

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



The Endothelium: The Vascular Information Exchange

Ran Wei, Stephanie E. Lunn, Stephen L. Gust,
Paul M. Kerr and Frances Plane

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.79897>

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 its 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 (PGI₂) 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 PGI₂ 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 its 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 Ca²⁺ concentration ([Ca²⁺]_i) drive these vasodilator pathways; NO is synthesized from L-arginine by the Ca²⁺-calmodulin-dependent enzyme NO synthase (NOS) [3], PGI₂ is generated by the actions of cyclooxygenase on arachidonic acid released by the actions of Ca²⁺-dependent phospholipase A₂ on membrane phospholipids [5, 6], and opening of Ca²⁺-activated K⁺ (K_{Ca}) channels causes hyperpolarization [3, 7]. Global changes in endothelial [Ca²⁺]_i have been widely studied [8, 9], but development of new technologies such as high-speed, high-resolution confocal Ca²⁺ imaging and generation of transgenic mice expressing genetically encoded Ca²⁺ indicators has allowed resolution of a wide range of transient Ca²⁺ 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 Ca²⁺ signaling patterns that occur independently of changes in bulk endothelial [Ca²⁺]_i [10–13].

Pulsars [10] and wavelets [11] are spontaneous, short-lived, (<0.03 s duration) spatially fixed Ca²⁺ events originating from distinct clusters of inositol 1,4,5-trisphosphate (InsP₃) 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}) Ca²⁺-activated K⁺ channels. Their dependence on InsP₃ provides a mechanism by which pulsars are linked to and regulated by G protein-coupled receptor (GPCR) signaling. Elevation of InsP₃ by endothelium-dependent vasodilators [10] or by flux of InsP₃ from smooth muscle cells following stimulation of α₁-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, InsP₃-dependent Ca²⁺ events similar to pulsars propagate into longer lasting Ca²⁺ waves (>8 s duration) facilitated by the longitudinal arrangement of ER/InsP₃ receptors to promote directional Ca²⁺-induced Ca²⁺ 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 Ca²⁺ 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 InsP₃-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 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 $[\text{Ca}^{2+}]_{\text{i}}$ 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 Ca²⁺-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 Ca²⁺ 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 [Ca²⁺]_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 [Ca²⁺]_i and activation of SK_{Ca} channels [34], indicating that there may be a direct link between TRPV4-mediated Ca²⁺ 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 Ca²⁺ 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 [Ca²⁺]_i promote recruitment of Ca²⁺-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 Ca²⁺ 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 Ca²⁺ influx through TRPV4 channels on the luminal surface of endothelial cells leads to spatially-restricted Ca²⁺ sparklets

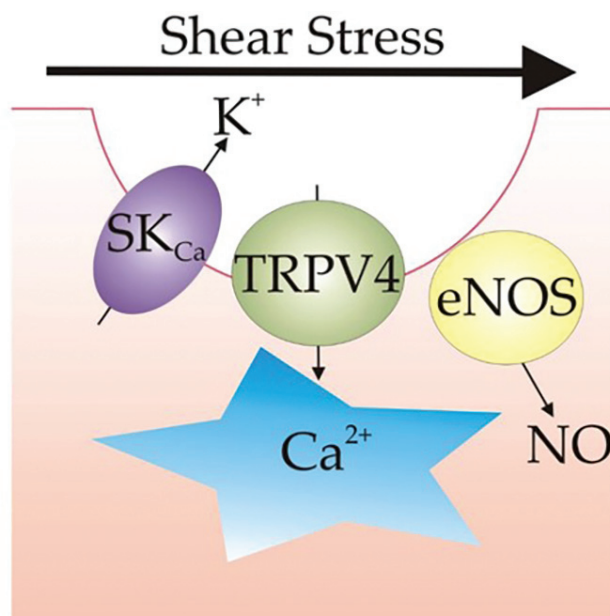


Figure 1. Model of localized endothelial Ca^{2+} signaling evoked by increases in shear stress. Shear stress-evoked Ca^{2+} influx through TRPV4 channels on the luminal surface of endothelial cells leads to spatially-restricted Ca^{2+} sparklets within a signaling microdomain to selectively activate 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 $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$ than for NO [82]. Measurements of global $[\text{Ca}^{2+}]_i$ 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

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 $[\text{Ca}^{2+}]_i$ 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

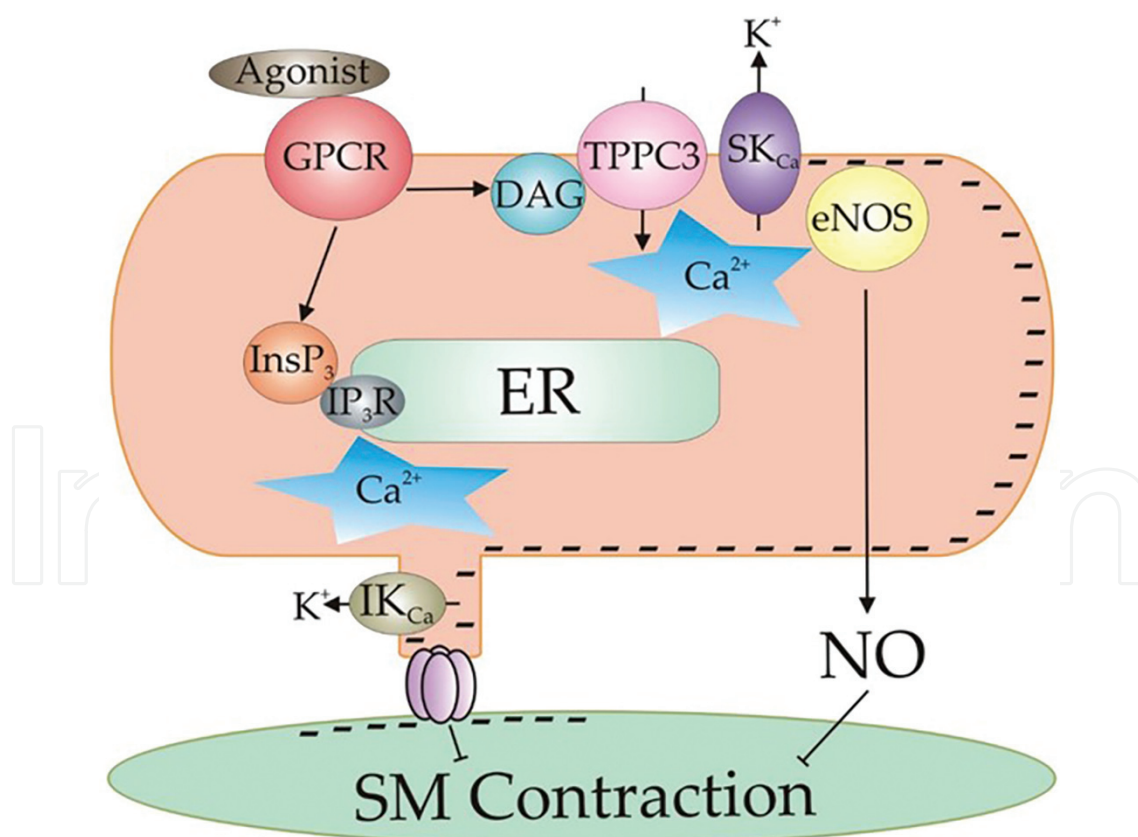


Figure 2. Schematic showing discrete Ca^{2+} signaling events elicited by agonists at endothelial GPCRs. InsP_3 -dependent Ca^{2+} pulsars are linked to activation of IK_{Ca} channels to evoke EDH whereas Ca^{2+} influx through DAG-activated TRPC channels is the primary source of Ca^{2+} for agonist 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 $[\text{Ca}^{2+}]_i$ 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 $[\text{Ca}^{2+}]_i$ 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 Ca²⁺ concentration and NO synthesis in rat cremaster arterioles, and elicit NO-mediated relaxation in mesenteric arteries [102–104].

Lacking voltage-operated Ca²⁺ channels, endothelial Ca²⁺ influx is mediated by TRP channels and so membrane hyperpolarization may be required to maintain an appropriate electrochemical driving force for agonist-induced Ca²⁺ influx and also to prevent channel inactivation and/or reduction in unitary conductance [105, 106]. Membrane depolarization does inhibit both agonist-induced increases in [Ca²⁺]_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 Ca²⁺ 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 Ca²⁺ entry raising the question of whether a small amplitude hyperpolarization will be insufficient to modulate Ca²⁺ entry. In rat mesenteric and cerebral arteries, that certainly appeared to be the case as changes in global endothelial [Ca²⁺]_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 Ca²⁺ influx through TRPV4 [112] and indicate that pharmacological activation of SK_{Ca} and IK_{Ca} channel may not only enhance Ca²⁺ entry to further amplify K_{Ca} channel activity, but also boost NO production [113]. In mouse mesenteric arteries, acetylcholine-evoked TRPV4-dependent Ca²⁺ signaling was inhibited in arteries from mice lacking IK_{Ca} channels indicating that in these arteries, endothelial stimulation drives sufficient IK_{Ca}-dependent Ca²⁺ entry through TRPV4 to enhance dynamics [13]. IK_{Ca} channel activity modestly augmented Ca²⁺ event amplitude but the most notable impact was in recruiting new Ca²⁺ 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 Ca²⁺ events but did promote increased amplitudes and durations indicating that SK_{Ca} channels may play a role in positive feedback Ca²⁺ regulation by shaping the size and time course of individual events. In porcine coronary arteries stimulation of NOS by InsP₃-dependent, large amplitude-low frequency Ca²⁺ 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 Ca²⁺ 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.

Acknowledgements

Work in the author's lab is supported by a grant-in-aid from the Heart and Stroke Foundation of Alberta, Nunavut and the North West Territories to F.P. (G130003076). P.M.K. received a RASCAF award from MacEwan University. Stipend support for graduate students was provided by the University of Alberta (QEII scholarships to S.E.L. and S.L.G., Walter H Johns Graduate Fellowship to S.E.L., 75th Anniversary award to R.W., Faculty of Medicine Recruitment Scholarship to S.L.G.), CIHR (Banting and Best Canada Graduate Scholarship, Masters Award to S.E.L.) and AIHS (Doctoral Studentship to S.E.L.).

Conflict of interest

The authors have declared no conflict of interest.

Author details

Ran Wei¹, Stephanie E. Lunn¹, Stephen L. Gust¹, Paul M. Kerr³ and Frances Plane^{1,2*}

*Address all correspondence to: fplane@ualberta.ca

1 Department of Pharmacology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

2 Cardiovascular Research Centre, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

3 Faculty of Nursing, Robbins Health Learning Centre, MacEwan University, Edmonton, Alberta, Canada

References

- [1] Vanhoutte PM, Shimokawa H, Feletou M, Tang EH. Endothelial dysfunction and vascular disease—A 30th anniversary update. *Acta Physiologica (Oxford, England)*. 2017; **219**(1):22-96
- [2] Bryan RM, You J, Golding EM, Marrelli SP. Endothelium-derived hyperpolarizing factor: A cousin to nitric oxide and prostacyclin. *Anesthesiology*. 2005; **102**:1261-1277
- [3] Sessa WC. Regulation of endothelial derived nitric oxide in health and disease. *Memórias do Instituto Oswaldo Cruz*. 2005; **100**:15-18
- [4] Emerson GG, Segal SS. Endothelial cell pathway for conduction of hyperpolarization and vasodilation along hamster feed artery. *Circulation Research*. 2000; **86**(1):94-100
- [5] Kudo I, Murakami M. Phospholipase A₂ enzymes. *Prostaglandins & Other Lipid Mediators*. 2002; **68-69**:3-58
- [6] Smith WL, Marnett LJ. Prostaglandin endoperoxide synthase: Structure and catalysis. *Biochimica et Biophysica Acta*. 1991; **1083**(1):1-17
- [7] Sandow SL, Hill CE. Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. *Circulation Research*. 2000; **86**:341-346
- [8] Laskey RE, Adams DJ, Cannell M, van Breemen C. Calcium entry-dependent oscillations of cytoplasmic calcium concentration in cultured endothelial cell monolayers. *Proceedings of the National Academy of Sciences of the United States of America*. 1992; **89**(5): 1690-1694
- [9] Blatter LA. Tissue specificity: SOCE: Implications for Ca²⁺ handling in endothelial cells. *Advances in Experimental Medicine and Biology*. 2017; **993**:343-361
- [10] Ledoux J, Taylor MS, Bonev AD, Hannah RM, Solodushko V, Shui B, Tallini Y, Kotlikoff MI, Nelson MT. Functional architecture of inositol 1,4,5-trisphosphate signaling in restricted spaces of myoendothelial projections. *Proceedings of the National Academy of Sciences of the United States of America*. 2008; **105**(28):9627-9632
- [11] Tran CHT, Taylor MS, Plane F, Nagaraja S, Tsoukias NM, Solodushko V, Vigmond EJ, Furstenhaupt T, Brigdan M, Welsh DG. Endothelial Ca²⁺ wavelets and the induction of myoendothelial feedback. *The American Journal of Physiology*. 2012; **302**(8):C1226-C1242
- [12] Francis M, Waldrup JR, Qian X, Solodushko V, Meriwether J, Taylor MS. Functional tuning of intrinsic endothelial Ca²⁺ dynamics in swine coronary arteries. *Circulation Research*. 2016; **118**(7):1078-1090
- [13] Qian X, Francis M, Köhler R, Solodushko V, Lin M, Taylor MS. Positive feedback regulation of agonist-stimulated endothelial Ca²⁺ dynamics by KCa3.1 channels in mouse mesenteric arteries. *The American Journal of Physiology*. 2016; **310**(9):H1151-H1163

- [14] Sonkusare SK, Bonev AD, Ledoux J, Liedtke W, Kotlikoff MI, Heppner TJ, Hill-Eubanks DC, Nelson MT. Elementary Ca^{2+} signals through endothelial TRPV4 channels regulate vascular function. *Science*. 2012;**336**(6081):597-601
- [15] Sullivan MN, Earley S. TRP channel Ca^{2+} sparklets: Fundamental signals underlying endothelium-dependent hyperpolarization. *The American Journal of Physiology*. 2013;**305**(10):C999-C1008
- [16] Bagher P, Beleznai T, Kansui Y, Mitchell R, Garland CJ, Dora KA. Low intravascular pressure activates endothelial cell TRPV4 channels, local Ca^{2+} events, and IK_{Ca} channels, reducing arteriolar tone. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**109**(44):18174-18179
- [17] Marziano C, Hong K, Cope EL, Kotlikoff MI, Isakson BE, Sonkusare SK. Nitric oxide-dependent feedback loop regulates transient receptor potential vanilloid 4 (TRPV4) channel cooperativity and endothelial function in small pulmonary arteries. *Journal of the American Heart Association*. 2017;**6**(12): pii: e007157
- [18] Sullivan MN, Gonzales AL, Pires PW, Bruhl A, Leo MD, Li W, Oulidi A, Boop FA, Feng Y, Jaggar JH, Welsh DG, Earley S. Localized TRPA1 channel Ca^{2+} signals stimulated by reactive oxygen species promote cerebral artery dilation. *Science Signaling*. 2015;**8**(358):ra2
- [19] Baeyens N, Bandyopadhyay C, Coon BG, Yun S, Schwartz MA. Endothelial fluid shear stress sensing in vascular health and disease. *The Journal of Clinical Investigation*. 2016;**126**(3):821-828
- [20] Chistiakov DA, Orekhov AN, Bobryshev YV. Effects of shear stress on endothelial cells: Go with the flow. *Acta Physiologica*. 2017;**219**(2):382-408
- [21] Thosar SS, Johnson BD, Johnston JD, Wallace JP. Sitting and endothelial dysfunction: The role of shear stress. *Medical Science Monitor*. 2012;**18**(12):RA173-RA180
- [22] Zhou J, Li YS, Chien S. Shear stress-initiated signaling and its regulation of endothelial function. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2014;**34**(10):2191-2198
- [23] Ghiadoni L, Salvetti M, Muiesan ML, Taddei S. Evaluation of endothelial function by flow mediated dilation: Methodological issues and clinical importance. *High Blood Pressure & Cardiovascular Prevention*. 2015;**22**(1):17-22
- [24] Yeboah J, Crouse JR, Hsu F-C, Burke GL, Herrington DM. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: The cardiovascular health study. *Circulation*. 2007;**115**(18):2390-2397
- [25] Phillips SA, Mahmoud AM, Brown MD, Haus JM. Exercise interventions and peripheral arterial function: Implications for cardio-metabolic disease. *Progress in Cardiovascular Diseases*. 2015;**57**(5):521-534
- [26] Son Y, Kim K, Jeon S, Kang M, Lee S, Park Y. Effect of exercise intervention on flow-mediated dilation in overweight and obese adults: Meta-analysis. *International Journal of Vascular Medicine*. 2017;**2017**:7532702

- [27] Green DJ, Dawson EA, Groenewoud HM, Jones H, Thijssen DH. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. *Hypertension*. 2014;**63**(2):376-382
- [28] Stoner L, Erickson ML, Young JM, Fryer S, Sabatier MJ, Faulkner J, Lambrick DM, McCully KK. There's more to flow-mediated dilation than nitric oxide. *Journal of Atherosclerosis and Thrombosis*. 2012;**19**(7):589-600
- [29] Uematsu M, Kitabatake A, Tanouchi J, Doi Y, Masuyama T, Fujii K, Yoshida Y, Ito H, Ishihara K, Hori M. Reduction of endothelial microfilament bundles in the low-shear region of the canine aorta. Association with intimal plaque formation in hypercholesterolemia. *Arteriosclerosis and Thrombosis*. 1991;**11**(1):107-115
- [30] Gündüz F, Koçer G, Ulker S, Meiselman HJ, Başkurt OK, Sentürk UK. Exercise training enhances flow-mediated dilation in spontaneously hypertensive rats. *Physiological Research*. 2011;**60**(4):589-597
- [31] Mendoza SA, Fang J, Gutterman DD, Wilcox DA, Bubolz AH, Li R, Suzuki M, Zhang DX. TRPV4-mediated endothelial Ca^{2+} influx and vasodilation in response to shear stress. *The American Journal of Physiology*. 2010;**298**(2):H466-H476
- [32] Brähler S, Kaistha A, Schmidt VJ, Wölflle SE, Busch C, Kaistha BP, Kacik M, Hasenau A-L, Grgic I, Si H, Bond CT, Adelman JP, Wulff H, de Wit C, Hoyer J, Köhler R. Genetic deficit of SK3 and IK1 channels disrupts the endothelium-derived hyperpolarizing factor vasodilator pathway and causes hypertension. *Circulation*. 2009;**119**(17):2323-2332
- [33] Wei R, Lunn SE, Tam R, Gust SL, Classen B, Kerr PM, Plane F. Vasoconstrictor stimulus determines the functional contribution of myoendothelial feedback to mesenteric arterial tone. *The Journal of Physiology*. 2018;**596**(7):1181-1197
- [34] Lu T, Wang XL, Chai Q, Sun X, Sieck GC, Katusic ZS, Lee HC. Role of the endothelial caveolae microdomain in shear stress-mediated coronary vasorelaxation. *The Journal of Biological Chemistry*. 2017;**292**(46):19013-19023
- [35] Chai Q, Wang X-L, Zeldin DC, Lee H-C. Role of caveolae in shear stress-mediated endothelium-dependent dilation in coronary arteries. *Cardiovascular Research*. 2013;**100**:151-159
- [36] Jalali S, del Pozo MA, Chen K, Miao H, Li Y, Schwartz MA, Shyy JY, Chien S. Integrin-mediated mechanotransduction requires its dynamic interaction with specific extracellular matrix (ECM) ligands. *Proceedings of the National Academy of Sciences of the United States of America*. 2001;**98**:1042-1046
- [37] Wang Y, Miao H, Li S, Chen KD, Li YS, Yuan S, Shyy JY, Chien S. Interplay between integrins and FLK-1 in shear stress-induced signaling. *The American Journal of Physiology*. 2002;**283**:C1540-C1547
- [38] Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA. A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*. 2005;**437**:426-431

- [39] Yamamoto K, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, Ohura N, Fukuda T, Sato T, Sekine K, Kato S, Isshiki M, Fujita T, Kobayashi M, Kawamura K, Masuda H, Kamiya A, Ando J. Impaired flow-dependent control of vascular tone and remodeling in P2X4-deficient mice. *Nature Medicine*. 2006;**12**:133-137
- [40] Xu J, Mathur J, Vessières E, Hammack S, Nonomura K, Favre J, Grimaud L, Petrus M, Francisco A, Li J, Lee V, Xiang FL, Mainquist JK, Cahalan SM, Orth AP, Walker JR, Ma S, Lukacs V, Bordone L, Bandell M, Laffitte B, Xu Y, Chien S, Henrion D, Patapoutian A. GPR68 senses flow and is essential for vascular physiology. *Cell*. 2018;**173**(3):762-775
- [41] Ahn SJ, Fancher IS, Bian JT, Zhang CX, Schwab S, Gaffin R, Phillips SA, Levitan I. Inwardly rectifying K⁺ channels are major contributors to flow-induced vasodilatation in resistance arteries. *The Journal of Physiology*. 2017;**595**(7):2339-2364
- [42] Zhang T, Chi S, Jiang F, Zhao Q, Xiao B. A protein interaction mechanism for suppressing the mechanosensitive Piezo channels. *Nature Communications*. 2017;**8**(1):1797
- [43] Li J, Hou B, Tumova S, Muraki K, Bruns A, Ludlow MJ, Sedo A, Hyman AJ, McKeown L, Young RS, Yuldasheva NY, Majeed Y, Wilson LA, Rode B, Bailey MA, Kim HR, Fu Z, Carter DA, Bilton J, Imrie H, Ajuh P, Dear TN, Cubbon RM, Kearney MT, Prasad RK, Evans PC, Ainscough JF, Beech DJ. Piezo1 integration of vascular architecture with physiological force. *Nature*. 2014;**515**(7526):279-282
- [44] Gao X, Wu L, O'Neil RG. Temperature-modulated diversity of TRPV4 channel gating: Activation by physical stresses and phorbol ester derivatives through protein kinase C-dependent and -independent pathways. *The Journal of Biological Chemistry*. 2003;**278**(29):27129-27137
- [45] Moccia F, Berra-Romani R, Tanzi F. Update on vascular endothelial Ca²⁺ signalling: A tale of ion channels, pumps and transporters. *World Journal of Biological Chemistry*. 2012;**3**:127-158
- [46] Reitsma S, Slaaf DW, oude Egbrink MG. The endothelial glycocalyx: Composition, functions, and visualization. *Pflügers Archiv*. 2007;**454**:345-359
- [47] Loot AE, Popp R, Fisslthaler B, Vriens J, Nilius B, Fleming I. Role of cytochrome P450-dependent transient receptor potential V4 activation in flow-induced vasodilatation. *Cardiovascular Research*. 2008;**80**(3):445-452
- [48] Hartmannsgruber V, Heyken WT, Kacik M, Kaistha A, Grgic I, Harteneck C, Liedtke W, Hoyer J, Köhler R. Arterial response to shear stress critically depends on endothelial TRPV4 expression. *PLoS One*. 2007;**2**(9):e827
- [49] Filosa JA, Yao X, Rath G. TRPV4 and the regulation of vascular tone. *Journal of Cardiovascular Pharmacology*. 2013;**61**(2):113-119
- [50] Bubolz AH, Mendoza SA, Zheng X, Zinkevich NS, Li R, Gutterman DD, Zhang DX. Activation of endothelial TRPV4 channels mediates flow-induced dilation in human

- coronary arterioles: Role of Ca^{2+} entry and mitochondrial ROS signaling. *The American Journal of Physiology*. 2012;**302**(3):H634-H642
- [51] Köhler R, Heyken WT, Heinau P, Schubert R, Si H, Kacik M, Busch C, Grgic I, Maier T, Hoyer J. Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2006;**26**(7):1495-1502
- [52] Addison MP, Singh TU, Parida S, Choudhury S, Kasa JK, Sukumaran SV, Darzi SA, Kandasamy K, Singh V, Kumar D, Mishra SKNO. Synthase inhibition attenuates EDHF-mediated relaxation induced by TRPV4 channel agonist GSK1016790A in the rat pulmonary artery: Role of TxA2. *Pharmacological Reports*. 2016;**68**(3):620-626
- [53] Gratton JP, Bernatchez P, Sessa WC. Caveolae and caveolins in the cardiovascular system. *Circulation Research*. 2004;**94**:1408-1417
- [54] Patel HH, Murray F, Insel PA. Caveolae as organizers of pharmacologically relevant signal transduction molecules. *Annual Review of Pharmacology and Toxicology*. 2008;**48**:359-391
- [55] Sandow SL, Neylon CB, Chen MX, Garland CJ. Spatial separation of endothelial small- and intermediate-conductance calcium-activated potassium channels (KCa) and connexins: Possible relationship to vasodilator function? *Journal of Anatomy*. 2006;**209**(5):689-698
- [56] Absi M, Burnham MP, Weston AH, Harno E, Rogers M, Edwards G. Effects of methyl β -cyclodextrin on EDHF responses in pig and rat arteries; association between SK_{Ca} channels and caveolin-rich domains. *British Journal of Pharmacology*. 2009;**151**(3):332-340
- [57] Saliez J, Bouzin C, Rath G, Ghisdal P, Desjardins F, Rezzani R, Rodella LF, Vriens J, Nilius B, Feron O, Balligand JL, Dessy C. Role of caveolar compartmentation in endothelium-derived hyperpolarizing factor-mediated relaxation- Ca^{2+} signals and gap junction function are regulated by caveolin in endothelial cells. *Circulation*. 2008;**117**(8):1065-1074
- [58] Goedicke-Fritz S, Kaistha A, Kacik M, Markert S, Hofmeister A, Busch C, Bänfer S, Jacob R, Grgic I, Hoyer J. Evidence for functional and dynamic microcompartmentation of Cav-1/TRPV4/KCa in caveolae of endothelial cells. *European Journal of Cell Biology*. 2015;**94**(7-9):391-400
- [59] Albinsson S, Nordström I, Swärd K, Hellstrand P. Differential dependence of stretch and shear stress signaling on caveolin-1 in the vascular wall. *The American Journal of Physiology*. 2008;**294**(1):C271-C279
- [60] Yu J, Bergaya S, Murata T, Alp IF, Bauer MP, Lin MI, Drab M, Kurzchalia TV, Stan RV, Sessa WC. Direct evidence for the role of caveolin-1 and caveolae in mechanotransduction and remodeling of blood vessels. *The Journal of Clinical Investigation*. 2006;**116**(5):1284-1291
- [61] Frangos JA, Eskin SG, McIntire LV, Ives CL. Flow effects on prostacyclin production by cultured human endothelial cells. *Science*. 1985;**227**:1477-1479

- [62] Hecker M, Mülsch A, Bassenge E, Busse R. Vasoconstriction and increased flow: Two principal mechanisms of shear stress-dependent endothelial autacoid release. *The American Journal of Physiology*. 1993;**265**(3 Pt 2):H828-H833
- [63] Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, Gutterman DD. Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. *Circulation Research*. 2003;**92**(2):e31-e40
- [64] Muller JM, Davis MJ, Kuo L, Chilian WM. Changes in coronary endothelial cell Ca^{2+} concentration during shear stress- and agonist-induced vasodilation. *The American Journal of Physiology*. 1999;**276**(5 Pt 2):H1706-H1714
- [65] Parnavelas JG, Kelly W, Burnstock G. Ultrastructural localization of choline acetyltransferase in vascular endothelial cells in rat brain. *Nature*. 1985;**316**:724-725
- [66] Yamamoto K, Sokabe T, Ohura N, Nakatsuka H, Kamiya A, Ando J. Endogenously released ATP mediates shear stress-induced Ca^{2+} influx into pulmonary artery endothelial cells. *The American Journal of Physiology*. 2003;**285**:H793-H803
- [67] Wilson C, Lee MD, McCarron JG. Acetylcholine released by endothelial cells facilitates flow-mediated dilatation. *The Journal of Physiology*. 2016;**594**(24):7267-7307
- [68] Ellinsworth DC, Sandow SL, Shukla N, Liu Y, Jeremy JY, Gutterman DD. Endothelium-derived hyperpolarization and coronary vasodilation: Diverse and integrated roles of epoxyeicosatrienoic acids, hydrogen peroxide, and gap junctions. *Microcirculation*. 2016;**23**(1):15-32
- [69] Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, Velicelebi G, Stauderman KA. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. *The Journal of Cell Biology*. 2005;**169**(3):435-445
- [70] Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, Meyer T. STIM is a Ca^{2+} sensor essential for Ca^{2+} -store-depletion-triggered Ca^{2+} influx. *Current Biology*. 2005;**15**(13):1235-1241
- [71] Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A, Hogan PG. Orai1 is an essential pore subunit of the CRAC channel. *Nature*. 2006;**443**(7108):230-233
- [72] Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature*. 2006;**441**(7090):179-185
- [73] Choi S, Maleth J, Jha A, Lee KP, Kim MS, So I, Ahuja M, Muallem S. The TRPCs-STIM1-Orai interaction. *Handbook of Experimental Pharmacology*. 2014;**223**:1035-1054
- [74] Freichel M, Suh SH, Pfeifer A, Schweig U, Trost C, Weissgerber P, Biel M, Philipp S, Freise D, Droogmans G, Hofmann F, Flockerzi V, Nilius B. Lack of an endothelial store-operated Ca^{2+} current impairs agonist-dependent vasorelaxation in TRP4 $^{-/-}$ mice. *Nature Cell Biology*. 2001;**3**(2):121-127

- [75] Zhang DX, Mendoza SA, Bubolz AH, Mizuno A, Ge ZD, Li R, Warltier DC, Suzuki M, Gutterman DD. Transient receptor potential vanilloid type 4-deficient mice exhibit impaired endothelium-dependent relaxation induced by acetylcholine in vitro and in vivo. *Hypertension*. 2009;**53**(3):532-538
- [76] Earley S, Pauyo T, Drapp R, Tavares MJ, Liedtke W, Brayden JE. TRPV4-dependent dilation of peripheral resistance arteries influences arterial pressure. *The American Journal of Physiology*. 2009;**297**(3):H1096-H1102
- [77] Fleming I, Rueben A, Popp R, Fisslthaler B, Schrodt S, Sander A, Haendeler J, Falck JR, Morisseau C, Hammock BD, Busse R. Epoxyeicosatrienoic acids regulate Trp channel dependent Ca^{2+} signaling and hyperpolarization in endothelial cells. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2007;**27**:2612-2618
- [78] Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature*. 1999;**397**(6716):259-263
- [79] Köhler R, Brakemeier S, Kühn M, Degenhardt C, Buhr H, Pries A, Hoyer J. Expression of ryanodine receptor type 3 and TRP channels in endothelial cells: Comparison of in situ and cultured human endothelial cells. *Cardiovascular Research*. 2001;**51**(1):160-168
- [80] Jacob R, Merritt JE, Hallam TJ, Rink TJ. Repetitive spikes in cytoplasmic calcium evoked by histamine in human endothelial cells. *Nature*. 1988;**335**(6185):40-45
- [81] Kansui Y, Garland CJ, Dora KA. Enhanced spontaneous Ca^{2+} events in endothelial cells reflect signalling through myoendothelial gap junctions in pressurized mesenteric arteries. *Cell Calcium*. 2008;**44**(2):135-146
- [82] Marrelli SP. Mechanisms of endothelial P2Y(1)- and P2Y(2)-mediated vasodilatation involve differential $[Ca^{2+}]_i$ responses. *The American Journal of Physiology*. 2001;**281**(4):H1759-H1766
- [83] Dedkova EN, Blatter LA. Nitric oxide inhibits capacitative Ca^{2+} entry and enhances endoplasmic reticulum Ca^{2+} uptake in bovine vascular endothelial cells. *The Journal of Physiology*. 2002;**539**(Pt 1):77-91
- [84] Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A. Sources of Ca^{2+} in relation to generation of acetylcholine-induced endothelium-dependent hyperpolarization in rat mesenteric artery. *British Journal of Pharmacology*. 1997;**120**(7):1328-1334
- [85] Nishimoto M, Mizuno R, Fujita T, Isshiki M. Stromal interaction molecule 1 modulates blood pressure via NO production in vascular endothelial cells. *Hypertension Research*. 2018 Apr 25 [Epub ahead of print]
- [86] Doughty JM, Plane F, Langton PD. Charybdotoxin and apamin block EDHF in rat mesenteric artery if selectively applied to the endothelium. *The American Journal of Physiology*. 1999;**276**(3 Pt 2):H1107-H1112

- [87] Pannirselvam M, Ding H, Anderson TJ, Triggle CR. Pharmacological characteristics of endothelium-derived hyperpolarizing factor-mediated relaxation of small mesenteric arteries from db/db mice. *European Journal of Pharmacology*. 2006;**551**(1–3):98-107
- [88] Yamanaka A, Ishikawa T, Goto K. Characterization of endothelium-dependent relaxation independent of NO and prostaglandins in Guinea pig coronary artery. *The Journal of Pharmacology and Experimental Therapeutics*. 1998;**285**(2):480-489
- [89] Marrelli SP, Eckmann MS, Hunte MS. Role of endothelial intermediate conductance K_{Ca} channels in cerebral EDHF-mediated dilations. *The American Journal of Physiology*. 2003;**285**(4):H1590-H1599
- [90] Chadha PS, Liu L, Rikard-Bell M, Senadheera S, Howitt L, Bertrand RL, Grayson TH, Murphy TV, Sandow SL. Endothelium-dependent vasodilation in human mesenteric artery is primarily mediated by myoendothelial gap junctions intermediate conductance calcium-activated K^+ channel and nitric oxide. *The Journal of Pharmacology and Experimental Therapeutics*. 2011;**336**(3):701-708
- [91] Sandow SL, Haddock RE, Hill CE, Chadha PS, Kerr PM, Welsh DG, Plane F. What's where and why at a vascular myoendothelial microdomain signalling complex. *Clinical and Experimental Pharmacology & Physiology*. 2009;**36**(1):67-76
- [92] Kerr PM, Wei R, Tam R, Sandow SL, Murphy TV, Ondrusova K, Lunn SE, Tran CHT, Welsh DG, Plane F. Activation of endothelial IK_{Ca} channels underlies NO-dependent myoendothelial feedback. *Vascular Pharmacology*. 2015;**74**:130-138
- [93] Dora KA, Gallagher NT, McNeish A, Garland CJ. Modulation of endothelial cell $KCa_{3.1}$ channels during endothelium-derived hyperpolarizing factor signaling in mesenteric resistance arteries. *Circulation Research*. 2008;**102**(10):1247-1255
- [94] Senadheera S, Kim Y, Grayson TH, Toemoe S, Kochukov MY, Abramowitz J, Housley GD, Bertrand RL, Chadha PS, Bertrand PP, Murphy TV, Tare M, Birnbaumer L, Marrelli SP, Sandow SL. Transient receptor potential canonical type 3 channels facilitate endothelium-derived hyperpolarization-mediated resistance artery vasodilator activity. *Cardiovascular Research*. 2012;**95**(4):439-447
- [95] Sonkusare SK, Dalsgaard T, Bonev AD, Hill-Eubanks DC, Kotlikoff MI, Scott JD, Santana LF, Nelson MT. AKAP150-dependent cooperative TRPV4 channel gating is central to endothelium-dependent vasodilation and is disrupted in hypertension. *Science Signaling*. 2014;**7**(333):ra66
- [96] Earley S, Gonzales AL, Garcia ZI. A dietary agonist of transient receptor potential cation channel V3 elicits endothelium-dependent vasodilation. *Molecular Pharmacology*. 2010;**77**(4):612-620
- [97] Earley S, Gonzales AL, Crnich R. Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca^{2+} -activated K^+ channels. *Circulation Research*. 2009;**104**(8):987-994

- [98] Kochukov MY, Balasubramanian A, Abramowitz J, Birnbaumer L, Marrelli SP. Activation of endothelial transient receptor potential C3 channel is required for small conductance calcium-activated potassium channel activation and sustained endothelial hyperpolarization and vasodilation of cerebral artery. *Journal of the American Heart Association*. 2014;**3**(4): pii: e000913
- [99] Yeon SI, Kim JY, Yeon DS, Abramowitz J, Birnbaumer L, Muallem S, Lee YH. Transient receptor potential canonical type 3 channels control the vascular contractility of mouse mesenteric arteries. *PLoS One*. 2014;**9**(10):e110413
- [100] Greenberg HZE, Carlton-Carew SRE, Khan DM, Zargaran AK, Jahan KS, Ho WS, Albert AP. Heteromeric TRPV4/TRPC1 channels mediate calcium-sensing receptor-induced nitric oxide production and vasorelaxation in rabbit mesenteric arteries. *Vascular Pharmacology*. 2017;**96-98**:53-62
- [101] Stankevicius E, Lopez-Valverde V, Rivera L, Hughes A, Mulvany MJ, Simonsen U. Combination of Ca²⁺-activated K⁺ channel blockers inhibits acetylcholine-evoked nitric oxide release in rat superior mesenteric artery. *British Journal of Pharmacology*. 2006; **149**(5):560-572
- [102] Sheng J, Braun AP. Small- and intermediate-conductance Ca²⁺-activated K⁺ channels directly control agonist-evoked nitric oxide synthesis in human vascular endothelial cells. *The American Journal of Physiology*. 2007;**293**(1):C458-C467
- [103] Stankevicius E, Dalsgaard T, Kroigaard C, Beck L, Boedtkjer E, Misfeldt M, Nielsen G, Schjorring O, Hughes A. Opening of small and intermediate calcium-activated potassium channels induces relaxation mainly mediated by nitric-oxide release in large arteries and endothelium-derived hyperpolarizing factor in small arteries from rat. *The Journal of Pharmacology and Experimental Therapeutics*. 2011;**339**(3):842-850
- [104] Sheng JZ, Ella S, Davis MJ, Hill MA, Braun AP. Openers of SK_{Ca} and IK_{Ca} channels enhance agonist-evoked endothelial nitric oxide synthesis and arteriolar vasodilation. *The FASEB Journal*. 2009;**23**(4):1138-1145
- [105] Oike M, Gericke M, Droogmans G, Nilius B. Calcium entry activated by store depletion in human umbilical vein endothelial cells. *Cell Calcium*. 1994;**16**:367-376
- [106] Wang X, Van Breeman C. Depolarization-mediated inhibition of Ca²⁺ entry in endothelial cells. *The American Journal of Physiology*. 1999;**277**:H1498-H1504
- [107] Adams DJ, Barakeh J, Laskey R, Van Breemen C. Ion channels and regulation of intracellular calcium in vascular endothelial cells. *The FASEB Journal*. 1989;**3**(12):2389-2400
- [108] Lückhoff A, Busse R. Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. *Pflügers Archiv*. 1990; **416**(3):305-311

- [109] Allen T, Iftinca M, Cole WC, Plane F. Smooth muscle membrane potential modulates endothelium-dependent relaxation of rat basilar artery via myoendothelial gap junctions. *The Journal of Physiology*. 2002;**545**(Pt 3):975-986
- [110] Clapham DE. Calcium signaling. *Cell*. 2007;**131**(6):1047-1058
- [111] McSherry IN, Spitaler MM, Takano H, Dora KA. Endothelial cell Ca^{2+} increases are independent of membrane potential in pressurized rat mesenteric arteries. *Cell Calcium*. 2005;**38**(1):23-33
- [112] Behringer EJ, Segal SS. Membrane potential governs calcium influx into microvascular endothelium: Integral role for muscarinic receptor activation. *The Journal of Physiology*. 2015;**593**(20):4531-4548
- [113] Behringer EJ. Calcium and electrical signaling in arterial endothelial tubes: New insights into cellular physiology and cardiovascular function. *Microcirculation*. 2017;**24**(3). DOI: 10.1111/micc.12328
- [114] Yap FC, Weber DS, Taylor MS, Townsley MI, Comer BS, Maylie J, Adelman JP, Lin MT. Endothelial SK3 channel-associated Ca^{2+} microdomains modulate blood pressure. *The American Journal of Physiology*. 2016;**310**(9):H1151-H1163
- [115] Westcott EB, Segal SS. Perivascular innervation: A multiplicity of roles in vasomotor control and myoendothelial signaling. *Microcirculation*. 2013;**20**(3):217-238
- [116] Kerr PM, Tam R, Ondrusova K, Mittal R, Narang D, Tran CH, Welsh DG, Plane F. Endothelial feedback and the myoendothelial projection. *Microcirculation*. 2012;**19**(5):416-422
- [117] Mutchler SM, Straub AC. Compartmentalized nitric oxide signaling in the resistance vasculature. *Nitric Oxide*. 2015;**49**:8-15
- [118] Biwer LA, Taddeo EP, Kenwood BM, Hoehn KL, Straub AC, Isakson BE. Two functionally distinct pools of eNOS in endothelium are facilitated by myoendothelial junction lipid composition. *Biochimica et Biophysica Acta*. 2016;**1861**(7):671-679
- [119] Segal SS, Jacobs TL. Role for endothelial cell conduction in ascending vasodilatation and exercise hyperaemia in hamster skeletal muscle. *The Journal of Physiology*. 2001;**536**(Pt 3): 937-946
- [120] Sinkler SY, Segal SS. Rapid versus slow ascending vasodilatation: Intercellular conduction versus flow-mediated signalling with tetanic versus rhythmic muscle contractions. *The Journal of Physiology*. 2017;**595**:7149-7165

