EC-dependent pathway [6], it has been used as a model GPCR agonist that reproducibly induces elevations in EC $[Ca^{2+}]_i$. Upon binding to muscarinic (M3) receptors on the plasma membrane of ECs, ACh activates phosphoinositide phospholipase C- β , which then hydrolyses phosphatidylinositol-4,5-bisphosphate to yield inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). In turn, IP_3 initiates release of Ca^{2+} from the endoplasmic reticulum (ER) through binding to IP_3 receptors (IP_3R), thereby elevating $[Ca^{2+}]_i$. Three isoforms of IP_3R (types 1, 2 and 3) have been identified, and upon binding of IP_3 , release Ca^{2+} from ER into the cytosol [5]. Refilling of ER Ca^{2+} content is dependent on the SERCA. The ER Ca^{2+} accounts for approximately 75% of the total Ca^{2+} storage in ECs with the remaining 25% of intracellular Ca^{2+} stored within the mitochondria [5]. Mitochondria may release Ca^{2+} following ER release, thereby amplifying Ca^{2+} signals detected within the cytosol [5]. Alternatively, as demonstrated in human umbilical vein ECs, mitochondrial Ca^{2+} flux may also contribute to refilling ER Ca^{2+} stores [7]. However, the role of mitochondria in Ca^{2+} homeostasis of microvascular ECs has not been studied mechanistically and this topic is primed for future investigation, particularly in light of blood flow control.

The second family of Ca^{2+} release channels on the ER encompasses the ryanodine receptors (RyR). Three isoforms of RyR have been identified (types 1, 2 and 3) and are highly expressed in skeletal muscle (predominantly RyR1), cardiac myocytes (mainly RyR2) and smooth muscle (all 3 isoforms) [8]. Whereas RyR have been identified in aortic ECs of rabbits, pigs and human beings, there is a distinct lack of evidence for the expression of RyR in ECs of the resistance vasculature. Caffeine, an activator of RyR, has been shown to have no effect on microvascular EC Ca^{2+} levels [9], suggesting that RyR may not be expressed in arteriolar ECs. Moreover, even in aortic ECs that express RyR, the predominant mechanism for ER Ca^{2+} release appears to be through IP_3R s [8]. Collectively, current evidence supports the conclusion that RyR do not play a major role in mobilizing Ca^{2+} from the ER within ECs.

Extracellular calcium influx secondary to intracellular release

Two mechanisms account for the influx of Ca^{2+} from the extracellular fluid across the plasma membrane into the cytosol of ECs: Receptor-activated calcium entry (RACE) and store-operated calcium entry (SOCE) [5]. While the intracellular release of Ca^{2+} in response to GPCR activation is well defined, Ca^{2+} influx in response to ACh in microvascular ECs remains an enigma. Evidence suggests that DAG may play a role in RACE. For example, in non-excitable cells, transient receptor potential canonical (TRPC) Ca^{2+} channels can be activated by DAG [10]. As microvascular ECs have been shown to express multiple isoforms of TRPC channels [10], DAG released in response to GPCR stimulation may activate TRPC channels, resulting in Ca^{2+} influx secondary to intracellular release. In turn, sustained activation of IP_3Rs leads to depletion of ER Ca^{2+} stores, thereby activating SOCE. One mechanism for ER Ca^{2+} store repletion involves stromal interaction molecule 1 (STIM1), a protein in the membrane of the ER that serves as a sensor of Ca^{2+} stores within the ER. In response to store depletion, STIM1 molecules translocate to form a complex in regions of the ER near the plasma membrane [11] where the Orai1 protein functions as a selective Ca^{2+} channel that is gated through interacting with STIM1, effectively serving as the store-operated channel. Analogous to IP_3Rs and RyRs, there are 3 isoforms of Orai1, and different combinations of respective isoforms may result in store-operated channels with distinct properties of regulation. In brief, with depletion of ER Ca^{2+} stores, STIM1 molecules form multimers on the ER membrane in proximity to Orai1 in the plasma membrane to enable Ca^{2+} influx that leads to refilling of ER Ca^{2+} stores [11]. While Orai1 proteins are capable of multimerizing and forming channels, evidence suggests that they may interact with TRPC channels or that STIM1 can interact directly with TRPC channels to facilitate SOCE [12]. It should be recognized that these relationships have been defined in model systems other than the microcirculation; thus, the role of STIM1 and Orai1 in regulating EC Ca^{2+} signaling in the resistance vasculature remains to be investigated.

Intercellular conduction through gap junction channels

In contrast to the circumferential orientation of SMCs and their paucity of GJCs in resistance vessels, the longitudinal orientation of ECs and their robust homocellular coupling through GJCs favours the endothelium as the cellular pathway by which signals travel along the vessel wall [13–15]. Indeed, in studies where the endothelium of FAs [14] or arterioles [15] was selectively damaged, vasodilation did not conduct beyond the area of damage. In contrast, selective disruption of SMCs had negligible effect on CVD [14]. Remarkably, ascending vasodilation in response to skeletal muscle contraction is similarly curtailed following EC damage [2]. Gap junctions typically exist as plaques of individual channels that are clustered between adjacent cells. Each GJC consists of two hemichannels (connexons) respectively comprised of 6 connexin (Cx) protein subunits. Thus, each cell contributes one connexon for each GJC and connexons from adjacent cells dock with each other to allow direct passage of ions and small molecules (<1 kDa; e.g., IP_3) between coupled cells. Four Cx isoforms have been identified in blood vessels: Cx37, Cx40, Cx43 and Cx45 [16, 17]. With some regional variation, arterioles and FAs express Cx37, Cx40 and Cx43 at the borders of ECs [15]. While these are the apparent isoforms responsible for EC: EC coupling, it remains to be resolved whether these GJCs exist as homomeric, heteromeric and/or heterotypic channels and each may have distinct properties for regulation (e.g., by phosphorylation, nitrosylation or voltage).

As shown in pressurized FAs isolated from the hamster retractor muscle, local stimulation (e.g., with ACh released from a micropipette) can induce a Ca^{2+} wave that travels along the endothelium and contributes to CVD through the release of nitric oxide (NO) [3]. The Ca^{2+} wave spreads more slowly (~ 100 $\mu\text{m/s}$) when compared to the more rapid (cm/s) electrical conduction of vasodilation mediated through the spread of hyperpolarization [3]. In turn, the

release of NO in the wake of hyperpolarization contributes to enhancing the duration of vasodilation along the vessel, albeit over shorter distances (< 1 mm) when compared to electrical signaling over several mm and multiple branches (fig. 1). Additional evidence for Ca^{2+} waves along microvascular endothelium come from intravital studies of arterioles in the cremaster muscle of $\text{Cx40}^{\text{BAC}}\text{-GCaMP2}$ transgenic mice [18]. These animals express a Ca^{2+} -sensitive fluorescent protein selectively in precapillary ECs through a genetically encoded calcium indicator molecule (GCaMP2) under endogenous transcriptional regulation of Cx40. Key questions underlying Ca^{2+} signaling along microvascular endothelium center on the contribution of Ca^{2+} influx *versus* Ca^{2+} release from internal stores along with the nature of the signal traveling between consecutive ECs. Discerning the role(s) of intercellular Ca^{2+} diffusion from IP_3 diffusion [19] will provide insight needed to resolve the signaling events contributing to this Ca^{2+} -dependent component of spreading vasodilation. In light of endothelial dysfunction during vascular disease [20–24], it also remains to be determined how these signaling events may be affected in such widespread conditions as obesity, diabetes and hypertension.

Electrical Signaling in Microvascular Endothelial Cells

Endothelium as an electrical conduit

Elevation of EC $[\text{Ca}^{2+}]_i$ (e.g., via stimulation with ACh) activates $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ to initiate hyperpolarization [16, 25, 26]. This change in membrane potential (V_m) then spreads along the endothelium and into SMCs via projections that penetrate the internal elastic lamina to form myoendothelial GJCs [17]. In turn, closure of voltage-gated (L-type) Ca^{2+} channels as SMCs hyperpolarize effectively lowers their $[\text{Ca}^{2+}]_i$ to produce vasodilation [16, 25]. In light of the endothelium serving as the predominant cellular pathway for electrical conduction [13–16], it should be recognized that individual ECs in resistance vessels are up to ~ 100 μm long [13], thus each EC may contact ~ 20 SMCs (each ~ 5 μm in diameter) along the vessel wall. In a complementary manner, each SMC (also ~ 100 μm long when relaxed) contacts up to 20 ECs (each ~ 5 – 7 μm wide) as it encircles the intima [4]. These morphological relationships promote the spread of hyperpolarization and vasodilation among cells of the vascular wall. Independent of receptor occupation or ion channel activation, intracellular injection of hyperpolarizing current evokes CVD [4, 27], substantiating the role of electromechanical coupling in rapidly promoting an increase in vascular conductance.

Conducted hyperpolarization and vasodilation: active versus passive

Electrical conduction appears to involve active or “regenerative” components that promote the spread of hyperpolarization and vasodilation along the vessel wall [28, 29]. Due to technical limitations in measuring V_m and diameter simultaneously at multiple sites along resistance networks, controversy remains as to whether active mechanisms indeed exist, as well as the nature of underlying signaling events. Nevertheless, evidence that vasomotor responses decay to an asymptote above resting baseline implicates a component of the conducted response that is not simply passive [28]. Insight to such components may be gained from knowing which ion channels are expressed that may contribute to this behaviour. For example, activation of inward rectifying K^+ channels (K_{IR}) in either ECs or SMCs would contribute additional outward current to the initial hyperpolarizing signal and thereby augment the spread of hyperpolarization along the vessel wall. Another contributing factor may involve enhanced Na^+/K^+ -ATPase activity in response to local elevations in $[\text{K}^+]_o$ [25, 30]. Irrespective of such events, the ability of hyperpolarization to travel along the endothelium reflects the biophysical properties of low axial resistance (through GJCs) between ECs relative to high membrane resistance. Further, owing to the high input

resistance of SMCs, little charge is required to move from the endothelium into SMCs (through myoendothelial GJCs) to promote hyperpolarization and relaxation [31].

The ability of a signal to travel along the endothelium may also be influenced by the nature of the initial stimulus. For example, in FAs of the hamster retractor muscle, the length constant describing the rate of decay for conducted hyperpolarization in response to current injection (~1.2 mm) was significantly less than that obtained for local stimulation with ACh (~1.9 mm) [27]. This difference implied that stimulation with ACh (local activation of muscarinic receptors) augmented the spread of hyperpolarization. Though the mechanism remains to be determined, it is tempting to suggest that muscarinic receptor stimulation (e.g., release of Ca^{2+} from the ER to activate $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$) is associated with an additional component of hyperpolarization (or other determinant of microvessel electrical properties) that is not manifest when V_m is changed passively by current injection. Moreover, current injection differs fundamentally from ACh delivery in that the stimulus is delivered into a single cell with a microelectrode independent of initial Ca^{2+} signaling, whereas muscarinic receptors of multiple ECs initiate hyperpolarization through GPCR-mediated signaling in response to ACh released from a micropipette. The activation of multiple cells (e.g., in response to muscle contraction) more likely represents physiological conditions encountered *in vivo*.

The nature of ion channels (e.g. $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$) and/or Cx isoform expression in the endothelium that govern the properties of electrical conduction have also been a subject of intense interest [26, 28]. Arterioles of the cremaster muscle in transgenic mice deficient in IK_{Ca} ($\text{K}_{\text{Ca}}3.1^{-/-}$) exhibited a significant decrease of CVD in response to ACh when compared to control animals or those in which $\text{K}_{\text{Ca}}2.3$ (SK_{Ca}) was inhibited pharmacologically [26]. Further, the amplitude of hyperpolarization in response to ACh was attenuated significantly in $\text{K}_{\text{Ca}}3.1^{-/-}$ mice *versus* controls, indicating that $\text{K}_{\text{Ca}}2.3$ alone could not compensate for the loss of $\text{K}_{\text{Ca}}3.1$ in producing a change in V_m in response to ACh. Thus, impaired CVD observed in $\text{K}_{\text{Ca}}3.1^{-/-}$ mice may reflect both attenuated initiation of hyperpolarization (and the ensuing vasodilation) as well as the conduction process itself (e.g., compromised ability to maintain hyperpolarization along the arteriolar endothelium). However, with V_m recorded only at the site of stimulation (i.e., initiation of hyperpolarization) [26], additional studies are required to see how the loss of $\text{K}_{\text{Ca}}3.1^{-/-}$ (or other ion channels) affects electrical signaling along arterioles. With respect to the role of GJCs, expression of Cx40 was required for regenerative CVD as deletion of this isoform resulted in conduction that decayed rapidly with distance; in contrast, deletion of Cx37 had no effect [28]. While these observations are intriguing, there were no complementary recordings of V_m . Thus, additional studies are required to resolve the underlying electrical events associated with targeted deletion of specific ion channels or Cx isoforms. While technically challenging, electrophysiology continues to be an important tool for gaining the mechanistic insight required to more completely understand the role of electrical signaling in governing CVD whether using pharmacological interventions or transgenic mice as model systems.

Resolving passive versus active electrical conduction

Recent work employing simultaneous measurements of diameter and V_m in arterioles of the mouse cremaster muscle has demonstrated the passive decay of hyperpolarization in response to a local ACh stimulus [32]. Remarkably, dilation was not attenuated along the same distance over which hyperpolarization decayed (from the local site of stimulation to 1.5 mm along arterioles). This behaviour was attributed to a restricted linear relationship between hyperpolarization and dilation, whereby the degree of hyperpolarization that decayed over this distance still met or exceeded the threshold necessary to maintain dilation [32]. In contrast to the proportionality demonstrated between changes in V_m and changes in

arteriolar diameter (~1 mV per 1 μm dilation) [4, 33], a non-linear relationship between these variables is consistent with the possibility of active mechanisms involved in electrical conduction from an endothelium-dependent stimulus. However, limitations to understanding properties intrinsic to the endothelium persist under conditions, either *in vivo* or *in vitro*, where the behaviour of the endothelium can be influenced by blood flowing through the vessel lumen and the presence of SMCs. Whether performed in isolated intact vessels or in microvascular networks of anaesthetized rodents, when SMCs (and vasomotor responses) are present, measurements of V_m are also limited with respect to duration as well as the number of sites that can be recorded from with sufficient resolution to accurately determine length constants for conducted responses.

Despite its widespread use as an intravital preparation, the question persists as to whether the cremaster muscle is representative of resistance microvessels throughout the body. To study the properties of electrical conduction intrinsic to the endothelium, investigators have developed models using cultured ECs [34]. While insightful relationships have been elucidated (e.g., how intercellular coupling is impacted by sepsis [34]), neither the morphology nor the phenotype of ECs maintained in culture reflect those of the intact microvessel. An alternative approach involves dissociating pericytes or SMCs [9] while leaving the underlying endothelium intact. Though the absence of SMCs and of blood flow remove important physiological influences, such models can provide unique insight to directly address specific mechanisms of signal initiation and determinants of intercellular coupling intrinsic to the endothelium. In light of earlier discussion, a key question centers on the dependence (or lack thereof) on changes in V_m for Ca^{2+} influx across the plasma membrane [9]. Continued studies of both *in vivo* and isolated microvascular endothelium are needed to provide greater mechanistic insight into the spatial and temporal distribution of electrical signals and vasomotor responses that underlie the coordinated regulation of tissue blood flow in healthy vessels and how these relationships may be altered with ageing and disease.

Interaction between Conducted Hyperpolarization and Endothelial Calcium

The interplay between Ca^{2+} signaling and hyperpolarization has been well-characterized in response to GPCR activation in ECs exposed to agonists. Whereas hyperpolarization enhances the electrochemical gradient for Ca^{2+} influx into ECs and may thereby promote NO synthesis [20, 35], agonist-induced elevation of $[\text{Ca}^{2+}]_i$ occurred independent of hyperpolarization in isolated arteriolar endothelium [9]. Nevertheless, a persisting question concerns the interplay between electrical responses and Ca^{2+} waves at local and remote sites along the resistance vasculature. Whereas local stimulation with ACh (via release from a micropipette) initiates both conducted hyperpolarization and Ca^{2+} waves [18, 36], it is unclear how these respective responses interact spatially and temporally in resistance networks. As hyperpolarization travels faster and further once initiated (fig. 1), SMC relaxation in response to a Ca^{2+} wave apparently reflects a complementary (albeit more spatially restricted) signaling pathway for CVD.

An intriguing possibility is that a Ca^{2+} wave may contribute to augmenting hyperpolarization with increasing distance from a site of stimulation [3, 27]. Another possibility is that electrical and Ca^{2+} signaling may differentially contribute to the regulation of blood flow within and along different arteriolar branch orders. For example, hyperpolarization may act more effectively to promote vasodilation along arterioles having a single layer of SMCs whereas dilation of resistance arteries with multiple layers of SMC may depend more on the diffusion of NO (or other autacoids) generated in response to Ca^{2+} waves. A complementary hypothesis is that the rapidly conducted hyperpolarization “primes” resistance networks with an initial dilation that is then sustained in more localized

regions by the ensuing Ca^{2+} wave [3]. Together, the differential rates of charge transfer and Ca^{2+} signaling along the endothelium may work to finely tune and integrate vasomotor responses underlying the local control of tissue perfusion. It remains controversial as to whether hyperpolarization at remote sites can increase $[\text{Ca}^{2+}]_i$ to sustain Ca^{2+} waves and/or conducted hyperpolarization (fig. 2). Early studies of cultured ECs indicated that either hyperpolarization or depolarization modulated $[\text{Ca}^{2+}]_i$ [37–39]. However, more recent studies of intact arteries and arterioles have found no increase in EC $[\text{Ca}^{2+}]_i$ in response to hyperpolarization [40–43]. Differences in experimental conditions and regional specialization of vascular beds may underlie such controversy and more work in this area is needed from studies of intact preparations of the resistance vasculature.

Interaction between TRP channels and $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$

It has been questioned whether microvascular ECs express functional voltage-gated ion channels [44]. Such proteins may be present in the plasma membrane of microvascular ECs and serve to influence V_m and $[\text{Ca}^{2+}]_i$ through signaling events that remain to be defined. Alternatively, TRP channels may play a major role in Ca^{2+} entering the cell to regulate V_m (e.g., via activating $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ in the plasma membrane) or to replete the ER for further intracellular Ca^{2+} release (fig. 2) [12]. In addition, the non-selective nature of TRP channels may contribute to depolarization through the entry of cations (e.g. Na^+ and Ca^{2+}) [12, 45]. This dichotomy in function depends upon the respective isoforms of TRP channels expressed in EC membranes, their permeability to different cations and the signaling complexes that exist between channel isoforms [12]. Indeed, recent evidence from $\text{TRPC1}^{-/-}$ mice suggests that TRPC1 contributes to depolarization, thereby dampening ACh-induced hyperpolarization of the EC membrane [45]. However, such behaviour was not observed in $\text{TRPC6}^{-/-}$ mice [45], highlighting differing functions of respective TRP channel isoforms in regulating the V_m of ECs. A key area for further research is how respective TRP channel isoforms expressed in ECs of arterioles and resistance arteries impact $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ function in light of homomeric and heteromeric TRP channel signaling complexes. In turn, understanding the interplay between TRP channels and $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ to shape Ca^{2+} waves and conducted hyperpolarization in the resistance vasculature may provide critical new insight for the treatment of vascular disease.

Roles of Calcium and Electrical Signaling in Cardiovascular Disease

Endothelial dysfunction is a common aspect of cardiovascular disease [46]; however, perturbations of EC Ca^{2+} signaling in such conditions has received little attention. Current evidence indicates that exposure of human ECs to hyperglycaemic conditions enhances SOCE and the induction of apoptosis with no difference in IP_3 -mediated Ca^{2+} release [47]. Stimulation of aortic ECs isolated from spontaneously hypertensive rats results in Ca^{2+} overload along with the production of reactive oxygen species [48]. Additionally, EC permeability is altered in cardiovascular disease as a result of changes in Ca^{2+} homeostasis and mobilization [49]. Whereas studies of microvascular EC dysfunction have been focused on the production and bioavailability of NO [46], dysfunction of $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ is implicated in the aetiology of cardiovascular disease and these channels are emerging as targets for therapeutic intervention [20, 22, 24]. Ageing is associated with endothelial dysfunction as demonstrated in human beings by the impaired ability to evoke endothelium-dependent vasodilation in response to ACh, to muscular exercise or to heating of the skin [21]. In addition to loss of NO bioavailability, impaired vasodilation with ageing has been attributed to reduced $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ function [21, 22]. With diet-induced obesity, studies of rat mesenteric arteries have shown an increase in IK_{Ca} function concomitant with a reduction in SK_{Ca} function resulting in impaired endothelium-derived hyperpolarization [23]. With ageing, CVD was found to decay more rapidly along arterioles of the gluteus maximus muscle of old (~20 month) *versus* young (~3 month) C57BL/6 mice [50]. Nevertheless, peak dilation

and constriction (during direct exposure to ACh and phenylephrine, respectively) did not differ across age groups [50], highlighting the need to consider spatial and temporal aspects of vascular coupling through GJCs in addition to more direct signaling events initiated by receptor activation.

Conclusion

Whereas EC dysfunction is consistently correlated with cardiovascular disease, the underlying mechanisms for Ca^{2+} and electrical signaling along the endothelium of resistance vessels and how these processes may be differentially altered with ageing and disease remain poorly understood. Continued progress in understanding the elementary events underlying electrical and Ca^{2+} signaling pathways and how these events interact with each other along the vessel wall promises to provide new insight for developing novel therapeutic approaches to treating vascular disease and improving the quality of life.

Acknowledgments

Research in the authors' laboratory is supported by United States Public Health Service through National Institutes of Health grants R37-HL041026 and R01-HL086483 (to SSS), F32-HL107050 (to MJS) and F32-HL110701 (to EJB). In accord with the limited number of references permitted for this MiniReview, many important papers could not be cited. No oversight or omission was intended by the authors.

Abbreviations

FA	feed artery
GJC	gap junction channel
ACh	acetylcholine
CVD	conducted vasodilation
SK_{Ca} ($\text{K}_{\text{Ca}2.3}$)	small conductance Ca^{2+} -activated K^{+} channel
IK_{Ca} ($\text{K}_{\text{Ca}3.1}$)	intermediate conductance Ca^{2+} -activated K^{+} channel
SMC	smooth muscle cell
EC	endothelial cell
SERCA	sarco/endoplasmic reticulum Ca^{2+} ATPase
GPCR	G protein coupled receptor
IP_3	inositol 1,4,5 trisphosphate
DAG	diacylglycerol
ER	endoplasmic reticulum
IP_3R	IP_3 receptor
RyR	ryanodine receptor
RACE	receptor-activated Ca^{2+} entry
SOCE	store-operated Ca^{2+} entry
TRP	transient receptor potential channel
STIM1	stromal interaction molecule 1
Cx	connexin

NO	nitric oxide
V _m	membrane potential
K _{IR}	inward rectifying K ⁺ channel

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Figure 1. Initiation and intercellular conduction of Ca^{2+} and electrical signals along endothelial cells

Initiation: A local stimulus (e.g., ACh, not shown) triggers intracellular Ca^{2+} release from the ER through IP_3R while Ca^{2+} enters the cell through the plasma membrane. Elevation of $[\text{Ca}^{2+}]_i$ activates $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ to increase K^{+} efflux and thereby generate hyperpolarization.

Conduction: Hyperpolarization (–) and Ca^{2+} waves pass through GJCs to neighbouring ECs. **Calcium:** Cytoplasmic Ca^{2+} stimulates NO production, a potent vasodilator. The Ca^{2+} -mediated component of conducted vasodilation is “slow” ($\sim 100 \mu\text{m/s}$) and restricted to $< 1 \text{ mm}$ from site of initiation. **Electrical:** Conduction of hyperpolarization through GJCs along the endothelium is rapid (cm/s) and encompasses several mm from the site of initiation.

Some charge is dissipated as the electrical signal travels from cell to cell; additional details omitted for simplicity. **Abbreviations:** GJC, gap junction channel; NO, nitric oxide; ER, endoplasmic reticulum.

Figure 2. Hypothetical interaction between $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ and TRP channels

A hypothesis for regenerative conduction independent from the activity of voltage-gated ion channels: Hyperpolarization (via conduction) enhances the electrochemical gradient for Ca^{2+} to enter ECs, e.g., through TRP channels. The influx of Ca^{2+} may directly activate $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$ along with increasing internal (ER) Ca^{2+} store content through SERCA. The Ca^{2+} released from the ER may also activate $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$. Both sources of Ca^{2+} may contribute to Ca^{2+} waves and promote NO synthesis (unexplored). Other signaling events (e.g., capacitive Ca^{2+} entry and STIM/Orai interaction) are also omitted for simplicity. Key interactions illustrated within a single EC may be occurring concomitantly along the endothelium. Though speculative, the reasons such interplay between hyperpolarization and Ca^{2+} entry have not been resolved may be attributable to the complexity of experiments required to investigate such interactions and the ability to simultaneously record these events during conducted vasodilation. Nevertheless, such a mechanism would serve to maintain ER Ca^{2+} stores and sustain signaling along the endothelium. *Abbreviations:* ER: endoplasmic reticulum; GJC: gap junction channel; $\text{IK}_{\text{Ca}}/\text{SK}_{\text{Ca}}$: intermediate and small conductance calcium-activated K^+ channels; IP_3R : inositol trisphosphate receptor; SERCA: sarco/endoplasmic reticulum Ca^{2+} ATPase; TRP: transient receptor potential channel.