Chapter 3

The Endothelium: The Vascular Information Exchange

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Abstract

Maintenance of adequate blood flow to tissues and organs requires that endothelial cells dynamically respond in a stimulus-specific manner to elicit appropriate changes in smooth muscle contractility and thus, arterial diameter. Endothelial cells can be stimulated directly by increases in blood flow and by humoral factors acting on surface receptors, as well as through flux of second messengers from smooth muscle cells activated by release of neurotransmitters from perivascular nerves. The ability of endothelial cells to generate stimulus-specific responses to these diverse inputs is facilitated by organization of ion channels and signaling proteins into microdomains that permit finely-tuned, spatially-restricted Ca^{2+} events to differentially activate key effectors such as nitric oxide (NO) synthase and Ca^{2+}-activated K^+ (K_{Ca}) channels. NO is a diffusible mediator which acts locally to cause vasodilation. Opening of K_{Ca} channels causes hyperpolarization of the endothelial membrane potential which spreads to surrounding smooth muscle cells to also cause local vasodilation. However, once initiated, hyperpolarization also spreads longitudinally through the endothelium to effect coordinated changes in blood flow within multiple arterial segments. Thus, the signaling pathways activated by a particular stimulus determine whether it's effects on arterial diameter are localized or can impact blood flow at the level of the vascular bed.

Keywords: endothelium, calcium, nitric oxide, microdomain, potassium channels

1. Introduction

Appropriate local control of blood flow through resistance arteries is critical to the functioning of tissues and organs, and to regulation of blood pressure. Lying at the interface between the blood and smooth muscle cells of the vessel wall, the endothelium plays a vital role in this dynamic process by transducing diverse chemical and mechanical stimuli into coordinated changes in arterial diameter. Endothelial cells respond to vasodilatory stimuli by releasing diffusible mediators such as nitric oxide (NO) and prostacyclin (PGI_2) and by initiating membrane hyperpolarization that spreads to surrounding smooth muscle cells via myoendothelial gap junctions (MEGJs) to inhibit contractility, a mechanism termed endothelium-dependent hyperpolarization (EDH) [1–3]. NO and PGI_2 are local mediators that diffuse to surrounding smooth muscle cells to cause relaxation. Once initiated, EDH spreads to surrounding smooth muscle cells to affect relaxation but conduction of hyperpolarization longitudinally through the endothelial layer means that EDH also contributes to coordination of changes in blood flow in multiple arterial segments within a vascular bed [4]. Thus, the ability of a stimulus to engage diffusible mediators versus EDH determines whether it's effects on arterial diameter and thus blood flow, are restricted to the local area or can impact blood flow at the level of the vascular bed.

Increases in endothelial intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) drive these vasodilator pathways; NO is synthesized from L-arginine by the Ca^{2+}-calmodulin-dependent enzyme NO synthase (NOS) [3], PGI_2 is generated by the actions of cyclooxygenase on arachidonic acid released by the actions of Ca^{2+}-dependent phospholipase A_2 on membrane phospholipids [5, 6], and opening of Ca^{2+}-activated K^+ (K_{Ca}) channels causes hyperpolarization [3, 7]. Global changes in endothelial $[Ca^{2+}]_i$ have been widely studied [8, 9], but development of new technologies such as high-speed, high-resolution confocal Ca^{2+} imaging and generation of transgenic mice expressing genetically encoded Ca^{2+} indicators has allowed resolution of a wide range of transient Ca^{2+} events within endothelial cells of intact arteries to provide a growing body of support for the concept of stimulus-specific engagement of effectors underpinned by spatially and temporally discrete Ca^{2+} signaling patterns that occur independently of changes in bulk endothelial $[Ca^{2+}]_i$ [10–13].

Pulsars [10] and wavelets [11] are spontaneous, short-lived, (<0.03 s duration) spatially fixed Ca^{2+} events originating from distinct clusters of inositol 1,4,5-trisphosphate ($InsP_3$) receptors on the membrane of endoplasmic reticulum (ER). First identified in mouse mesenteric artery and hamster skeletal muscle arteriolar endothelial cells, these events predominantly occur close to endothelial projections that abut or form MEGJs with smooth muscle cells [10, 11] and exert a basal vasodilator influence through activation of intermediate conductance (IK_{Ca}) Ca^{2+}-activated K^+ channels. Their dependence on $InsP_3$ provides a mechanism by which pulsars are linked to and regulated by G protein-coupled receptor (GPCR) signaling. Elevation of $InsP_3$ by endothelium-dependent vasodilators [10] or by flux of $InsP_3$ from smooth muscle cells following stimulation of α_1-adrenoceptors [11] increases pulsar size and/or frequency through recruitment of new sites and a reduction in the interval between pulsars at a given site. In porcine coronary arteries, $InsP_3$-dependent Ca^{2+} events similar to pulsars propagate into longer lasting Ca^{2+} waves (>8 s duration) facilitated by the longitudinal arrangement of ER/$InsP_3$ receptors to promote directional Ca^{2+}-induced Ca^{2+} release along the endothelial cell axis and are associated with activation of both NOS and K_{Ca} channels [12].

Sparklets are generated by spatially restricted Ca^{2+} influx through members of the transient receptor potential (TRP) ion channel family [14, 15]. Sparklets were first identified in mouse mesenteric arteries under experimental conditions in which $InsP_3$-mediated pulsars were eliminated [14]. Exposure of the endothelium to TRPV4 agonists and/or acetylcholine increased the activity of these discrete Ca^{2+} signals which were linked to activation of both IK_{Ca} and small conductance (SK_{Ca}) Ca^{2+}-activated K^+ channels, effects which were absent in arteries from mice lacking TRPV4 [14]. In rat cremaster arterioles, clustering of TRPV4-mediated sparklets in endothelial projections was linked exclusively to activation of IK_{Ca} channels [16] and in mouse small pulmonary arteries, shear stress-stimulated TRPV4 activity was linked to NO production [17]. Larger endothelial sparklets mediated by simultaneous opening of two TRPA1 and leading to activation of IK_{Ca} channels were shown to underlie dilation to reactive oxygen species in rat cerebral arteries [18]. We will now discuss how grouping of Ca^{2+} signaling and effector proteins into microdomains allows dynamic, stimulus-specific Ca^{2+} events which determine the recruitment of effectors thus the degree to which blood flow is impacted.

2. Stimulus-specific endothelial Ca^{2+} signaling

2.1. Shear stress

In vivo, endothelial sensing of laminar shear stress, the tangential frictional force exerted by blood flowing across the cell surface, plays a dominant role in acute modulation of vascular tone and therefore, tissue perfusion [19–21]. In the majority of resistance arteries, increases in blood flow stimulate endothelium-dependent relaxation of surrounding smooth muscle cells and increase arterial diameter, a response termed flow-mediated dilatation. Flow also influences gene expression and structural remodeling with areas of disturbed flow and reduced shear stress is associated with development of atherosclerotic plaques [22]. Measurement of acute responses to increases in shear stress is the most widely used clinical index of endothelial function and vascular health with attenuation of flow-induced dilation associated with increased risk of cardiovascular diseases [23, 24]. Indeed, reductions in shear stress are a likely mechanism by which endothelial function is altered with inactivity, an effect which can be overcome by exercise interventions [25, 26].

In animals and humans, acute shear stress-induced vasodilation can be mediated by both NO and EDH [27–31]. Although endothelial cells express both SK_{Ca} and IK_{Ca} channels, data from isolated arteries indicate that it is SK_{Ca} channels that play a predominate role in mediating the EDH component of this response. Deletion of SK_{Ca} but not IK_{Ca} channels impaired both NO and EDH-mediated dilation to shear stress stimulation in mouse isolated carotid arteries [32]. In rat isolated perfused mesenteric beds, shear stress-induced modulation of sympathetic vasoconstriction was prevented by both the NOS inhibitor L-N^G-nitroarginine methyl ester (L-NAME) and apamin, a selective blocker of SK_{Ca} channels, but not by the IK_{Ca} channel inhibitor TRAM-34 [33]. Similarly, shear stress-evoked dilation of mouse isolated coronary arteries was inhibited by apamin [34] and L-NAME [35].

Mechanotransduction, the conversion of increases in shear stress into changes in arterial diameter, is reliant upon rises in endothelial $[Ca^{2+}]_i$ mediated by Ca^{2+} entry. In vitro studies have identified multiple candidates as potential endothelial mechanosensors including integrins [36], tyrosine kinase receptors [37], intercellular junction proteins [38], and P2X4 receptors which are cation channels activated by adenosine triphosphate (ATP) [39]. Work with transgenic mice has implicated GPR68, a proton-sensing rhodopsin-like GPCR [40], inwardly rectifying K^+ channels [41] and PIEZO1, a Ca^{2+}-permeable, non-selective cation channel [42, 43]. However, recently, it is the role of TRPV4 in endothelial responses to increases in shear stress that has received particular attention. This Ca^{2+} permeable channel can be directly activated by shear stress [34, 44] via membrane deformation or through a lever-like action involving cytoskeletal linkages to molecules embedded in the glycocalyx [45, 46], the layer of proteoglycans and glycoproteins that covers the luminal surface of the endothelium, or indirectly through upstream production of arachidonic acid metabolites [47]. Genetic deletion of TRPV4 results in blunted flow-mediated dilation of mouse carotid and mesenteric arteries [31, 47, 48] and pharmacological inhibition of these channels blocked flow-evoked increases in endothelial $[Ca^{2+}]_i$ in isolated mouse mesenteric, human coronary, and rat carotid and gracilis arteries [31, 49–51]. In bovine coronary endothelial cells block of TRPV4 inhibited both shear stress-evoked increases in $[Ca^{2+}]_i$ and activation of SK_{Ca} channels [34], indicating that there may be a direct link between TRPV4-mediated Ca^{2+} influx and SK_{Ca} channel activity. This idea is supported by the demonstration that in rat pulmonary arteries, vasodilation to the TRPV4 agonist GSK1016790A was mediated by activation of SK_{Ca} channels [52]. In the same vessels, and in mouse small pulmonary arteries, shear stress-stimulated TRPV4 activity was also linked to NO production [17] suggesting a further link between TRPV4 and NOS.

Building on these findings, several lines of evidence now support the notion that acute increases in shear stress cause Ca^{2+} influx through TRPV4 channels to selectively activate both SK_{Ca} channels and NOS, and that this pathway is enabled by organization of TRPV4, NOS, SK_{Ca} channels and the caveolae scaffold protein caveolin-1 into microdomains within caveolae, flask-shaped structures on the endothelial cell surface rich in signaling proteins [34, 53, 54]. SK_{Ca} channels are localized to the luminal membrane of endothelial cells in rat mesenteric arteries [55] and SK_{Ca} channel protein was co-immunoprecipitated with caveolin-1 from endothelial cells of the same arteries and from porcine coronary arteries [56]. It is well established that endothelial NOS is localized to caveolae where it is negatively regulated through its interaction with caveolin-1 [57]. Increases in $[Ca^{2+}]_i$ promote recruitment of Ca^{2+}-calmodulin to displace caveolin-1 from NOS thereby activating it [53]. In bovine coronary and human microvascular endothelial cells, SK_{Ca} channels were co-localized with caveolin-1, NOS and TRPV4 channels within microdomains at the luminal endothelial cell surface [34, 57, 58]. Furthermore, in mesenteric arteries from mice lacking caveolin-1, endothelial TRPV4 channel activity was impaired indicating that a direct interaction between TRPV4 and caveolin-1 may be functionally important for Ca^{2+} entry in response to shear stress [57]. Caveolin-1 has been shown to initiate downstream signaling in response to increases in shear stress [59] leading to the suggestion that caveolae act as mechanosensors to elicit a cascade of events that promote vasodilation. In line with this proposal, shear stress-induced dilation is defective and endothelial SK_{Ca} current reduced in coronary and carotid arteries of mice lacking caveolin-1, an effect rescued by re-introduction of endothelial specific caveolin-1 [58, 60].

Together these findings, gathered using a range of approaches and from a number of different arteries, support an elegant model in which shear stress-evoked Ca^{2+} influx through TRPV4 channels on the luminal surface of endothelial cells leads to spatially-restricted Ca^{2+} sparklets

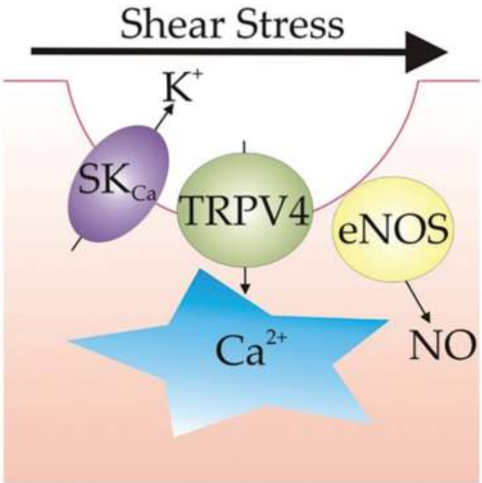

Figure 1. Model of localized endothelial Ca^{2+} signaling evoked by increases in shear stress. Shear stress-evoked Ca^{2+} influx through TRPV4 channels on the luminal surface of endothelial cells leads to spatially-restricted Ca^{2+} sparklets within a signaling microdomain to selectively activate SK_{ca} channels and endothelial NOS (eNOS).

within a signaling microdomain to selectively activate SK_{Ca} channels and NOS to elicit vasodilation (**Figure 1**). However, a number of questions remain to be addressed. Shear stress increases PGI_2 production in bovine coronary and human umbilical vein endothelial cells [34, 61] and rabbit isolated femoral arteries [62], and hydrogen peroxide (H_2O_2) contributes to flow-mediated dilation in coronary arterioles from patients with heart disease [63] but the functional role of these factors in acute flow-mediated vasodilation has not fully been explored. A significant component of flow-induced dilation remained in isolated mesenteric arteries of mice lacking TRPV4 [31] which could indicate that, as suggested in earlier reports, Ca^{2+}-independent processes may also contribute to this response [64] or the involvement of another route for Ca^{2+} influx. The possibility that flow-induced increases in endothelial cell $[Ca^{2+}]_i$ are stimulated by localized release endothelium-derived paracrine mediators such as ATP, substance P or acetylcholine, first proposed over 30 years ago [65, 66], has recently received renewed support with the demonstration that endothelial organic cation transporters release acetylcholine in response to increases in shear stress in rat isolated carotid arteries [67]. The same study suggests that $InsP_3$-mediated Ca^{2+} release from ER stores and Ca^{2+} entry through TRPC but not TRPV4 contributes to flow-induced endothelial Ca^{2+} signaling in these vessels. This finding highlights the fact that further work is required to elucidate the differential signaling networks underlying endothelial responses to acute increases in shear stress in different arteries.

2.2. Agonists at endothelial GPCRs

Many endogenous and exogenous chemicals bind to GPCRs leading to stimulation of EDH and production of NO, PGI_2 and other diffusible mediators such as epoxyeicosatrienoic acids and H_2O_2, to cause vasodilation [1, 2, 68]. Measurements of bulk endothelial $[Ca^{2+}]_i$ established the role of $InsP_3$-mediated Ca^{2+} release and subsequently store-operated Ca^{2+} entry (SOCE) in this process [9]. The mechanism underlying endothelial SOCE has been controversial but recent evidence supports a model in which Ca^{2+} store depletion allows spatial reorganization of Ca^{2+} sensor protein stromal interaction molecules (STIMs) so that they can aggregate into clusters that physically interact with Ca^{2+}-selective Orai channels at the ER-plasma membrane junction [69–72]. TRPC and TRPV4 can also interact with STIMs [73] and studies of knock-out mice have provided evidence for a role for TRPC4 in acetylcholine-evoked SOCE in aortae [74] and for TRPV4 in SOCE in mesenteric [75] and carotid arteries [76]. A receptor-operated Ca^{2+} entry mechanism can also be mediated by DAG-induced activation of TRPC and TRPV channels. For example, in human umbilical vein endothelial cells, bradykinin stimulated both translocation of DAG-sensitive TRPC6 to the cell membrane and Ca^{2+} influx [77, 78]. Expression of mRNA for another ER Ca^{2+} release channel, the ryanodine receptor (RyR), has been detected in endothelial cells of human mesenteric arteries [79], and RyRs have been suggested to mediate Ca^{2+} oscillations in cultured bovine aortic and human umbilical vein endothelial cells [80] but to date, ryanodine has been shown to have no effect on endothelial Ca^{2+} signaling or vasodilation [12, 81]. There is significant variation in the reported contribution of EDH, NO and other mediators to agonist-evoked dilation, both in terms of differences between agonists and arteries. Thus, for the purposes of this chapter we will limit our discussion to three agents commonly used to stimulate endothelium-dependent vasodilation in experimental studies, acetylcholine, ATP and substance P.

The first evidence that differential endothelial Ca^{2+} signaling underlies agonist-evoked EDH and NO came from a study of rat isolated middle cerebral arteries in which EDH-dependent vasodilation to purinergic agonists required a larger increase in $[Ca^{2+}]_i$ than for NO [82]. Measurements of global $[Ca^{2+}]_i$ indicated that different sources of Ca^{2+} contributed to agonist-stimulated production of NO and EDH; NO production is associated with SOCE [83] whereas EDH is linked to both $InsP_3$-mediated Ca^{2+} release and SOCE [84]. Similarly, both agonist evoked SOCE and NO production are suppressed in isolated aortae from mice lacking STIM1, the primary endothelial STIM [85]. Building on these findings, data accrued over the past 15 years from functional, histological, Ca^{2+} imaging and immunohistochemical studies of intact arteries and endothelial-smooth muscle co-cultures support agonist-evoked EDH and NO release being mediated by distinct Ca^{2+} signaling within specialized domains.

2.2.1. EDH

EDH is mediated by opening of both IK_{Ca} and SK_{Ca} channels but their relative contribution to agonist-evoked vasodilation, based on the effects of selective pharmacological inhibitors, displays significant variation between agonists and arteries. Simultaneous block of both IK_{Ca} and SK_{Ca} channels is required to inhibit acetylcholine-evoked EDH in mesenteric arteries from rats and mice, and guinea-pig coronary arteries [86–88] whereas in rat cerebral and human mesenteric arteries the same response is largely reliant on IK_{Ca} channels [89, 90].

IK_{Ca} and SK_{Ca} channels display a differential spatial distribution within endothelial cells and their contribution to agonist-evoked EDH appears to be mediated by different signaling pathways. In mesenteric arteries from rats, mice and humans, IK_{Ca} channels are localized within

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regions associated with MEGJs in which ER membrane, $InsP_3$ receptors, gap junction connexins, TRPC3 and TRPV4 have also been identified [10, 11, 14, 55, 90–93]. In mouse and rat mesenteric arteries, acetylcholine increased the frequency and number of $InsP_3$-dependent Ca^{2+} pulsars in this region which were linked to activation of IK_{Ca} channels to evoke EDH [10, 93] (**Figure 2**). TRPC3 may support this process by providing Ca^{2+} entry for refilling of $InsP_3$-sensitive ER stores, and/or direct activation of both SK_{Ca} and IK_{Ca} channels [91, 94]. However, in other reports acetylcholine exclusively stimulates TRPV4 in the vicinity of MEGJs to generate Ca^{2+} sparklets and which in turn activate IK_{Ca} channels in mouse mesenteric arteries [14, 95]. This occurs via a mechanism dependent on the anchoring protein AKAP, and is consistent with deletion of TRPV4 resulting in blunting of acetylcholine-evoked increases in endothelial $[Ca^{2+}]_i$ and loss of EDH in mouse mesenteric arteries [75, 76]. TRPV3 [96] and TRPA1 [97] are also expressed in endothelial cells and activators of these channels can certainly initiate increases in Ca^{2+} signaling and EDH in cerebral arteries, but a role for these channels in agonist-stimulated EDH has yet to be demonstrated.

In contrast to IK_{Ca} channels, SK_{Ca} channels are associated with caveolae and are diffusely distributed across the cell membrane with a higher level expression at endothelial-endothelial cell borders [12, 55, 90, 93]. Also, unlike IK_{Ca} channels, evidence is lacking for a direct link between agonist-evoked, $InsP_3$-mediated Ca^{2+} events and SK_{Ca} channel activity. Instead, it

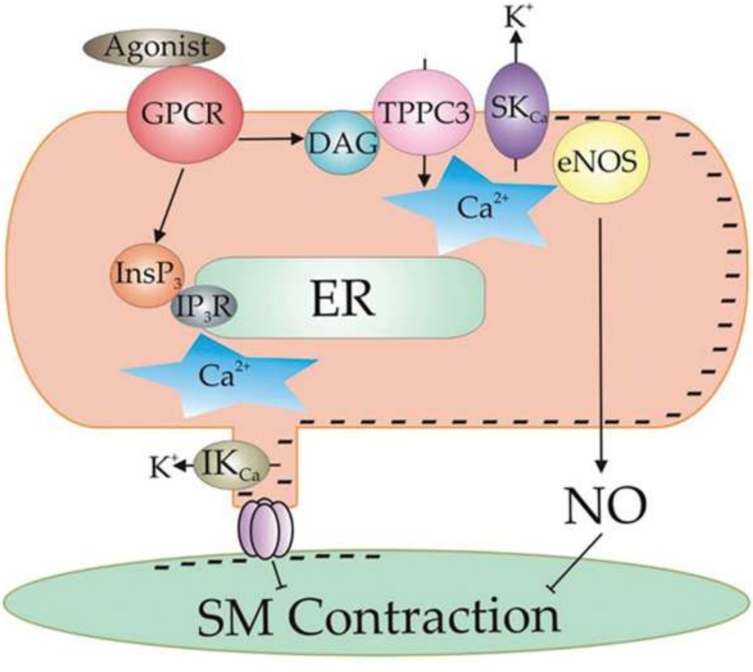

Figure 2. Schematic showing discrete Ca^{2+} signaling events elicited by agonists at endothelial GPCRs. $InsP_3$-dependent Ca^{2+} pulsars are linked to activation of IK_{Ca} channels to evoke EDH whereas Ca^{2+} influx through DAG-activated TRPC channels is the primary source of Ca^{2+} for agonist stimulation of endothelial SK_{Ca} channels and eNOS.

appears that Ca^{2+} influx through TRP channels is the primary source of Ca^{2+} for agonist stimulation of endothelial SK_{Ca} channels [94, 98]. In mouse cerebral artery, ATP caused rapid trafficking of TRPC3 to the plasma membrane to provide Ca^{2+} influx to selectively activate SK_{Ca} channels to cause EDH [98] (**Figure 2**). As described earlier, TRPV4 are also associated with caveolae and are a source of Ca^{2+} for SK_{Ca} channel activation in response to increases in shear stress but whether this relationship accounts for engagement of SK_{Ca} channels by agonists has not been explored.

2.2.2. NO

In contrast to EDH, the role of localized Ca^{2+} signaling in agonist-evoked NO release has received little attention. NOS, TRPV4 and TRPC3 are located in caveolae microdomains, and deletion of either channel blunts acetylcholine-evoked NO release and NO-mediated relaxation in mouse mesenteric and carotid arteries [75, 99] suggesting they may provide a source of Ca^{2+} for agonist-driven NOS activation. Heteromultimers of TRPV4-TRPC1 channels mediate vasorelaxation of rabbit mesenteric arteries in response to stimulation of the Ca^{2+}-sensing receptor through NO production [100] but the underlying Ca^{2+} dynamics were not assessed. A recent study has shown that TRPV4-mediated sparklets underlie ATP driven activation of endothelial NOS in mouse small pulmonary arteries. The resulting NO initiates vasodilation and also guanylyl cyclase-protein kinase G signaling in the endothelium that limits TRPV4 channel cooperativity and serves as a negative feedback signal to regulate TRPV4 channel function [17]. This description of ATP-evoked, spatially distinct TRPV4 sparklets and localized TRPV4-NOS signaling support a novel paradigm that NOS can be activated by spatially restricted Ca^{2+} signals, and identifies TRPV4 channels as a key regulator of NOS activity in the pulmonary microcirculation.

In contrast, in porcine isolated coronary arteries, substance P increased the occurrence of discrete $InsP_3$-dependent endothelial Ca^{2+} events in a concentration-dependent manner; low concentrations primarily increased the number of Ca^{2+} events and at higher concentrations the number of Ca^{2+} events saturated while the magnitude of individual events increased [12]. This pattern correlated with a greater role for NO in vasorelaxation at lower concentrations suggesting subtle Ca^{2+} signal expansion at low stimulation levels may preferentially target NOS. A key finding of this study was that idiosyncratic Ca^{2+} signal expansion corresponded with coronary artery vasorelaxation whereas global changes in $[Ca^{2+}]_i$ did not highlighting that frequency modulation of discrete Ca^{2+} signals is the primary driver of this functional response and that measurement of changes in bulk $[Ca^{2+}]_i$ do not adequately describe the Ca^{2+} signaling pathways that underlie endothelium-dependent vasodilation.

2.2.3. Membrane potential and Ca^{2+} microdomain signaling

Production of NO and stimulation of EDH have long been regarded as separate mechanisms for agonist-evoked vasodilation but several lines of evidence indicate that there may be a facilitatory relationship between endothelial SK_{Ca} and IK_{Ca} channel activity and NO. SK_{Ca} channel activity has been linked to NO-mediated vasodilation to agonists with deletion of these channels causing impaired NO-mediated dilation to acetylcholine in mouse carotid arteries and increased expression enhancing NO-mediated dilation of cremaster arterioles

[32]. In rat mesenteric arteries, block of SK_{Ca} and IK_{Ca} channels reduces agonist-evoked, NO-mediated vasorelaxation and NO release [101]. Conversely, activators of endothelial K_{Ca} channels can enhance NO release from cultured endothelial cells, enhance ATP-induced increases in cytosolic Ca^{2+} concentration and NO synthesis in rat cremaster arterioles, and elicit NO-mediated relaxation in mesenteric arteries [102–104].

Lacking voltage-operated Ca^{2+} channels, endothelial Ca^{2+} influx is mediated by TRP channels and so membrane hyperpolarization may be required to maintain an appropriate electrochemical driving force for agonist-induced Ca^{2+} influx and also to prevent channel inactivation and/or reduction in unitary conductance [105, 106]. Membrane depolarization does inhibit both agonist-induced increases in $[Ca^{2+}]_i$ and NO release in cultured endothelial cells [107, 108], and in rat isolated basilar arteries, endothelial depolarization was associated with a reduction in NO-mediated relaxation to acetylcholine [109]. Nonetheless, the ability of hyperpolarization to regulate Ca^{2+} entry by increasing the electrical driving force is controversial. The large concentration gradient (~20,000-fold for extracellular versus intracellular) [110] and driving force for Ca^{2+} entry raising the question of whether a small amplitude hyperpolarization will be insufficient to modulate Ca^{2+} entry. In rat mesenteric and cerebral arteries, that certainly appeared to be the case as changes in global endothelial $[Ca^{2+}]_i$ were independent of changes membrane potential [89, 111]. However, more recent work with endothelial cell tubes isolated from resistance arteries has provided renewed support for hyperpolarization enhancing acetylcholine-evoked Ca^{2+} influx through TRPV4 [112] and indicate that pharmacological activation of SK_{Ca} and IK_{Ca} channel may not only enhance Ca^{2+} entry to further amplify K_{Ca} channel activity, but also boost NO production [113]. In mouse mesenteric arteries, acetylcholine-evoked TRPV4-dependent Ca^{2+} signaling was inhibited in arteries from mice lacking IK_{Ca} channels indicating that in these arteries, endothelial stimulation drives sufficient IK_{Ca}-dependent Ca^{2+} entry through TRPV4 to enhance dynamics [13]. IK_{Ca} channel activity modestly augmented Ca^{2+} event amplitude but the most notable impact was in recruiting new Ca^{2+} firing sites as well as increasing firing frequencies at pre-existing sites. In the same study, increasing or decreasing SK_{Ca} expression had little additional effect on the occurrence of Ca^{2+} events but did promote increased amplitudes and durations indicating that SK_{Ca} channels may play a role in positive feedback Ca^{2+} regulation by shaping the size and time course of individual events. In porcine coronary arteries stimulation of NOS by $InsP_3$-dependent, large amplitude-low frequency Ca^{2+} waves [12], exactly the types of events which were lost in mesenteric arteries from mice with an endothelial specific knockout of SK_{Ca} channels [114], suggests that SK_{Ca} channels are required for their development. As mentioned above, deletion of SK_{Ca} channels impaired NO-mediated dilation to acetylcholine [32] and together, these findings support the notion that their role in protraction of Ca^{2+} events may be important in stimulation NOS.

2.3. Myoendothelial feedback

The sympathetic nervous system regulates total peripheral resistance and is a key modulator of resistance artery diameter through release of noradrenaline and co-transmitters such as ATP and neuropeptide Y [115]. Noradrenaline causes vasoconstriction through activating α_1-adrenoceptors on vascular smooth muscle cells, a process which is limited by engagement of endothelial mechanisms through myoendothelial feedback. The current model of myoendothelial feedback involves flux of $InsP_3$ from smooth muscle to endothelial cells to elicit localized increases in Ca^{2+}, activation of IK_{Ca} channels and possibly NOS, to limit smooth muscle contractility [11, 91, 116]. This model is supported by ultrastructural and histochemical studies showing that in rat mesenteric and basilar, and hamster retractor feed arteries, MEGJ connexins and IK_{Ca} channels are in close spatial association with ER and $InsP_3$ receptors within endothelial projections that extend through the internal elastic lamina to make contact with smooth muscle cells [11, 55, 91, 94]. In hamster retractor feed arteries, myoendothelial feedback is fully accounted for by EDH. The α_1-adrenoceptor agonist phenylephrine induced localized, $InsP_3$-mediated Ca^{2+} signaling events within endothelial projections and block of endothelial IK_{Ca} channels enhanced smooth muscle depolarization and vasoconstriction [11]. In rat basilar arteries in which NO makes a major contribution to myoendothelial feedback, smooth muscle depolarization to 5-HT was accompanied by IK_{Ca} channel-mediated endothelial hyperpolarization. Inhibition of IK_{Ca} channels, gap junctional communication, TRPC3 or NOS potentiated smooth muscle depolarization to 5-HT in a non-additive manner indicating that rather being distinct pathways, NO and endothelial IK_{Ca} channel activity are part of an integrated mechanism for the regulation of agonist-induced vasoconstriction [91]. In the latter study, Ca^{2+} signaling was not investigated and the link between IK_{Ca} channel activity and NO production was not defined. However, NOS has now been localized close to MEGJs [117] and in co-cultures stimulation of smooth muscle cells with phenylephrine leads to MEGJ specific NOS phosphorylation within endothelial cells to increase NO [118]. Also, in mouse mesenteric vessels, phenylephrine stimulated endothelial TRPV4 sparklets in an $InsP_3$-dependent manner, to engage SK_{Ca} and IK_{Ca} channels as well as, to a lesser extent, NOS [17]. Thus, given the ability of IK_{Ca} channels to modulate endothelial Ca^{2+} dynamics [12, 113, 114], it may be proposed that activation of IK_{Ca} channels at MEGJs following stimulation of smooth muscle cells by GPCR agonists, may amplify dynamic Ca^{2+} signals to enhance NO production.

3. Local versus conducted responses

The majority of studies described in this chapter have been conducted on isolated resistance arteries which in in vivo would be part of branching network of resistance vessels supplied by feed arteries in which effective control of blood flow requires coordinated behaviour amongst arterial segments [119]. As described above, diffusible mediators such as NO act locally to increase arterial diameter. In contrast, K_{Ca} channel-mediated hyperpolarization leads to both local dilation and conduction of the response through the endothelium for distances of several millimeters. This conduction allows for coordination of changes in arterial diameter in multiple vessel segments and so optimizes blood flow [4, 119, 120]. That is not to say that diffusible mediators do not play a role in global blood flow regulation within vascular beds. A recent study of the vascular bed of the mouse gluteus maximus muscle revealed that NO and EDH provide complementary endothelial pathways for ascending vasodilatation to optimize oxygen delivery to the muscle. EDH of downstream arterioles conducts along the endothelium into proximal feed arteries to cause dilation, and NO is released in response to luminal shear stress which increases secondary to downstream dilatation [120].

4. Conclusion

It has become apparent over the past 15 years that endothelial Ca^{2+} signaling patterns underlie the engagement of effectors such as NOS and/or K_{Ca} channels. The physiological significance of these stimulus-specific signaling pathways is not just that they determine the mediator of vasodilation, but also the scope of the impact of each stimulus on blood flow. Stimuli which predominantly elicit release of diffusible mediators will elicit local vasodilation whereas those that initiate EDH have the potential to dilate multiple arterial segments and so affect tissue perfusion. Further work is required to determine if the patterns of Ca^{2+} signaling described here have widespread applicability, and how they are impacted by age, sex and cardiovascular risk factors. Investigation of how changes in the components of signaling microdomains contribute to the etiology of endothelial dysfunction in conditions such as diabetes and hypertension may lead to the identification of new therapeutic targets.

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Conflict of interest

The authors have declared no conflict of interest.

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