WJBC World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
doi:10.4331/wjbc.v3.i7.127

World J Biol Chem 2012 July 26; 3(7): 127-158
ISSN 1949-8454 (online)
© 2012 Baishideng. All rights reserved.

REVIEW

Update on vascular endothelial Ca^{2+} signalling: A tale of ion channels, pumps and transporters

Francesco Moccia, Roberto Berra-Romani, Franco Tanzi

Francesco Moccia, Franco Tanzi, Department of Biology and Biotechnologies "Lazzaro Spallanzani", Laboratory of Physiology, University of Pavia, Via Forlanini 6, 27100 Pavia, Italy
Roberto Berra-Romani, Department of Biomedicine, School of Medicine, Benemérita Universidad Autónoma de Puebla, 3 Sur 2702, Colonia Volcanes Puebla 72410, México
Author contributions: Moccia F wrote the manuscript; Berra-Romani R revised the manuscript, Tanzi F wrote the manuscript.
Correspondence to: Dr. Francesco Moccia, Department of Biology and Biotechnologies "Lazzaro Spallanzani", Laboratory of Physiology, University of Pavia, Via Forlanini 6, 27100 Pavia, Italy. francesco.moccia@unipv.it
Telephone: +39-382-987169 Fax: +39-382-987527
Received: May 17, 2012 Revised: July 4, 2012
Accepted: July 11, 2012
Published online: July 26, 2012

Abstract

A monolayer of endothelial cells (ECs) lines the lumen of blood vessels and forms a multifunctional transducing organ that mediates a plethora of cardiovascular processes. The activation of ECs from as state of quiescence is, therefore, regarded among the early events leading to the onset and progression of potentially lethal diseases, such as hypertension, myocardial infarction, brain stroke, and tumor. Intracellular Ca^{2+} signals have long been know to play a central role in the complex network of signaling pathways regulating the endothelial functions. Notably, recent work has outlined how any change in the pattern of expression of endothelial channels, transporters and pumps involved in the modulation of intracellular Ca^{2+} levels may dramatically affect whole body homeostasis. Vascular ECs may react to both mechanical and chemical stimuli by generating a variety of intracellular Ca^{2+} signals, ranging from brief, localized Ca^{2+} pulses to prolonged Ca^{2+} oscillations engulfing the whole cytoplasm. The well-defined spatiotemporal profile of the subcellular Ca^{2+} signals elicited in ECs by specific extracellular inputs depends on the interaction between Ca^{2+} releasing channels, which are located both on the plasma membrane and in a number of intracellular organelles, and Ca^{2+} removing systems. The present article aims to summarize both the past and recent literature in the field to provide a clear-cut picture of our current knowledge on the molecular nature and the role played by the components of the Ca^{2+} machinery in vascular ECs under both physiological and pathological conditions.

© 2012 Baishideng. All rights reserved.

Key words: Endothelial cells; Ca^{2+} signalling; Plasma membrane; Endoplasmic reticulum; Intracellular Ca^{2+} release; Ca^{2+} entry; Ca^{2+} removal; Ca^{2+} oscillations

Peer reviewers: Saobo Lei, Associate Professor, Department of Pharmacology, Physiology and Therapeutics, University of North Dakota, 501 N Columbia Road, Grand Forks, ND 58203, United States; Claudio F Perez, PhD, Department of Anesthesia, Brigham and Women's Hospital, GW Thorn Building, TH-726B, 20 Shattuck Street, Boston, MA 02115, United States

Moccia F, Berra-Romani R, Tanzi F. Update on vascular endothelial Ca^{2+} signalling: A tale of ion channels, pumps and transporters. *World J Biol Chem* 2012; 3(7): 127-158 Available from: URL: http://www.wjgnet.com/1949-8454/full/v3/i7/127.htm DOI: http://dx.doi.org/10.4331/wjbc.v3.i7.127

INTRODUCTION

It has long been known that an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) plays a key role in the intricate network of signal transduction pathways exploited by endothelial cells (ECs) to maintain cardiovascular homeostasis[1]. Due to its strategic location at the interface between the vascular wall and bloodstream, the endothelium is exposed to a myriad of transmitters (released by autonomic and sensory nerves or platelets), circulating hormones, autacoids, cytokines, growth factors, and drugs, as well as to mechanical stimuli, such as pulsatile

stretch, shear stress, and changes in the local osmotic pressure[2,3]. Moreover, vascular ECs might serve as thermosensors and modulate peripheral vasoconstriction or vasodilation depending on the environmental temperature[4]. A remarkable blend of membrane receptors, transporters, and ion channels, which are located both on the plasma membrane and within the intracellular Ca^{2+} reservoirs (namely, endoplasmic reticulum, lysosomes, and Golgi), may be utilized by ECs to detect and react selectively to the incoming stimulus with the most suitable Ca^{2+} signal[1,4-8]. A number of excellent reviews have recently outlined features and activation mechanisms of Ca^{2+}-permeable channels in vascular endothelium[1,7,8]. The present article will survey the Ca^{2+} signaling toolkit whereby ECs shape their Ca^{2+} responses to chemical and mechanical stimuli by summarizing the most recent developments in the field. Recent work has shown that endothelial progenitor cells (EPCs), a subpopulation of mononuclear cells that are mobilized from bone marrow upon an ischemic insult, impinge on Ca^{2+} signals to proliferate, traffic to the injured tissue, and form capillary-like structures. However, we refer to a recent review from our laboratory for an exhaustive description of our current knowledge about the functional role and the molecular underpinnings of Ca^{2+} signalling in human EPCs[9].

Ca^{2+} SIGNALLING IN VASCULAR ECs

In resting, i.e., non-stimulated cells, $[Ca^{2+}]_i$ is very low (around 100 nmol/L) due to the activity of various active mechanisms that remove Ca^{2+} out of the cytosol[10]. More specifically, the plasma membrane Ca^{2+}-ATPase (PMCA) and the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) extrude Ca^{2+} by direct ATP hydrolysis, while the Na^+/Ca^{2+} exchanger (NCX) clears cytosolic Ca^{2+} by exploiting the Na^+ gradient across the plasma membrane[10]. An increase in $[Ca^{2+}]_i$ up to 1 μmol/L is the key signal to activate vascular ECs following recruitment of either tyrosine-kinase linked receptors (TRKs) or G-protein coupled receptors (GPCRs) by growth factor and vasoactive agents, respectively[1,6,7,9,11,12]. The endothelial Ca^{2+} response may comprise an initial Ca^{2+} spike, shaped by Ca^{2+} mobilization from the intracellular Ca^{2+} reservoir, followed by an intermediate plateau level due to Ca^{2+} entry across the plasma membrane[6,7,9]. However, at low-dose agonist stimulation, the Ca^{2+} signal may adopt an oscillatory pattern driven by the interplay between intracellular Ca^{2+} release and Ca^{2+} influx from the extracellular space[1,6,13]. Responding cells with an intermediate behavior between the extremes described above can also be found. Accordingly, in most microvascular ECs, chemical stimulation does not elicit a remarkable Ca^{2+} inflow and results in either a transient Ca^{2+} spike or localized, high frequency Ca^{2+} oscillations, which are attributable to Ca^{2+} mobilization from the intracellular store[14-16]. Conversely, when the Ca^{2+} signal is uniquely modelled by Ca^{2+} entry, a sustained and monotonic increase in $[Ca^{2+}]_i$ occurs[17,18]. Mechanical stimulation may also affect vascular endothelium by causing an elevation in $[Ca^{2+}]_i$. Indeed, exposure of ECs to fluid shear stress, pulsatile stretch, and cell swelling may evoke both Ca^{2+} discharge from intracellular Ca^{2+} pools and Ca^{2+} inflow through mechanosensitive channels on the plasmalemma[4,7,19]. Recent studies have argued that ion channels serve as direct sensor of mechanical stimuli, but rather as a relay that is engaged by a yet to identify upstream receptor[19]. Unlike the Ca^{2+} response to extracellular ligands, mechanosensitive Ca^{2+} signals normally lack the biphasic kinetics and exhibit either a transient[20,21] or an oscillatory pattern[21-25]. In a few cases, a dose-dependent stepwise elevation in $[Ca^{2+}]_i$ was observed in cultured ECs exposed to laminar shear stress[24,26,27]. The contact between air microbubbles and cultured ECs may also result in an intracellular Ca^{2+} wave[28]. Finally, an increase in $[Ca^{2+}]_i$ arises in vascular ECs upon contact with circulating leukocytes[29] or metastatizing tumor cells[30,31].

Ca^{2+} RELEASE FROM INTRACELLULAR STORES

The endoplasmic reticulum (ER) houses the largest intracellular Ca^{2+} reservoir in ECs, amounting to approximately 75% of the total cellular storage capacity[32]. A recent ultrastructural analysis has revealed that the endothelial ER consists of tubulovesicular structures that are interconnected at various sites to form a widespread network within the ECs. In the periphery of the cells, they lie immediately adjacent to the plasma membrane and the caveolae at the apical, lateral, and basal surfaces of the cells and occasionally run closely parallel to the plasma membrane itself[33-35]. Intriguingly, the ER-plasmalemma coupling is tighter (around 8 nm) in macrovascular than in microvascular (around 87 nm) ECs[33]. As Ca^{2+} entry may be governed by the physical interaction between an ER Ca^{2+}-sensor and the plasmalemmal Ca^{2+}-permeable channels (see below), this feature might help explain why microvascular ECs normally lack the plateau phase[33]. In the middle of the cells, the tubulovesicular structures may be in close contact with the mitochondria, sometimes enveloping them[34-36], and are in continuity with the nuclear membrane[34]. Ca^{2+} is sequestered into the ER lumen, where it attains a concentration of about 100-500 μmol/L, by high-affinity (K_m around 1 μmol/L) SERCA pumps, which can be inhibited by pharmacological agents, such as the marine toxin thapsigargin and cyclopiazonic acid (CPA)[37,38]. Inhibition of the SERCA pump with either drug leads to Ca^{2+} store depletion, which indicates that ER membrane is constitutively leaky to Ca^{2+}. Candidates to mediate the leak channel in a variety of cell types include polycystin-2, presenilins, and the ribosome-translocon complex[37]. No study has, however, hitherto addressed the molecular underpinnings of passive Ca^{2+} leak from ER in ECs[1]. Calreticulin represents the major Ca^{2+} buffer within the ER lumen in all non-muscle cells[38], as well as in vascular endothelium[34,35]. Maintenance of a constant luminal level of Ca^{2+} in ECs

is also fulfilled by a number of additional Ca^{2+}-binding proteins, including calnexin, glucose-regulated protein (GRP)-78, and GRP-94[38]. All these Ca^{2+} buffers also serve as molecular chaperones and their protein folding activity is regulated by ER Ca^{2+} concentration ($[Ca^{2+}]_{ER}$)[38]. Monitoring of intraluminal Ca^{2+} levels in ECs transiently transfected with a cameleon-based Ca^{2+} probe targeted to the ER, $D1_{ER}$, has shown that, in the presence of extracellular Ca^{2+}, extracellular autacoids do not induce a massive decline in $[Ca^{2+}]_{ER}$[39-42]. Consequently, Ca^{2+}-dependent protein folding is largely maintained during physiological cell stimulation and does not activate any stress signaling pathway[38,42]. Conversely, a rapid drop in ER Ca^{2+} content occurs when ECs are stimulated in the absence of extracellular Ca^{2+} entry[43]. Owing to the large electrochemical gradient existing between ER lumen and the cytosol, rapid release of Ca^{2+} from the intracellular organelle occurs upon the opening of two largely homologous receptor types on its membrane, which are named for their affinity for either the cell metabolite inositol-1,4,5-trisphosphate ($InsP_3$) or the plant alkaloid ryanodine[1,6,11]. Ryanodine receptors (RyRs), in particular, may be engaged by either a local increase in intracellular Ca^{2+} levels trough a process called Ca^{2+}-induced Ca^{2+} release (CICR) or the second messenger, cyclic ADPribose (cADPr)[44-48]. The remaining 25% of the total stored Ca^{2+} in ECs is loaded in non-ER intracellular pools, mainly mitochondria, which contribute to agonists-induced intracellular Ca^{2+} signaling[32,36,40,41]. Ca^{2+} enters the organelles through a uniporter, present in the inner membrane, and can be exported *via* the stimulation of a mitochondrial NCX (NCX_{mito})[36,41], thus creating a Ca^{2+} cycling system for the control of mitochondrial Ca^{2+} concentration that, under resting conditions, is equal to around 200 nmol/L in ECs[49]. Mitochondria give rise to an impressive network in ECs, which is in tight apposition with either Ca^{2+} release channels on the ER or Ca^{2+}-permable pathways, mainly store-operated Ca^{2+} channels (SOCs), on the plasma membrane[34-36]. Acidic, lysosome-like vesicles may also serve as Ca^{2+} storage organelles in vascular endothelium[50,51]. Unlike the large network that is the ER, lysosomes constitute relatively small, discrete and mobile vesicles with a limited Ca^{2+} content, which can be discharged by NAADP[50,51]. An attempt to quantify the relative lysosomal Ca^{2+} content suggested the latter to amount to around 79% of the ER Ca^{2+} pool[52]. Such a value, which is at odds with a previous analysis of the relative capacities of intracellular Ca^{2+} storage organelles[32], does not truly reflect lysosomal Ca^{2+} levels. Indeed, unlike the large network that is the ER, lysosomes generally constitute relatively small, discrete and mobile vesicles with a limited Ca^{2+} content[53]. However, such acidic stores may be situated very close to the Ca^{2+}-releasing channels on ER. As a consequence, NAADP induces scattered, local Ca^{2+} signals that are capable of generating a global Ca^{2+} wave only by triggering further Ca^{2+} mobilization from $InsP_3Rs$ and/or RyRs *via* the CICR mechanism[53] (see below). Along with $InsP_3$, cADPr, and NAADP, ECs may liberate intracellular Ca^{2+} in response to other second messengers, such as arachidonic acid (AA)[54], whose intracellular target remains to be elucidated, and reactive oxygen species (ROS), such as H_2O_2[55,56] and superoxide anion (O_2^-), which directly evoke Ca^{2+} release from $InsP_3Rs$[57]. As to the sphingolipid-derived messenger, sphingosine-1-phosphate (S1P), that may elicit intracellular Ca^{2+} release independently from $InsP_3Rs$ and RyRs[10], it has been shown that, when endogenously synthesized in autacoids-stimulated ECs, it modulates SOCs, but does not stimulate Ca^{2+} mobilization[58]. In blood vessels, however, plasma S1P may be produced and released by erythrocytes and platelets and induce Ca^{2+} mobilization in ECs upon binding to the G_i protein-coupled S1P1 receptor[59]. The latter, in turn, recruits phospholipase C (PLC) to generate $InsP_3$ and, thus, stimulates $InsP_3Rs$ and activates SOCs[60,61].

Phospholipase C, $InsP_3$, and $InsP_3$-receptors

The intracellular second messenger $InsP_3$ liberates Ca^{2+} ions stored within the ER and, in ECs, is synthesized following activation of either GPCRs or TRKs[1,6,11,62]. $InsP_3$ is a highly diffusible hydrophilic messenger that is produced by the cleavage of phosphatidylinositol-4,5-bisphosphate (PIP_2). This reaction is catalyzed by a family of PLC isozymes, which, depending on the subtype, can be activated by G-proteins ($G_{q/11}$), phosphorylation or Ca^{2+} itself[63,64]. The other product of PIP_2 hydrolysis is diacylglycerol (DAG), which stays within the plasma membrane where it either activates protein kinase C (PKC) or gates members of the transient receptor potential (TRP) canonical (TRPC) subfamily of Ca^{2+}-permeable channels[10]. The 13 mammalian PLC isozymes (excluding alternatively spliced forms) identified to date are all single polypeptides and can be divided into five types: PLCβ (β1-4), PLCδ (δ1-4), PLCγ (γ1 and γ2), PLCε, PLCζ, and PLCη (η1 and η2)[63,64]. The amino acid sequences of PLC isozymes are relatively nonconserved with the exception of two regions, known as the X and Y domains, that form the catalytic core and are associated to various combinations of regulatory domains that are common to many other signalling proteins. In more detail, in γ-type isozymes, this region contains two additional Src homology 2 (SH2) domains and one SH3 domain, which bind phosphorylated tyrosine residues and proline-rich sequences, respectively[63,64]. All PLC isoforms, with the exception of PLCε, are endowed with at least one potential polyphosphoinositides binding site, the pleckstrin homology (PH) domain, which is located in the NH_2-terminal region preceding the X domain[63,64]. Moreover, the COOH-terminal region possesses a C2 domain which confers a strong Ca^{2+} sensitivity to the enzymatic activity of PLC. The C2 domain is, however, absent in the γ-type[63,64]. In this view, four EF domains are located between the PH and X domains of all PLC subclasses but their functional significance is yet to be fully elucidated[63,64]. PLCε, finally, exhibits a NH_2-terminal Ras guanine nucleotide exchange factor (RasGEF)-like domain and at least one COOH-

terminal Ras binding (RA) domains that are involved in its activation[63,64]. ECs express a variety of PLC isoforms. PLCβ1 is enlisted following GPCR binding to their specific agonists, which include a plethora of circulating autacoids: acetylcholine, ATP, ADP, thrombin, bradykinin, serotonin, angiotensin II, and anandamide[1,7,65]. Receptor occupancy results in the activation of the pertuxis toxin (PTX)-insensitive heterotrimeric $G_{q/11}$ protein family. As a consequence, the GTP-bound $G_{\alpha q/11}$ subunit dissociates from the $G_{\beta\gamma}$ dimer and activates the enzyme[1,7,63,64]. A remarkable exception is provided by the proteinase-activated receptor 1, which utilizes both $G_{\alpha q}$[66,67] and $G_{\alpha o}$[68] to trigger Ca^{2+} signals in thrombin-stimulated ECs[69], albeit only the former leads to $InsP_3$ production[70]. The $G_{\alpha o}$-dependent activation is mediated by the $G_{\beta\gamma}$ dimer[68], which may recruit all PLC-β subtypes with the exception of the β4 isoform[63,64]. This mechanism is also involved in PLCβ engagement by the PTX-sensitive family of G_i proteins[63,64], as observed in ECs exposed to S1P[59-61]. PLCγ1 is the main γ-type expressed in ECs[9], with PLCγ2 being primarily restricted to cells of the hematopoietic lineage[71]. PLCγ1 is the isozyme mediating the increase in $[Ca^{2+}]_i$ elicited in vascular endothelium by growth factors, such as epidermal growth factor (EGF)[72], vascular endothelial growth factor (VEGF)[73-75], platelet-derived growth factor (PDGF)[76], and basic fibroblast growth factor (bFGF)[73,77]. Upon growth factor binding to its cognate receptor, PLC-γ1 is recruited to the activated receptors *via* SH2-domain-mediated phosphotyrosine interaction and then subjected to phosphorylation by receptor tyrosine kinase[63,64]. It has, however, been reported that PLC-γ1 may be also stimulated by GPCR in ECs. For instance, it may associate with the COOH-terminal intracellular domain of the bradykinin BR receptor (B2), which results in a transient phosphorylation and $InsP_3$ production[78,79]. On the other hand, VEGF has been recently shown to elicit PLCβ3 lipase activity upon binding to VEGFR-2 (KDR), which in turn physically interacts with $G_{q/11}$ proteins to induce $G_{\beta\gamma}$ dissociation from G_α subunits[80]. $G_{\beta\gamma}$ may thus stimulate PLCβ3 to generate $InsP_3$ and trigger an intracellular Ca^{2+} wave[81]. PLCδ1 has been so far detected in a limited number of vascular beds, although its ability to evoke $InsP_3$-dependent Ca^{2+} mobilization has been probed only in human umbilical vein ECs (HUVECs) transfected with PLCδ1-containing plasmids[82]. While there is no report of PLCη expression in ECs and PLCζ is present almost exclusively in mammal sperms[6], PLCε may promote angiogenesis in a mouse model of adenocarcinoma, but its role in Ca^{2+} signaling has not been explored[83]. It is, however, remarkable that β2-adrenergic excitation results to $InsP_3$-dependent Ca^{2+} discharge upon recruitment of the exchange protein activated by cAMP-1 (Epac-1)[84], which has long been known to stimulate PLCε[64]. In agreement with PLC activation by both GPCRs and TKRs, $InsP_3$ production has been evaluated in a variety of stimulated ECs[1]. In $[^3H]$myoinositol-labeled cultured cells, both thrombin and histamine-induced increases in $InsP_3$ occurred in less than 15 s, and were temporally correlated with the Ca^{2+} signals[70,85-87]. Bradykinin and ATP also evoked an elevation in $InsP_3$ concentration, which attained a peak value at 15 s after agonist application and then decayed to a plateau level with kinetics similar to those of the ensuing Ca^{2+} signals[70,88,89]. The same methodology revealed that platelet activating factor (PAF)-induced $InsP_3$ formation is maximal after 1 min of stimulation[90]. Similarly, a radioreceptor assay of $InsP_3$ accumulation in ECs revealed that VEGF induced a rapid increase in $InsP_3$ levels, which achieved a peak after 4 min of exposure[91]. Due to the objective difficulties entailed by these pioneering measurements, it is unlikely that they truly reflect the dynamics of agonists-induced $InsP_3$ accumulation in vascular ECs. A more recent investigation, based on an innovative analytical method, revealed that $InsP_3$ levels peak within < 1 s from ATP stimulation and then rapidly subside due to degradation to lower order inositol phosphates[92]. Besides agonist stimulation, $InsP_3$ production in vascular ECs may be stimulated by reactive oxygen species (ROS), such as $O_2^{·-}$[93] and H_2O_2[94].

In addition to extracellular agonists, mechanical stimuli may induce $InsP_3$ formation in vascular endothelium. Cyclic strain was found to elicit a transient spike of immunoreactive $InsP_3$ in bovine aortic ECs (BAECs): peak levels occurred 10 s after the initiation of strain and decayed to control values 25 s later[95]. Moreover, BAEC exposure to shear stress induced a biphasic increase in intracellular $InsP_3$ concentration, whereas a transient $InsP_3$ peak arising within the first 15 s was followed by a major peak observed at 15 min after the onset of the stimulus. $InsP_3$ levels returned to pre-shear stress level within the following 15 min[96]. Recent studies have demonstrated that the vascular system is endowed with mechanosensitive GPCRs translating mechanical stimuli into an $InsP_3$-dependent Ca^{2+} mobilization[97]. Consistently, shear stress induces bradykinin B2 receptor to undergo a conformational transition that triggers the downstream signaling cascades, i.e., $InsP_3$ and DAG production, even in the absence of the agonist[97,98]. The kinetics of $InsP_3$ synthesis under these conditions, however, has not been determined.

Once produced by PLC isozymes, $InsP_3$ diffuses into the cytosol and evokes a short-lasting Ca^{2+} efflux from ER[99]. $InsP_3$-induced Ca^{2+} mobilization from ER has been characterized both in saponin-permeabilized ECs loaded with Ca^{2+} and intact ECs perfused with caged $InsP_3$ *via* a patch pipette[45,99-101]. The release of Ca^{2+} by this second messenger displayed an EC_{50} around 1 μmol/L and was independent of $InsP_3$ metabolism[99,101]. $InsP_3$ acts on a tetrameric receptor characterized by a very long cytosolic NH_2-terminal tail that contains the $InsP_3$-binding site, which has been crystallized recently[102]. Three $InsP_3$ receptor subtypes have been identified that may assemble to form either homo- or hetero-tetrameric Ca^{2+}-releasing channels. $InsP_3$ binds to the very long cytosolic NH_2-terminal tail, while the proper Ca^{2+} channel is located in the COOH-terminal portion of the molecule, which contains

Table 1 Pattern of expression of InsP3Rs, RyRs and TPCs in vascular endothelial cells as detected by reverse transcription/polymerase chain reaction, immunostaining for cultured cells, immunohistochemistry, Western blotting, and immunoprecipitation

	InsP3R1	InsP3R 2	InsP3R 3	RyR1	RyR2	RyR3	TPCs1-2
Human brain microvascular ECs[113]	+(RT-PCR, WB, IC)						
Human umbilical vein ECs[106]	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)				
Human microvascular dermal ECs[213]			+(IP)				
Human mesenteric artery ECs[127]	+(RT-PCR)			-(RT-PCR)	-(RT-PCR)	+(RT-PCR)	
EA.hy926 cells[51,127]	+(RT-PCR)			-(RT-PCR)	-(RT-PCR)	+(RT-PCR)	+(WB)
Mouse cremasteric ECs[108]	+(WB, IC)	+(WB, IC)	+(WB, IC)				
Rat hippocampus ECs[413]	-(IHC)	+(IHC)	-(IHC)				
Rat aortic ECs[106,107]	+(RT-PCR, IC, IHC)	+(RT-PCR, IC, IHC)	+(RT-PCR, IC, IHC)				
Rat mesenteric artery ECs[107]	+(RT-PCR, IC, IHC)	+(RT-PCR, IC, IHC)	+(RT-PCR, IC, IHC)				
Rat basilar artery ECs[107]	+(RT-PCR, IC, IHC)	+(RT-PCR, IC, IHC)	+(RT-PCR, IC, IHC)				
Rat adrenal medulla microvascular ECs[106]	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)				
Rat splenic sinus ECs[35]				-(IC)	-(IC)	+(IC)	
Bovine aortic ECs[104,109]	+(WB, IC)	+(WB, IC)	+(WB, IC)				
Ovine uterine artery ECs[414]		+(IP)					

RT-PCR: Reverse transcription/polymerase chain reaction; WB: Western blotting; IC: Immunostaining for cultured cells; IHC: Immunohistochemistry; ISH: *In situ* hybridization; IP: Immunoprecipitation; ECs: Endothelial cells.

the sixth transmembrane™ domain motif and the loop connecting helices 5 and 6 folding back into the membrane[102]. The molecular mass of each InsP$_3$R isoform is around 260 kDa[103,104], which indicates that the native protein is around 1 MDa[102]. The Hill coefficient for InsP$_3$-elicited Ca^{2+} release is around 3.8 and suggests that InsP$_3$ binding to all subunits is required to induce channel opening[99]. All InsP$_3$R subtypes have been detected in ECs (Table 1), both under culture conditions[104-106] and in intact vessels[107,108]. The expression pattern of InsP$_3$R isoforms in both freshly isolated and cultured ECs is: InsP$_3$R-3 > InsP$_3$R-2 > InsP$_3$R-1[106]. This feature becomes even more remarkable in proliferating cells, which suggests that distinct isoforms may impact on different aspects of EC physiology[106]. In this view, recent work has revealed that InsP$_3$Rs forms mostly, albeit not exclusively, homotetramers in vascular ECs[109]. The subcellular localization of InsP$_3$R subtypes may vary with cell isolation and culture. In cultured ECs, InsP$_3$R-1 is mainly localized to the ER, InsP$_3$R-2 resides within the nucleus, and InsP$_3$R-3 is detected at the perinuclear region[104], while, in the intact vascular wall, all isoforms are tightly associated to F-actin fibers and InsP$_3$R-1 and InsP$_3$R-2 lie along with InsP$_3$R-3 near the edge of the nucleus[107]. None of the three subtypes has been detected in close association with the plasma membrane[104,107], although some studies have reported a protein with high homology to the InsP$_3$R-1 in endothelial caveolae *via* binding to F-actin[34,110]. Such plasmalemmal InsP$_3$ might mediate the InsP$_3$-gated Ca^{2+} entry originally described by Vaca and Kunze[111]. Moreover, InsP$_3$R-1 may be associated to the EC side of myoendothelial junctions *in vivo* and thus plays a key role in transmitting intracellular Ca^{2+} waves from vascular endothelium to the adjoining smooth muscle cell layer[108]. InsP$_3$R expression may be altered under pathological conditions, such as hypertension, whereas ECs isolated from spontaneously hypertensive rats display an increase in InsP$_3$R-2 transcripts and a decrease in InsP$_3$R-1 mRNA[112]. Moreover, InsP$_3$R levels augment as a consequence of ethanol metabolism and oxidative stress[113]. InsP$_3$R gating is regulated by both InsP$_3$ and Ca^{2+}. InsP$_3$ is able to activate InsP$_3$Rs when the surrounding Ca^{2+} is between 50 nmol/L and 200 nmol/L, whereas higher Ca^{2+} levels inhibit Ca^{2+} release even at saturating InsP$_3$ concentrations[102]. Such a biphasic dependence on ambient Ca^{2+} is due to two independent Ca^{2+}-binding sites that mediate channel activation or closure, respectively. More specifically, InsP$_3$ tunes InsP$_3$R sensitivity to cytosolic Ca^{2+}: it favors Ca^{2+} binding to the stimulatory site, which is the trigger for pore opening, and inhibits Ca^{2+} binding to the inhibitory one[102,114]. Consistent with this model, InsP$_3$-induced Ca^{2+} mobilization is enhanced at lower (200 nmol/L) than at higher (2 μmol/L) Ca^{2+} concentrations in saponin-permeabilized BAECs[99,115]. The large modulatory domain between the InsP$_3$-binding site and the channel-forming region presents consensus sequences for numerous protein kinases, including PKC, protein kinase A (PKA), cGMP-dependent protein kinase (PKG), Akt kinase, calmodulin-dependent protein kinase II (CaMKII), tyrosine kinases, and cyclin-dependent kinase 1/cyclin B (cdc2/CyB) complex[102]. Additionally, InsP$_3$R channel activity is sensitive to a variety of modulators, including ATP, redox balance, and luminal Ca^{2+}, and interacting proteins, such as calmodulin, Ca^{2+}-binding proteins (CaBPs), chromogranins, InsP$_3$R binding protein released with inositol-1,4,5-trisphosphate (IRBIT), RACK1 and $G_{\beta\gamma}$, ERp44, FKBP12, cytochrome C, Bcl-2 proteins, huntingtin, adapter proteins (ankyrin, homer, protein 4.1N), and proteases (caspase-3 and calpain)[102]. As to vascular ECs, PKC and CaMKII block InsP$_3$-dependent Ca^{2+} release *via* ATP-dependent phosphorylation of InsP$_3$Rs[116-118]. Conversely, PKA and mammalian target of rapamycin (mTOR) phosphorylate InsP$_3$Rs to render them more sensitive to ligand binding and, thereby, increase the velocity of InsP$_3$-dependent Ca^{2+} waves[118]. In addition, an InsP$_3$R-ankyrin complex has been detected in cells exposed to hyaluronic acid (HA). Such stimulation favors the ankyrin-mediated recruitment of ER In-

sP_3Rs into plasmalemmal caveolae; an event that triggers Ca^{2+} release independently on agonist binding[119]. $InsP_3R$ modulation in ECs may be further accomplished by the gasotransmitters, NO and H_2S. While the former induces Ca^{2+} release likely *via* receptor S-nitrosylation[120,121], the latter inhibits $InsP_3$-induced Ca^{2+} discharge probably through receptor S-sulfhydration[122,123]. An additional post-translational modification of endothelial $InsP_3Rs$ is provided by *S*-glutathionylation, which might sensitize the receptor to the basal level of $InsP_3$[124]. In this view, the $InsP_3R$ may be directly regulated by ROS, which may oxidize the two highly conserved thiol groups located in the COOH-tail of the protein[102]. It has, indeed, been shown that Ca^{2+} release from endothelial $InsP_3Rs$ may be evoked by H_2O_2[55-57], which may increase the sensitivity of the intracellular Ca^{2+} pool to $InsP_3$[56].

RyRs and cADPr

Although $InsP_3R$ is the predominant ER Ca^{2+}-releasing channels in ECs, RyR-dependent signalling has also been reported in vascular endothelium. Three RyR isoforms have been identified (RYR-1, RyR-2, RyR-3) which assemble as homo-tetrameric channels consisting of four 560-kDa polypeptides[125]. They possess a very large cytosolic NH2-domain comprising around 90% of the receptor amino acid sequence. This region is part of a macromolecular complex and serves as a scaffold for numerous regulatory or modulatory proteins, such as calmodulin, calstabin, sorcin, and presents binding sites for cytosolic ions and modulators, including Ca^{2+}, Mg^{2+}, and adenine nucleotides. RyRs may also be modulated PKA- and CamKII-mediated phosphorylation, may undergo oxidative/nitrosative modifications, and are subject to pH modulation[126]. In addition, RyRs are modulated by SR luminal Ca^{2+} *via* a signaling pathway that includes calsequestrin (CSQ), triadin and junction[126]. Comparative studies carried out by either single-cell reverse transcription/polymerase chain reaction (RT-PCR) or immunocytochemistry have revealed that ECs mainly express RyR-3[34,127], but not RyR-1 and RyR-2 (Table 1) . Immunogold labeling with anti-RyR indicates that RyR-3 is located in the nuclear membranes, the ER tubulovesicular system, and the cristae membrane of mitochondria[34]. In the light of the ability of RyRs to modulate sub-plasmalemmal Ca^{2+} signaling in ECs (see below), it is remarkable that RyR-3 is largely expressed in the ER structures facing the plasma membrane[34]. Ca^{2+} release from RyRs in ECs, as well as in both excitable and non-excitable cells[126], is triggered by CICR[44,45], that occurs when 1-10 μmol/L Ca^{2+} nearby the receptor and is prevented when Ca^{2+} is 1-10 mm[126]. RyRs may be pharmacologically activated by the methylxanthine derivative, caffeine, which sensitizes the receptor to resting Ca^{2+} levels, and by ryanodine, which binds to and locks the receptor in an open state[128]. In a number of ECs from different vascular districts, caffeine/ryanodine may evoke either fast Ca^{2+} transients[46,129,130] or slow Ca^{2+} release from ER[131,132]. Other studies, however, failed to report any increase in bulk $[Ca^{2+}]_i$ in ECs exposed to either caffeine[72,130] or ryanodine[132,133]. Similarly, RyR contribution to agonists-induced Ca^{2+} signals has been detected in some[44,47,132,134,135], but not all ECs[72,136,137]. RyR-dependent signaling might be masked by SERCA activity, so that Ca^{2+} ions liberated into the cytosol by RyR opening are immediately sequestered into the $InsP_3$-sensitive region of ER lumen by its Ca^{2+}-ATPase[130]. In addition, monitoring of subplasmalemmal and perinuclear Ca^{2+} levels revealed that RyR-sensitive Ca^{2+} mobilization may selectively occur beneath the plasma membrane, but not in the bulk cytosol[133]. Indeed, RyRs may be part of a subplasmalemmal Ca^{2+} control unit (SCCU), as confirmed by their localization in the ER membrane facing the cytosolic leaflet of the plasma membrane (see above). The SCCU enables a functional coupling between RyRs expressed on superficial ER and plasmalemmal NCX, so that Ca^{2+} released from RyRs is vectorially pumped out by NCX without affecting $[Ca^{2+}]_i$. Under these conditions, RyR contribution to endothelial Ca^{2+} signaling may be appreciated only upon NCX inhibition[133,138,139] or by monitoring the local activation of large conductance Ca^{2+}-dependent K^+ channels[140,141]. Besides CICR, RyRs may be stimulated by cADPr[10], a second messenger that can be generated in response to either agonist stimulation[142] or oxidative stress through the synthesis of H_2O_2[143,144]. The ADP ribosyl cyclase, CD38, which is located both on the cell surface and on intracellular membranes, catalyzes nicotinamide adenine dinucleotide (NAD) conversion into cADPr by a cyclization reaction occurring at neutral pH[142]. cADPr, in turn, acts by displacing the FK506 binding protein 12 (FKBP12 or calstabin1) or its related isoform FKBP12.6 (FKBP12.6 or calstabin2) from RyRs[48], which increases their open probability and leads to Ca^{2+} release[125]. A recent study has demonstrated that bradykinin induces Ca^{2+} release by stimulating cADPr synthesis and RyR activation in human coronary artery ECs[47]. Moreover, genetic ablation of FKBP12/12.6 from endothelial RyRs caused an intracellular Ca^{2+} leak associated with the impairment of NO production and endothelial dysfunction[145,146]. It is, however, yet to demonstrate that cADPr triggers Ca^{2+} release by dissociating FKBP12/12.6 from RyRs in ECs. It should also be noticed that a number of studies have failed to find direct activation of RyRs by cADPr, as discussed previously[48]. For instance, cADPr has been reported to promote the activity of the SERCA pump, thereby increasing the rate of ER refilling and Ca^{2+} release *via* the luminal excitation of RyRs[142]. Mechanical stimulation may also lead to RyR activation in vascular ECs. More specifically, shear stress may induce oscillations in $[Ca^{2+}]_i$ by stimulating the interplay between mechano-sensitive Ca^{2+} influx and RyRs through the CICR mechanism[25].

NAADP and two-pore channels

NAADP is the newest and most efficacious member of the family of Ca^{2+}-mobilizing messengers, being active already at nanomolar concentrations[53]. NAADP is synthe-

sized from NADP by CD38 in a base-exchange reaction occurring at acidic pH and targets acidic organelles, such as the endolysosomal Ca^{2+} storage compartment[53,142], although other signaling pathways may control NAADP production[53]. Ca^{2+} is sequestered into lysosomal lumen by a putative Ca^{2+}/H^+ exchanger, which is driven by a vacuolar-H^+ ATPase[53]. NAADP-gated Ca^{2+} release is mediated by TPC2, whose primary sequence places them in the superfamily of voltage-gated cation channels. Hydropathy analysis has revealed that the full-length protein contains 12 putative TM (TM) α helices that are organized into two homologous domains of six TM segments, each with a membrane re-entrant pore loop between the fifth and sixth segments. Although three TPCs have been identified (TRPC1-3), only TPC2 is endowed with both high (5 nmol/L) and low (10 μmol/L) affinity binding sites for NAADP and is selectively targeted to lysosomes[53]. Due to the limited amount of Ca^{2+} in the endolysosomal compartment, which consists of small vesicles (0.2-1 μm in diameter) moving throughout the cytosol, NAADP-induced Ca^{2+} signals *per se* appear as scattered, discrete events[53]. The relatively small quantity of Ca^{2+} mobilized by endolysosomes may, however, be amplified into a global Ca^{2+} wave by recruitment of $InsP_3Rs$ and RyRs *via* CICR[53,147]. This feature requires the physical proximity between the NAADP-sensitive Ca^{2+} pool and the Ca^{2+}-releasing channels on ER, that has been documented in a number of cell types[53]. NAADP has recently been involved in endothelial Ca^{2+} signaling (Table 1). A cell-permeable NAADP analog induced an increase in $[Ca^{2+}]_i$ in human aortic ECs, which was associated with membrane hyperpolarization and NO production. Importantly, NAADP-dependent Ca^{2+} release triggered the Ca^{2+} response to acetylcholine and thrombin, but not ATP[50]. Importantly, NAADP-dependent Ca^{2+} release triggered the Ca^{2+} response to acetylcholine and thrombin, but not ATP[50]. Consistent with these data, it was subsequently shown that histamine binding to H1 receptors caused an increase in intracellular NAADP levels in EA.hy926 cells, which evoked a TPC2-mediated Ca^{2+} signal and secretion of von Willebrand factor[51]. Unlike human aortic ECs, however, the Ca^{2+} response to thrombin was insensitive to the inhibition of NAADP-dependent signalling[51].

Ca^{2+} ENTRY FROM THE EXTRACELLULAR SPACE

Ca^{2+} influx across the plasma membrane sustains the Ca^{2+} response to both chemical and mechanical stimuli in ECs, as well as most other nonexcitable cells. In addition to refilling the intracellular Ca^{2+} stores, Ca^{2+} inflow is responsible for the activation of Ca^{2+}-sensitive effectors which are strategically located near the inner mouth of endothelial Ca^{2+} channels[6,9,11,62]. Ca^{2+} entry in vascular endothelium may be mediated by voltage-operated Ca^{2+} channels (VOCs), agonist-operated Ca^{2+} channels (AOCs), and mechanosensitive channels (MSCs)[7,11,13,62]. AOCs, in turn, comprise at least three types of Ca^{2+}-permeable membrane pathways, which may be gated by distinct signaling mechanisms: (1) receptor-operated channels (ROCs), which are ionotropic receptors activated by direct binding of the ligand; (2) second-messenger-operated channels (SMOCs), which are stimulated by intracellularly generated mediators, such as DAG, AA and its derivatives (eicosanoids and anandamide), ADPr, and cyclic nucleotides; and (3) SOCs, which open in response to a depletion of the ER Ca^{2+} pool. Most, but not all, endothelial SMOCs, as well as MSCs, belong to the TRP family of nonselective cation channels[4,8,19,148-150], whereas the molecular nature of SOCs is still debated[151,152]. The inrush of Ca^{2+} ions consequent to the opening of the Ca^{2+}-permeable membrane route is governed by the following equation:

$$J_{Ca} = 2 \times F \times N \times p \times \gamma_{Ca} \times (V_M - E_{Ca})$$

where J_{Ca} is Ca^{2+} influx, V_M is the membrane potential, E_{Ca} the is equilibrium potential for Ca^{2+}, F is the Faraday constant, N is the number of channels, p and γ_{Ca}, respectively, are their open probability and conductance[7]. Ca^{2+} entry may, therefore, be modulated by any change in V_M according to the following general rule: hyperpolarization increases the driving-force pushing Ca^{2+} into the cytosol, while depolarization reduces Ca^{2+} influx across the plasmalemma[7,153]. This rule, however, may not apply to microvascular ECs, which may be endowed with VOCs, as depicted below. We refer the reader interested in the ion channels controlling endothelial V_M to a number of exhaustive and relatively recent reviews[7,153]. The description of the Ca^{2+}-permeable membrane pathways in ECs will be addressed by focussing on the following topics: (1) VOCs; (2) ROCs; (3) cyclic-nucleotide-gated channels, which belong to SMOCs; (4) TRP channels, which comprise SMOCs, SOCs, and SACs; and (5) Orai1 and Stim1, which mediate SOCs.

Voltage-gated Ca^{2+} influx in ECs

Although classically considered as non-excitable cells, ECs from capillaries, but not from large vessels, may express both L- and T-type VOCs[154-159]. VOCs have been detected in microvascular ECs from bovine adrenal glands (BAMCECs)[154-156], rat and pig brain[160,161], rat and mice lungs[157,162-165]. The following features suggest that the voltage-gated currents measured in BAMCECs belong to the L- and T-type described in endocrine secretory cells: their kinetic and gating properties, current-voltage relationships, sensitivity to BAY K 8644, nifedipine, and amiloride, and their different selectivity for Ba^{2+} and Ca^{2+}[156]. The T-type transient current displayed a conductance of 8 pS and may contribute to depolarisation-induced Ca^{2+} influx[155,166], while the L-type large current manifests a single-channel conductance of 20 pS and is affected by dihydropyridines[154]. Besides T- and L-type VOC, BAMCECs express a voltage-dependent Ca^{2+} channel (termed SB) that shows a single-channel conductance of 2.8 pS in 100 mmol/L Ba^{2+} and is sensitive to BAY K 8644, but not nicardipine[154]. Such a path-

way is operative at negative resting potentials and might contribute to low-threshold Ca^{2+} inflow[154]. Pulmonary microvascular vascular ECs (PMVECs) exhibit a $Ca_v3.1$ (α_{1G}) voltage-gated T-type Ca^{2+} current that is transiently activated at ⩾ -60 mV and peaks at around -10 mV[158,159]. Although α_{1G} may mediate T-type voltage-dependent Ca^{2+} currents without any interaction with its ancillary proteins (β-, γ-, and $\alpha_2\delta$ subunits), $\alpha_2\delta_2$-, β_1- and β_3-, but not β_2- and β_4-subunits are expressed in PMVECs[158]. The window current of T-type Ca_v 3.1 channels occurs at membrane potentials ranging from -60 mV to -30 mV, a voltage interval that may be achieved in ECs challenged with extracellular agonists when their V_M is close to the K^+ equilibrium potential, i.e., -70/-80 mV[7]. Thrombin, in particular, utilize SOCs to depolarize PMVECs and exploit T-type $Ca_v3.1$-mediated Ca^{2+} entry to drive the exocytosis of von Willebrand factor[162]. Exposure of PMVECs to shear stress may upregulate $Ca_v3.1$ (α_{1G}) expression, so that flow cessation results in an abrupt Ca^{2+} entry through T-type Ca^{2+} channels[157]. Ca^{2+} entry through T-type Ca_v 3.1 channels has also been observed in mouse pulmonary capillary endothelium, where it selectively drives P-selectin expression[163]. An R-type VOC has been, finally, described in canine aortic ECs, that may contribute to PAF-induced Ca^{2+} entry[167]. This channel does not inactivate during long-lasting depolarizations, therefore, it is well suited to mediate prolonged Ca^{2+} inflow[7]. It is, finally, noticeable that angiotensin II causes α_{1G} though AT1 receptors, Ras and MEK[168]. This study, however, did not assess the appearance of voltage-gated Ca^{2+} signals, although it showed that mibefradil, a T-type Ca^{2+} channel inhibitor, prevented the regeneration of an injured monolayer[168].

ROCs in ECs

Vascular ECs may express at least two distinct ROCs, namely the nicotinic acetylcholine (ACh) receptor (nAChR) and the purinergic P_{2X} receptor, which are gated by ACh and ATP, respectively. These pathways are also permeable to K^+ and Na^+ and gate an inward current at physiological V_M. Therefore, in capillary ECs endowed with VOCs, ROCs may also affect Ca^{2+} signaling by depolarizing the cells and inducing a voltage-dependent Ca^{2+} inrush.

Neuronal nAChRs: nAChRs are ionotropic receptors that mediate fast cationic currents in response to their selective agonist, ACh. They are named after nicotine, a psychoactive ingredient of tobacco that can mimic the action of ACh in opening these channels. The neuronal nAChRs are pentameric channels comprising either combinations of two different types of subunit (α and β) or five copies of the same α subunit symmetrically arranged in a barrel-like configuration[169]. Each subunit consists of four α-helical spanning domains (TM1-TM4), with TM2 of each subunit lining the receptor pore[169,170]. Ten α isoforms (α1-α10) and four β subtypes [β1-β4], which may assemble in either homomeric or heteromeric configurations, have been identified[170,171]. The diverse stoichiometric combinations may result in ion channels displaying different pharmacological [ligand affinity and α-bungarotoxin (BTX) sensitivity] and biophysical (mean open time and Ca^{2+} permeability) features[172]. The neuronal α-BTX-sensitive nAChRs are formed by α7-α9 homopentamers, are inhibited by α-BTX, and display the highest Ca^{2+}:Na^+ permeability ratio (P_{Ca}/P_{Na}), while the non-α-BTX nAChRs are heteropentamers containing at least one α (out of α2-α6) and one β (out of β2-β4) subunits and exhibit a much lower P_{Ca}/P_{Na}[172]. As reviewed by Arias *et al*[173] and Gahring and Rogers[170], ECs from both macro- and microvascular origin present a pattern of α [α2-α5, α7] and β [β2 and β4] isoforms consistent with the expression of both nAChR7 and nAChR3. Activation of the latter does not elicit any detectable Ca^{2+} influx in vascular endothelium[174], while the former triggers a proangiogenic Ca^{2+} entry that might be exploited to enhance tissue revascularization[173,175]. Moreover, nAChRs-mediated membrane depolarization induces VOCs-dependent Ca^{2+} inrush in BAMCECs[166].

P_{2X} receptors: The ionotropic P_{2X} receptors are channels that respond to extracellular ATP to induce membrane depolarization and an influx of Ca^{2+}. Seven P_{2X} receptor subtypes (P_{2X1-7}) have been identified: each of them possesses two TM domains (TM1 and TM2) connected by a large extracellular loop and with the NH_2- and COOH-terminal tails extending into the cytoplasm[176]. The extracellular loop contains the ATP binding site, while TM1 and TM2 are thought to line the channel pore[176]. The P_{2X} receptors assemble as homo- or heterotrimers with a range of biophysical features and functions, including different P_{Ca}/P_{Na} values (which may range from 1 to 71) and rates of inactivation[177]. For instance, $P2_{X3}$-mediated currents rapidly decay during prolonged exposition to ATP, whereas P_{2X7} remains open for several minutes[176]. Vascular ECs express all P_{2X} receptor isoforms in both arteries and veins[178,179], although P_{2X4} levels are higher in the latter[179]. P_{2X4} expression in the arterial endothelial layer undergoes a dramatic increase following balloon injury, which hints at this subunit as a key player in EC regeneration[179]. The electrophysiological analysis showed that channels with the electrophysiological and pharmacological properties of both P_{2X4} (fast inactivation, inward rectification, conductance of around 36 pS, insensitivity to reactive blue) and P_{2X7} (slow inactivation, conductance of 9 pS, linear current-to-voltage relationship, sensitivity to reactive blue) are functionally present on EC plasma membranes[180]. P_{2X5} subunits may assemble with P_{2X4} subtypes and form a heterotrimer associated to a current displaying the characteristics of a P_{2X4} current[180]. Ca^{2+} imaging recordings demonstrated that P_{2X4} is the main mediator of ATP- and flow-induced Ca^{2+} inflow in vascular endothelium[181,182]. Shear stress induces ATP release from ECs through different mechanisms, including exocytosis or ATP binding of cassette transporters[183,184], or it may stimulate a cell surface ATP-synthase[185]. ATP, in turn, causes a step-like elevation in $[Ca^{2+}]_i$[181,182], which

leads to NO production and vasorelaxation *in vivo*[186]. ATP-dependent Ca^{2+} entry is linearly correlated with the amplitude of the mechanical stimulation[187].

Cyclic nucleotide-gated Ca^{2+} channels

Cyclic nucleotide-gated channels (CNGs) are nonselective cation channels gated by direct binding of the cyclic nucleotides, cAMP and cGMP[188]. There are six members of the CNGC family: four α subunits (CNGA1-4) and two β subunits (CNGB1 and CNGB3). They all share structural homology with voltage-gated channels and have six TM domains with both the NH_2- and COOH-termini facing the cytoplasm. A pore region lies between TM5 and TM6. The CNGA1-3 subunits form channels whereas CNGA4 and the two CNGB isotypes have a modulatory function. The cyclic nucleotide binding site is located at the COOH-terminal domain of each subunit[188]. All CNGs conduct Ca^{2+} better than Na^+, a feature which enables these conductances to affect significantly intracellular Ca^{2+} signaling[189]. Both CNGA1, which is selectively gated by cGMP, and CNGA2, which opens in response to cAMP, are widely expressed by ECs from several vascular beds, although cultured cells undergo downregulation of both subunits[190,191]. Both cAMP and cGMP levels may increase in ECs challenged with β-receptor agonists, such as epinephrine, noradrenaline and isoprenaline[192,193]. Moreover, intracellular levels of both cyclic nucleotides may be raised when ECs are stimulated with adenosine and ATP[192-194], which are coupled, respectively, to A_{2B} and P_{2Y1} receptors[194-196], and VEGF, which acts through VEGFR-2[196]. Finally, SOCs-mediated Ca^{2+} entry may lead to the intracellular accumulation of cGMP, which in turn leads to CNG opening and membrane depolarization, thus establishing a negative feedback responsible for SOC closure[197]. CNGA2 is the main contributor to cyclic-nucleotide-induced Ca^{2+} inflow in vascular endothelium stimulated with epinephrine[198], ATP[195] or adenosine[194]. CNGA2-mediated Ca^{2+} influx, in turn, results in NO production and vasorelaxation[194]. Moreover, NO may upregulate CNGA2 levels in ECs[199].

TRP channels

The super-family of TRP channels comprises 28 members, which have been subdivided in six categories based on the homology of their amino-acid sequence: the TRPC (canonical; TRPC1-TRPC7), TRPV (vanilloid; TRPV1-TRPV6), TRPM (melastatin; TRPM1-TRPM8), TRPP (polycystin; TRPP1-TRPP3), TRPML (mucolipin; TRPML1-TRPML3), and the TRPA (ankyrin; TRPA1) families[200]. They all are nonselective cation channels, with the exception of TRPM4 and TRPM5, which are only permeable to Na^+ and K^+ ($P_{Ca}/P_{Na} < 0.05$), and TRPV5 and TRPV6, which are Ca^{2+} selective ($P_{Ca}/P_{Na} > 100$)[200]. All the family members have the same basic structure consisting of six TM domains (TM1-TM6) with the NH_2- and COOH-termini located in the cytoplasm. Between TM5 and TM6, which are highly conserved among all the homologs, there is a re-entrant pore loop constituted by a short hydrophobic stretch lining the channel pore. This molecular architecture resembles that of the archetypal 6-TM voltage-gated channels, although TM4 lacks the conserved block of arginine residues forming the voltage sensor of transmembrane electrical potential[201]. Most of the differences between the three families are found within the NH_2- and COOH-terminal tails. The TRPC and TRPV family members contain three or four ankyrin repeats at their NH_2 terminus, while TRPA1 contains 14 ankyrin repeats. All mammalian TRP families present a homologous sequence of around 25 amino acids immediately COOH-terminal to TM6, which has been termed TRP domain and consists of a highly conserved six-amino-acid TRP box (EWKFAR) and a proline-rich motif. The TRP domain is, however, lacking in TRPA and TRPP[201]. TRPC proteins contain a number of calmodulin/$InsP_3R$ binding domains (CIRB) at both their NH_2- and COOH-tails, while a predicted coil:coil region may be present in both locations of TRPC, TRPM, and TRPP[19]. The TRPM family is characterized by having very long N and C termini. The latter contain enzymatic activity in some of the channels, e.g., TRPM2 has an ADP-ribose pyrophosphatase, whereas TRPM7 contains an atypical protein kinase[200]. TRP subunits within one subfamily may assemble in either homo- or heterotetramers and this gives rise to large variability in their biophysical properties and activating mechanisms[19,200,201]. Vascular ECs have been shown to express TRP channels from all subfamilies[8,150]. More specifically, all the TRPC, all TRPV, all TRPM except TRPM5, TRPP1, TRPP2 and TRPA1 are present in ECs (Tables 2-4). Endothelial TRP channels serve as polymodal cell sensors, as each TRP subunit may be engaged by more than one signaling pathway. The selective coupling to distinct downstream Ca^{2+}-sensitive decoders enables TRP-mediated Ca^{2+} influx to govern a plethora of processes in ECs[4,19,148,149]. For sake of clarity, we will separately address the activating mechanisms and the main functions fulfilled by each TRP isoform in vascular endothelium.

TRPC channels

The TRPC family may be subdivided in four subfamilies according to their amino acid homology: TRPC1, TRPC2, TRPC3/6/7 (around 80% homology), and TRPC4/5 (around 60% homology). TRPC2 is a pseudogene in humans and does not fulfil any functional role in vascular ECs. TRPC channels are nonselective cation channels whose P_{Ca}/P_{Na} may vary from 0.9 to 9[202]. The TRPC subunits may assemble as homo- and heterotetramers. In the latter case, they may combine with members of their own subfamily or TRPC1 may form heteromers with TRPC1, TRPC4/5 and TRPC3[200]. Endothelial TRPC channels are activated by GPCRs and TKRs coupled to PIP_2 hydrolysis by PLC activation and, depending on their stimulating mechanism, may be part of either SMOCs or SOCs.

TRPC1: TRPC1 is widespread in both cultured and native ECs (Table 2), where it mainly serves as SOC and controls transendothelial permeability[203,204], although

Table 2 Pattern of expression of transient receptor potential canonical channels in vascular endothelial cells as detected by reverse transcription/polymerase chain reaction, immunostaining for cultured cells, immunohistochemistry, Western blotting, northern blots, and in situ hybridization

	TRPC1	TRPC2	TRPC3	TRPC4	TRPC5	TRPC6	TRPC7
PCa/PNa and conductance (pS)[202]	Non-selective, 16	2.7, 42	1.6, 60-66	1.1, 30-42	9, 47-66	5, 28-37	0.5-5.4, 25-50
Human pulmonary artery ECs[210,211,219,222,249,261,415,416]	+(RT-PCR, NB, WB, IC)		+/-(RT-PCR)	+(RT-PCR, WB, IC)	-(RT-PCR)	+/-(RT-PCR) +(WB)	
Human coronary artery ECs[234,237]	+(RT-PCR, WB, ISH, IHC)		+(RT-PCR, WB, ISH, IHC)	+(RT-PCR, WB, ISH, IHC)	+(RT-PCR, WB, ISH, IHC)	+(RT-PCR, WB, ISH, IHC)	+(RT-PCR, WB, ISH)
Human cerebral artery ECs	+(RT-PCR, ISH, IHC)		+(RT-PCR, ISH, IHC)	+(RT-PCR, ISH, IHC)	+(RT-PCR, ISH, IHC)	+(RT-PCR, ISH, IHC)	+(RT-PCR, ISH)
Human intact mesenteric artery[127]	+(RT-PCR)		+(RT-PCR)	-(RT-PCR)	-(RT-PCR)		
Human glomerular ECs						+(WB, IC)	
Human preglomerular ECs[244,245]			+(RT-PCR, WB, IHC)			+(IHC)	
Human dermal microvascular ECs[18,212,221,222,264]	+(RT-PCR, WB)	-(RT-PCR)	+(RT-PCR)	+(RT-PCR, WB)	-(RT-PCR)	+/-(RT-PCR) +(WB)	+/-(RT-PCR)
HUVECs[127,205,212,215,218,220-223,227,231]	+(RT-PCR, WB)	-(RT-PCR)	+/-(RT-PCR)	+/-(RT-PCR)	+/-(RT-PCR)	+/-(RT-PCR) +(WB)	+(RT-PCR)
EA.hy926 cells[127,243,269]	+(RT-PCR)		+/-(RT-PCR) +(WB)	+/-(RT-PCR)	+(RT-PCR, WB)	+/-(RT-PCR)	
Mouse aortic ECs[216,228,248,258]	+(RT-PCR, WB, IC)		+(WB)	+(RT-PCR, WB)	+(WB)	+(WB)	
Mouse pulmonary artery ECs[218,251,259,262]	+(RT-PCR, WB)	-(RT-PCR)	+/-(RT-PCR)	+(RT-PCR, WB)	-(RT-PCR)	+(RT-PCR, WB)	-(RT-PCR)
Mouse brain capillary ECs	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)
Bovine pulmonary artery ECs[232]	+(RT-PCR)		-(RT-PCR)	+(RT-PCR)			
Bovine aortic ECs[207-209,248,267,268]	+(RT-PCR, WB, IC)		+/-(RT-PCR) +(WB)	+(RT-PCR, WB)	+(RT-PCR, WB)	+(RT-PCR, WB)	
Bovine brain capillary ECs	+(RT-PCR)	-(RT-PCR)	+(RT-PCR)	-(RT-PCR)	+(RT-PCR)	-(RT-PCR)	-(RT-PCR)
Bovine adrenal capillary ECs[292]							
Rat aortic ECs[233,239]	-(WB)		+(WB)	-(WB)	+(WB)	+(WB)	
Rat pulmonary artery ECs[260]	+(RT-PCR)	+(RT-PCR)	+/-(RT-PCR)	+(WB, IC) -(RT-PCR)		-(RT-PCR)	-(RT-PCR)
Rat coronary arterioles ECs	+(RT-PCR, IHC)						
Rat cerebral artery ECs[311]	+(RT-PCR)						
Rat mesenteric artery ECs[227,312]	+(RT-PCR, WB, IHC)		+(WB, IHC)	+(WB, IHC)	+(WB, IHC)		
Rat descending vasa recta ECs[254]				+(RT-PCR, WB, IC)	-(RT-PCR)		
Rat splenic sinus ECs[35]	+(IHC)						
Porcine aortic ECs[241,242]			+(RT-PCR, WB)	+(WB)			
Porcine coronary artery ECs[235]			+(RT-PCR, WB)				
Zebrafish aortic ECs[225]	+(IHC, ISH)					+(ISH)	
Ovine uterine artery ECs[414]	+(WB)		+(WB)	+(WB)	+(WB)	+(WB)	

RT-PCR: Reverse transcription/polymerase chain reaction; WB: Western blotting; IC: Immunostaining for cultured cells; IHC: Immunohistochemistry; ISH: *In situ* hybridization; NB: Northern blotting; ECs: Endothelial cells; HUVECs: Human umbilical vein ECs.

recent studies have argued against this conclusion[205,206]. TRPC1 is engaged by a number of inflammatory agonists, such as ATP, thrombin, tumor necrosis factor (TNF)-α, and angiotensin II (AngII), and by growth factors, such as VEGF and bFGF[5,12,148,207-209]. TRPC1 may act as a SOC due to its ability to interact with $InsP_3Rs$ through several signaling cascades. The first model implies RhoA-dependent actin rearrangement, which leads $InsP_3Rs$ to couple to and gate TRPC1[210]. RhoA activation, in turn, is induced by TRPC6 *via* PKCα-mediated phosphorylation[211]. At the same time, PKCα also phosphorylates TRPC1 to enhance RhoA-dependent stimulation[212]. Alternatively, thrombin utilizes caveolin-1 (Cav-1) to trigger TRPC1-mediated Ca^{2+} entry: Cav-1 scaffolding domain (CSD) enables the physical and functional interaction of TRPC1 and of $InsP_3R3$ with Cav-1 protein within the caveolae[213]. In this view, a recent work has demonstrated that polyunsaturated fatty acids, such as docosahexaenoic acid, may reduce store-operated Ca^{2+} entry (SOCE) by displacing TRPC1 from membrane caveolae lipid rafts[214]. Finally, VEGF recruits TRPC1 *via* direct coupling to $InsP_3R2$ in an angiopoietin-1-regulated

Table 3 Pattern of expression of transient receptor potential vanilloid channels in vascular endothelial cells as detected by reverse transcription/polymerase chain reaction, immunostaining for cultured cells, immunohistochemistry, Western blotting, and in situ hybridization

	TRPV1	TRPV2	TRPV3	TRPV4	TRPV5	TRPV6
PCa/PNa and conductance (pS)[202]	10, 35-80	1-3, NM	12, 172	6, 90	> 100, 75	> 100, 40-70
Human coronary artery ECs[300]				(+RT-PCR, WB, IHC)		
Human pulmonary artery ECs[415]	+(RT-PCR)	+(RT-PCR)		+(RT-PCR)		
Human pulmonary microvascular ECs	+(RT-PCR)					
Human cerebral microvascular ECs[272]	+(RT-PCR, IC)					
Human cerebral arterioles ECs[305]			+(RT-PCR, IHC)			
Human dermal microvascular ECs[319]				+(RT-PCR, WB)		
Breast cancer derived microvascular ECs[319]				+(RT-PCR, WB)		
Human umbilical vein ECs[227,277,296,301]	+(RT-PCR)			+(WB, IHC)		
Mouse aortic ECs[280,285,299,306]	+(WB)			+(RT-PCR, WB, NM, IHC)		
Mouse pulmonary artery ECs[313]				+(WB, IHC)		
Mouse mesenteric artery ECs[27,280,302]	+(RT-PCR, WB, IC)			+(RT-PCR, IC)		
Mouse cerebral microvascular ECs		+(RT-PCR)		+(RT-PCR, WB)		+(RT-PCR)
Mouse dermal microvascular ECs[307]				+(RT-PCR, WB)		
Mouse carotid artery ECs[290,296]				+(IHC)		
Rat mesenteric artery ECs[226,275,302]	+(WB)			+(WB, IC, IHC)		
Rat femoral artery ECs[312]				+(RT-PCR, IC)		
Rat pulmonary artery ECs[303]				+(WB, IHC)		
Rat renal artery ECs[303]				+(IHC)		
Rat cardiac microvascular ECs[303,316]				+(IHC)		
Rat cerebral artery ECs[287,297,311]		+(RT-PCR)	+(RT-PCR, IC)	+(RT-PCR, IC)		
Rat carotid artery ECs[291]				+(RT-PCR)		
Porcine aortic ECs[312,317]				+(RT-PCR, IC, WB)		
Porcine coronary artery ECs[273]	+(IHC)					
Bovine aortic ECs[271]	+(WB)					
Bovine adrenal capillary ECs[292]				+(RT-PCR, WB)		
Zebrafish ventricular ECs				+(ISH)		

RT-PCR: Reverse transcription/polymerase chain reaction; WB:Western blotting; IC: Immunostaining for cultured cells; IHC: Immunohistochemistry; ISH: *In situ* hybridization; NM: Not measured; ECs: Endothelial cells.

fashion[215]. The circulating protein, klotho, may bind to both VEGFR-2 and TRPC1 to promote their co-internalization, reduce Ca^{2+} influx and maintain endothelial integrity[216]. A number of studies have, however, reported that TRPC1 must combine with TRPC4 to be operated by intracellular Ca^{2+} stores[217,218]. This issue will be addressed below. TRPC1-mediated Ca^{2+} entry enlists calmodulin to activate the myosin light chain kinase (MLCK) and induce EC retraction, thus disassembling intercellular junctions and increasing endothelial permeability[204]. Moreover, TRPC1-gated Ca^{2+} entry is capable of stimulating nuclear factor (NF)-κB *via* AMP-activated protein kinase and PKCδ, so that NF-κB may initiate the transcriptional programme involved in the host defense to inflammatory stimuli and in EC resistance to apoptosis[219,220]. NF-κB, in turn, increases TRPC1 expression in TNF-α-stimulated cells, thus establishing a feed-forward loop that amplifies agonist-elicited SOCE and the increase in transendothelial permeability[221,222]. Similarly, AngII upregulates TRPC1 levels in an NF-κB-dependent manner in ECs[223]. Finally, TRPC1 may be involved in endothelial tube formation both *in vitro*[224] and *in vivo*[225]. The expression of TRPC1 in vascular ECs may be increased by high glucose levels, with a consequent increase in the amplitude of agonist-induced Ca^{2+} influx and a dramatic impact on endothelial function in diabetic patients[209]. TRPC1 not only acts as an SOC in vascular endothelium, but may also serve as an MSC by assembling with TRPV4 to mediate flow-induced Ca^{2+} entry in vascular endothelium[226]. A drop in luminal Ca^{2+} favors the translocation of the TRPC1-TRPV4 heteromeric channel from the ER to plasmalemmal caveolae[227], where the complex amplifies shear-stress-induced Ca^{2+} influx and augments the ensuing vascular dilation[227]. In addition, such an enhanced vesicular trafficking of TRPC1-TRPV4 channels may contribute to endothelial SOCs[228]. Similarly, TRPC1 may mediate stretch-induced Ca^{2+} entry after traumatic injury in microvessel endothelial cells, thereby resulting in NO synthesis and actin stress fiber formation[229].

TRPC3/6/7: TRPC3, TRPC6 and TRPC7 are Ca^{2+}-permeable channels that may be activated by DAG in a PKC-independent manner and may thus be regarded as SMOCs[200,201]. TRPC3 and TRPC6 are widely expressed both in cultured ECs and *in situ* endothelium, while TRPC7 is far less abundant (Table 2). DAG stimulates TRPC3- and TRPC6-gated Ca^{2+} entry into ECs exposed to a number of agonists, including VEGF[18,230,231], ATP[232-234], and bradykinin[232,235]. TRPC3- and TRPC6-induced Ca^{2+} inflow underpin the stimulatory effect of VEGF on endothelial proliferation, migration and permeability[18,231,236], while ATP exploits TRPC3 to activate NF-κB and increase vascular cell adhesion molecule-1 expression[237]. TRPC3-driven NO synthesis and vasore-

Table 4 Pattern of expression of transient receptor potential melastatin channels in vascular endothelial cells as detected by reverse transcription/polymerase chain reaction, Western blotting, and in situ hybridization

	TRPM1	TRPM2	TRPM3	TRPM4	TRPM5	TRPM6	TRPM7	TRPM8
PCa/PNa and conductance (pS)[202]	NM, NM	0.5-1.6, 52-80	0.1-10, 65 (Ca^{2+})-130	Not permeable, 25	Not permeable, 16-25	PCa/PNa~6, NM	3, 40-105	1-3, 83
Human Pulmonary artery ECs[320]	+(RT-PCR)	+/-(RT-PCR) +(WB)	+(RT-PCR)	+(RT-PCR)	-(RT-PCR)	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)
Human umbilical vein ECs[325,327,328]				+(RT-PCR, WB)		-(RT-PCR)	+(RT-PCR, WB)	
Mouse aortic ECs[329]				+(RT-PCR, WB)			+(RT-PCR)	
Human dermal microvascular ECs[327]							+(RT-PCR, WB)	
Mouse cerebral microvascular ECs	-(RT-PCR)	+(RT-PCR)	+(RT-PCR)	+(RT-PCR)	-(RT-PCR)	-(RT-PCR)	+(RT-PCR)	-(RT-PCR)
Mouse cardiac microvascular ECs[8]		+(RT-PCR, WB)					+(RT-PCR)	
Rat cerebral artery ECs[311]				+(RT-PCR)		+(RT-PCR)	+(RT-PCR)	
Rat spinal cord capillary ECs[324]				+(ISH)				
Rat mesenteric artery ECs[312]							+(RT-PCR)	

RT-PCR: Reverse transcription/polymerase chain reaction; WB: Western blotting; IHC: Immunohistochemistry; ISH: *In situ* hybridization; NM: Not measured; ECs: Endothelial cells.

laxation may be attenuated by the hypoxia-reoxygenation injury of vascular ECs, which prevents TRPC3 trafficking to the plasma membrane[235]. Moreover, TRPC3 supports both spontaneous and histamine-evoked Ca^{2+} oscillations arising, respectively, during and after EC tubule formation[224]. Finally, TRPC3-mediated Ca^{2+} inflow may stimulate flow-dependent endothelium-dependent vasodilation[238]. Endothelial TRPC3 and TRPC6 may be inhibited by NO in a protein-kinase-G-dependent fashion[239,240], while TRPC3 may also be inhibited by a PKC-mediated phosphorylation on Ser-712[239]. Both TRPC3 and TRPC6 may be recruited by signaling mechanisms other than DAG in vascular ECs. TRPC3 may provide a leakage Ca^{2+} entry pathway in unstimulated ECs[234] or behave as an SOC owing to its ability to physically interact with $InsP_3R2$ in uterine artery ECs[105]. Connexin-43-mediated intercellular communication between adjacent ECs favors TRPC3/$InsP_3R2$ association, although *via* a yet to be identified mechanism[105]. Moreover, endothelial TRPC3 may assemble with its distant relative, TRPC4, and form a heteromeric channel sensitive to oxidative stress and with biophysical properties distinct from both TRPC3 and TRPC4 homomers[241,242]. Finally, TRPC3 may be activated by thapsigargin-induced ER Ca^{2+} store depletion following PLC-dependent DAG synthesis and PKCη activation. PKCη, in turn, engages Src tyrosine kinase to phosphorylate and gate TRPC3[243]. TRPC3 levels in vascular ECs may be downregulated by VEGF in a PI3K-sensitive manner, and upregulated by an increase in blood pressure[244,245]. TRPC6, on the other hand, may be translocated from intracellular sites to the caveolae compartment of the plasma membrane by cytochrome P450 (CYP)-derived epoxyeicosanotrienoic acids (EETs). This process enhances bradykinin-induced Ca^{2+} entry and thus prolongs the activation of small and intermediate Ca^{2+}-activated K^+ channels responsible for endothelial hyperpolarization[246]. The functional consequence of TRPC6 insertion into the plasmalemma is related to the ability of ECs to transmit their negative shift in V_M to the adjacent smooth muscle cells and trigger vasorelaxation (the so-called endothelial-dependent hyperpolarizing factor, EDHF)[247]. TRPC6 externalization may also be induced by lysophosphatidylcholine (LPC): the following Ca^{2+} influx induces TRPC5 translocation to the plasma membrane, thus allowing sustained Ca^{2+} entry into ECs[248]. TRPC5-mediated Ca^{2+} inflow, in turn, is essential to restrict endothelial movement and might be relevant in impairing the healing process of the intimal layer in atherosclerotic arteries, where LPC is abundant[248]. TRPC6 externalization requires the phosphatase and tensine homolog (PTEN), whose tail 394-403 residues associate with the channel and favour its plasmalemmal localization[249]. It has yet to be elucidated whether EETs and LPC utilize PTEN to induce the intracellular movements of TRPC6 in ECs. Recent studies have shown that TRPC6 plays a key role in the onset of the lung ischemia-reperfusion edema (LIRE) as a consequence of endothelial NOX2-dependent ROS, which, in turns, leads to PLCγ activation, DAG kinase inhibition and an increase in submembranal DAG levels[250]. Consistently, PAF-induced lung edema depends upon the activation of acid sphingomyelinase (ASM), which engages TRPC6 to caveolae, thereby resulting in Ca^{2+} influx and following increase in endothelial permeability[251]. This process is inhibited by NO donors[251].

TRPC4/5: TRPC4 and TRPC5 form a subfamily with the closest homology with TRPC1[201]. Both channels open in response to the Gq/11 family GPCRs and TKRs, although neither $InsP_3$ nor DAG alone are able to activate them[200,201,252]. It has, however, been demonstrated that TRPC4 opening is favored by PIP_2 hydrolysis, although it also requires G_i recruitment and intracellular Ca^{2+}[253]. The scenario is further complicated by the necessity for TRPC4 to interact with the adaptor NHERF, ezrin/radixin/moesin ERM proteins, and cortical actin

via its COOH-terminal PDZ-binding domain to undergo PIP_2 modulation[253,254]. Similarly, TRPC5 activation may be potentiated by intracellular Ca^{2+} release and is modulated by the scaffolding ERM-binding phosphoprotein-50 (EBP50)[255,256]. TRPC4 and TRPC5 may assemble either as homo- or heterotetramers *via* their first ankyrin repeat in the NH2-terminal[257] and are abundantly expressed in both cultured cells and naive tissues. TRPC4 has long been considered as the key component of SOC in vascular endothelium[203], because TRPC4-deficient mice lack SOCE and exhibit impaired NO production and increased vascular permeability[258,259]. According to this model, the extent of store depletion is conveyed to PM by protein 4.1N, which links the cytoskeletal protein, spectrin, to TRPC4[260]. The latter, in turn, contributes pore-forming subunits to the store-dependent channel along with TRPC1 according to the following stoichiometry, 1 TRPC1 and 3 TRPC4[203,217,260,261]. The association between the two subunits is favoured by Cav-1, which also recruits $InsP_3R1$ into the membranal signalplex[262]. $InsP_3R1$, in turn, may interact with protein 4.1N, a feature which would explain the dependence of the TRPC1-TRPC4 complex on intracellular Ca^{2+} stores[263]. An alternative model proposes that this heterotetramer is activated by a protein termed stromal interaction molecule-1 (Stim1)[218], which senses intraluminal ER Ca^{2+} levels, as more widely illustrated below. A recent study, however, questioned TRPC1 and TRPC4 involvement in endothelial SOCE[205,206]. The controversy was explained by the finding that TRPC4 is externalized to the plasma membrane upon binding to β-catenin only in subconfluent, migrating ECs, but not in quiescent, barrier-forming cells, which is the typical condition for Ca^{2+} signaling studies[264]. Therefore, TRPC4 might serve as an agonist-recruited SOC during endothelial cell-cycle transitions from a proliferating to a quiescent phenotype[265]. Whether TRPC4 contributes to the repetitive Ca^{2+} spikes arising in HUVECs both before (spontaneous) and after (histamine-evoked) tubule formation in a store-dependent or independent manner has yet to be elucidated[224]. A single nucleotide polymorphism in the TRPC4 gene has been associated with protection from myocardial infarction and results in a larger current density when the mis-sense channel is expressed in heterologous cell systems[266]. This feature is likely due to a less bulky Val-957, which favors TRPC4 insertion into the plasma membrane and its interaction with the catalytic site of the tyrosine kinase that phosphorylates the channel at Tyr-959[266]. Less information is available on the role played by TRPC5 in ECs, where it may be constitutively open and also participate to ATP-induced Ca^{2+} entry[267]. TRPCs have been suggested to be activated also by NO *via* direct S-nitrosylation[186], but this mode of regulation is absent in vascular endothelium, where TRPC5 is actually inhibited by NO[267]. Conversely, the endothelial TRPC5 channel may be potentiated by the plant-derived isoflavone, genistein, independently on tyrosine kinases[268] and is upregulated by erythropoietin[269].

TRPV channels

The TRPV family consists of four subfamilies on the basis of their structure and function: TRPV1/2, TRPV3, TRPV4, and TRPV5/6[201]. The latter are highly Ca^{2+}-selective pathways, while TRPV1-4 are nonselective cation channels whose P_{Ca}/P_{Na} ranges between 1 and 15 pS[202]. All TRPV channels are endowed with 3-6 NH_2-terminal ankyrin repeats, which enable the protein to traffic to the plasma membrane, are required for tetramerization, and control protein-protein interactions[270]. TRPV channels may assemble either as homo- or heterotetramers, although their exact stoichiometry *in situ* is still unknown[270]. TRPV1-4, which are the only isoforms expressed in vascular ECs, may be gated by a number of stimuli, including G_q signaling, endocannabinoids, dietary agonists, changes in temperature and osmolality, pulsatile stretch and shear stress[270]. TRPV channels are, therefore, a remarkable example of polymodal channels and may serve as both MSCs and SMOCs.

TRPV1: TRPV1 is a nonselective cation channel with a preference for calcium that can be expressed in both cultured and native ECs (Table 3). Endothelial TRPV1 channels may be activated by the dietary agonist, capsaicin, and the endogenous cannabinoids, anandamide and 2-arachidonoyl-glycerol (2-AG)[149,270-272]. TRPV1 may, therefore, serve as an SMOC, although the physiological ligands that are capable of recruiting this channel in ECs are yet to be elucidated. Pharmacological stimulation of TRPV1, either by capsaicin or anandamide, causes vascular relaxation upon release of several vasodilators, including NO[273-275], calcitonin gene-related peptide (CGRP)[276,277], and EDHF[273]. CGRP may also afford a protective effect against LPC- and lipopolysaccharide (LPS)-dependent injury[276,277]. Furthermore, TRPV1 mediates the vasorelaxing effects of the synthetic, cannabinoid-like compound, VSN16, albeit it is still unknown whether TRPV1 is engaged by VSN16 directly or *via* the "abnormal-cannabidiol receptor"[278]. The signaling cascade responsible for endothelial NO synthase (eNOS) activation by TRPV1 has been unravelled. TRPV1 may trigger Ca^{2+}-dependent PI3K/Akt/CaMKII signaling, which leads to TRPV1 phosphorylation, increases TRPV1-eNOS complex formation, eNOS stimulation, and NO synthesis[271]. This signaling pathway might involve the AMP-activated protein kinase (AMPK), a multifunctional regulator of energy homeostasis that is phosphorylated upon TRPV1-mediated Ca^{2+} influx[279]. TRPV1-induced vasodilation is impaired in obese Ossabaw swine with the metabolic syndrome and thus could be a mechanism involved in endothelial dysfunction and development of coronary disease[273]. Chronic stimulation of TRPV1 by capsaicin enhances PKA and eNOS phosphorylation, which, in turn, results in vasorelaxation, reduces blood pressure and delays the onset of brain stroke in spontaneously hypertensive rats[280]. Moreover, dietary capsaicin reduces the high-salt-diet-induced endothelial dysfunction and nocturnal hypertension by pre-

venting the generation of O_2^- anions and NO reduction through vascular TRPV1 activation[281]. Baseline mean arterial pressures are, however, not statistically different in wild-type and TRPV1$^{-/-}$ mice[282,283]. Besides chemical agonists, TRPV1 may be gated by temperature higher than 40 ℃ in ECs[277,284]. This feature gives support to the hypothesis that TRPV1 contribute to peripheral vascular dilatation and high temperatures[8]. Conversely, a recent report described TRPV1-induced endothelin production by cultured ECs, which might cause the activation of vascular ET_A receptors and increase the mean arterial pressure *in vivo*[285]. Finally, capsaicin opposes angiogenesis by causing G1 arrest of ECs *via* downregulation of cyclin D1 and inhibited VEGF-triggered signaling pathways[286]. Whether this effect depends on TRPV1 stimulation is yet to be demonstrated.

TRPV2: TRPV2 has been mainly located in vascular smooth muscle cells (VSMCs), where it may be activated by cell swelling and act as an MSC[270]. Only a few studies have reported TRPV2 expression in ECs (Table 3) and, therefore, its function in vascular endothelium is still unclear. Due to its ability to mediate Ca^{2+} inflow in ECs exposed to moderate heat, however, it might aid TRPV1 to stimulate NO synthesis and peripheral vasorelaxation at > 43 ℃[8,284].

TRPV3: TRPV3 is a polymodal channel that may be gated by either natural compounds, such as carvacrol, eugenol, and camphor, or innocuous warm temperatures (> 30 ℃)[200]. TRPV3 expression has recently been shown in both *in situ* and cultured ECs (Table 3). In intact tissue, TRPV3 stimulation by carvacrol induces VSMC hyperpolarization *via* the Ca^{2+}-dependent opening of intermediate- (IK_{Ca}) and small-conductance (SK_{Ca}) K^+ channels. The consequent K^+ efflux activates smooth muscle inwardly rectifying potassium channels (K_{IR}) and amplifies the negative shift in VSMC V_M, thereby promoting arterial relaxation[287]. In human corneal ECs, TRPV3-induced Ca^{2+} entry is evoked by raising bath temperature from 25 to > 44 ℃[284]. If present also in vascular endothelium, this feature would render the channel capable of sensing high temperatures in peripheral circulation along with TRPV1 and TRPV2.

TRPV4: TRPV4 is the most polymodal TRP channel, being a molecular integrator of both chemical and physical stimuli[200,270], and may be expressed in both native and cultured ECs (Table 3). More specifically, endothelial TRPV4 may be activated by non-noxious temperatures (> 24 ℃)[288], hypo-osmotic cell swelling[289], shear stress[290,291], cyclic strain[292,293], and pharmacologically, by the non-PKC-activating phorbol ester, 4α-phorbol-12,13-didecanoate (4αPDD)[294] and by the plant-derived flavone, apigenin[295]. Cell swelling and shear stress do not gate TRPV4 directly, but *via* the PLA_2-dependent synthesis of AA, and its subsequent metabolization to 5',6'-epoxyeicosatrienoic acid (EET) through a CYP epoxygenase-dependent pathway[290,291,296-299]. A unique mechanism has been described in human coronary arterioles isolated from patients suffering from coronary artery disease, where the endothelial TRPV4 stimulate mitochondria to release ROS, which in turn cause VSMCs to relax[300]. Conversely, TRPV4 signaling induced by mechanical strain is mediated by direct force transfer from β1 integrins to the integrin-associated transmembrane CD98 protein within focal adhesions[293]. This feature renders TRPV4 a pure MSC that can be activated within only 4 ms by external force application[293]. TRPV4-mediated Ca^{2+} responses to heat and 4αPDD require an aromatic amino acid at the NH_2-terminal of the third transmembrane domain[289]. Recent work proposed that Cav-1 governs TRPV4 activity and location to the plasma membrane in ECs[301]. NO, PGI_2, and IK_{Ca}/SK_{Ca}/MaxiK-mediated EDHF are vasodilatory pathways that are activated following by pharmacological activation of TRPV4[27,291,302-304]. An alternative mechanism has been unravelled in human coronary arterioles from patients with coronary artery disease, where flow-induced TRPV4 activation causes ECs to release mitochondrial ROS, thereby relaxing the adjoining VSMCs[300]. Under physiological conditions, TRPV4-mediated vasodilation, mainly *via* NO and EDHF production, is induced by both shear stress[27,290,291,296] and extracellular autacoids, such as Ach and UTP[297,301,305,306]. Although the cellular mechanisms linking membrane receptors to TRPV4 opening are still unclear, UTP utilizes PLA_2 to gate the channel[297], while PKCα mediates Ach-dependent activation[307]. Despite the evidence for TRPV4-evoked vasodilation, the baseline blood pressure is not affected in TRPV4$^{-/-}$ mice[305,306]. Nevertheless, systemic activation of the channel may induce vasorelaxation *in vivo*[302,303,308,309] and, eventually, lead to circulatory collapse[303]. The vasorelaxing effect of TRPV4 is more evident in rodent models of N^{ω}-nitro-l-arginine (L-NNA)- and salt-induced hypertension[305,308,309]: this feature suggests that its main function might be to adverse excessive vasoconstricting stimuli and protect the vasculature during times of extreme stress[270]. It is, therefore, intriguing that TRPV4 is downregulated in a genetic model of salt-susceptible hypertensive rats exposed to a salt-enriched diet, whereas its function and expression are enhanced in salt-resistant rat strains[308]. In this view, apigenin might afford a protective effect on the development of cardiovascular diseases by stimulating TRPV4 to release EDHF[295]. An additional role for TRPV4-dependent Ca^{2+} signals consists in driving the shear stress-induced growth of collateral vessels (arteriogenesis) which occurs as a consequence of arterial occlusion[310-312]. Ca^{2+} entry may, indeed, recruit several Ca^{2+}-sensitive transcription factors, including nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1 (NFATc1), Kv channel interacting protein 3, calsenilin (KCNIP3/CSEN/DREAM), and myocyte enhancer factor 2C (MEF2C)[310]. On the other hand, TRPV4-mediated Ca^{2+} influx elicited by cyclic strain triggers cytoskeletal remodeling and movement necessary for cell

realignment in the vasculature[292]. TRPV4 channels are extensively expressed in alveolar septal capillaries[163,313], where they control lung endothelial permeability and barrier integrity in response to intravascular and airway pressures[314-316]. More specifically, TRPV4-gated Ca^{2+} entry increases vascular permeability to such an extent that barrier disruption and alveolar flooding may occur[313]. Finally, TRPV4 may enable ECs to sense body temperature, because the threshold for the heat-dependent activation for this channel is set at 25-27 ℃ and it is activated by moderate heat in ECs[4,288,317,318]. The results provided by knocked out mice, however, suggest that the steady state release of NO from endothelium is not governed by such a mechanism. A recent study implicated TRPV4 in tumor angiogenesis, as Florio Pla and coworkers reported an increase in TRPV4 levels in breast tumor ECs (b-TECs) as compared to control cells belonging to a human dermal microvascular endothelial cell line (HMVEC). In particular, AA-dependent TRPV4-mediated Ca^{2+} influx selectively drives cell migration *via* remodeling of the actin cytoskeleton in b-TECs, but not in HMVECs[319].

TRPM channels

The melastatin-related TRP subfamily was named based on the first discovered member, melastatin 1 (TRPM1), the gene of which was identified from melanomas. Members of the TRPM family are divided into four groups: TRPM1/3, TRPM2/8, TRPM4/5, and TRPM6/7[200,201]. TRPM channels exhibit highly varying cation permeability, from Ca^{2+} impermeable (TRPM4/5) to highly Ca^{2+} and Mg^{2+} permeable (TRPM6 and TRPM7)[202]. All the TRPM isoforms except TRPM5 are expressed in ECs (Table 4), although only the role of TRPM2 and TRPM7 has been extensively elucidated in these cells[270]. These channels are regarded as chanzymes, as they result from the fusion of an ion-channel pore-forming domain with an enzymatic domain[200].

TRPM2: TRPM2 is a non-elective cation channel whose predominant feature is the so-called Nudix box, a consensus region for pyrophosphatases that is localized in the cytoplasmic COOH-terminal tail of the channel protein and confers a unique activation mechanism, i.e., gating by ADPr, on the channel[144]. ADPr arises from breakdown of β-NAD, CD38, or other enzymes acting on cADPr and hydrolysis of ADP polymers by poly-ADP ribose glycohydrolase (PARP). TRPM2 is also activated by oxidative or nitrosative stress (e.g., H_2O_2)[4,144], perhaps mediated by mitochondrial ADPr. Therefore, TRPM2 serves as a sensor for the intracellular redox status by mediating oxidative stress-induced Ca^{2+} entry and triggering the subsequent Ca^{2+}-dependent elevation in endothelial permeability[4,200,320]. This chain of reactions is suppressed by a truncated TRPM2 isoform (TRPM2-S), generated by alternative splicing of the full-length protein (TRPM2-L)[144]. Moreover, TRPM2 may underlie neutrophil-elicited increase in endothelial $[Ca^{2+}]_i$, which is a prerequisite for transendothelial migration and, consequently, for sepsis-induced increase in lung vascular permeability[321]. The NH_2-terminal domain possesses a calmodulin-binding site which renders the channel sensitive to changes in sub-plasmalemmal Ca^{2+}, which shifts the dose-response curve to ADPr to the left[144]. In addition, the NH_2-terminal cytosolic tail of TRPM2 also contains a high affinity binding site for PKCα, which rapidly colocalizes with TRPM2-S in ECs challenged with H_2O_2[4,321]. PKCα might, thus, increase TRPM2-mediated Ca^{2+} influx and increase in endothelial permeability by phosphorylating TRPM2-S[4,321].

TRPM4: TRPM4, which is expressed as two splice variants, TRPM4a (non functional) and TRPM4b (functional, traditionally referred to as TRPM4), which is selective for monovalent cations, with an order of permeability $Na^+ > K^+ > Cs^+ > Li^+$, but not for Ca^{2+}[200,202]. TRPM4 is present in both intact vessels and cultured ECs (Table 4) and is gated by cytosolic Ca^{2+} with a K_D ranging from 0.4 to 9.8 μmol/L[322]. Therefore, TRPM4 represents the long-sought Ca^{2+}-activated nonselective cation channels[200]. A short stretch of six acidic amino acids in the pore loop determines its monovalent selectivity[202]. We refer the reader to other recent and comprehensive reviews for the molecular details of TRPM4 modulation in cell types other than ECs[322,323]. Briefly, the Ca^{2+}-dependent activation is regulated by ATP, Ca^{2+}-calmodulin, and PKC. The current-voltage exhibits a large outward rectification, indicative of a voltage-dependent behavior which is further enhanced by increasing the temperature between 15 and 35 ℃. PIP_2 also modulates channel activity by influencing its Ca^{2+} and voltage sensitivity[322]. The main function attributed to TRPM4 in ECs is that, when activated, it protects against Ca^{2+} overload by depolarizing the cell, thereby decreasing the driving force for Ca^{2+} entry. It has, however, recently been shown that TRPM4 plays a crucial role in promoting EC death. TRPM4 is upregulated in capillary ECs of injured spinal cord, which leads to microvessel fragmentation, formation of satellite (petecchial) hemorrhagic lesions, and secondary hemorrhages[324]. In addition, TRPM4 mediates LPS-induced EC death by a yet to be unveiled mechanism[325].

TRPM7: TRPM7 is a nonselective cation channel that exhibits moderate permeability to Mg^{2+} (albeit at very low conductances) and is, therefore, involved in intracellular Mg^{2+} homeostasis[200]. In addition, TRPM7 is permeable to Zn^{2+}, Co^{2+}, and Mn^{2+}, thereby providing a membrane pathway for metal ions[323]. TRPM7 might form either homo- or heteromultimers with its close homolog TRPM6, and has been found both in *in situ* and *in vitro* ECs (Table 4). Similar to TRPM2, TRPM7 serves as a chanzyme, its primary structure resulting from the fusion of a cation-selective ion channel pore with a COOH-terminal region possessing homology to a unique family of serine/threonine kinase domains, named the α-kinase family[200,326]. The enzymatic activity of the α-kinase domain is not required for pore opening, although evidence

exists that it may have an alternative means of influencing channel gating[323,326]. The mechanism underlying the constitutive activation of the channel is, therefore, still unresolved[323]. The phosphotransferase activity of the kinase domain is, nevertheless, able to respond to local changes in free Mg^{2+} occurring as the result of Mg^{2+} flux through the channel. TRPM7 might, therefore, act as "Mg^{2+}-sensor"[323,326]. Consistently, silencing TRPM7 mimics the effect of lowering extracellular Mg^{2+} in human microvascular ECs by impairing cell migration and arresting the cells in G0/G1 and G2/M phases[327]. Similarly, TRPM7 may inhibit EC proliferation *via* an extracellular signal-regulated kinase (ERK)-dependent reduction in eNOS levels[328]. This finding has been supported by *in vivo* observations performed in mice with inherited hypomagnesemia, TRPM7 upregulation is associated with endothelial dysfunction consequent to a decrease in eNOS levels and an increase in systolic blood pressure, to an augmented expression of vascular cell adhesion molecule-1 and plasminogen activator inhibitor-1, and to the downregulation of annexin-1, a downstream target of TRPM7[329]. Unlike VSMCs, the endothelial TRPM7 is insensitive to laminar shear stress[330].

TRPP channels or polycystins

The TRPP family consists of eight members, only two of which, i.e., polycystic kidney disease 2 (PKD2 or TRPP2) and PKD2-like (or TRPP3), form Ca^{2+}-permeable channels[200,323]. TRPP2 may, however, assemble with the non-channel protein, TRPP1 (or PKD1), in order to form a heterocomplex able to transduce laminar shear stress in intracellular $[Ca^{2+}]_i$ signals[200,323]. Both TRPP1 and TRPP2 have been found in cultured and *in situ* vascular ECs (Table 5). TRPP1 is an integral membrane glycoprotein with 11 transmembrane domains, an extensive NH_2-terminal extracellular tail involved in cell-cell, cell-matrix interaction and signaling pathways, and a cytosolic COOH-terminal endowed with a coiled-coil domain[331]. TRPP1 interacts with TRPP2, a six-transmembrane domain protein of the TRP ion channel family[200,323]. Polycystins also interact with multiple partners, including the TRP channel subunits TRPC1 and TRPV4, as well as several elements of the cytoskeleton[331]. By the coiled-coil COOH-terminal domain, TRPP2 and TRPP1 can form a functional polycystin complex in the apical cilia of both cultured and native ECs[332,333]. Due to its large extracellular NH_2-terminal tail, TRPP1 has been suggested to be a mechanical sensor regulating the opening of the associated calcium-permeable channel TRPP2[323]. Shear-stress-induced and TRPP1/TRPP2-dependent Ca^{2+} entry, in turn, triggers NO production by recruiting PKC, Ca^{2+}-calmodulin and the PI3K/Akt pathway[332,333]. Moreover, TRPP2 may aid TRPC1 in mediating Ca^{2+} influx, NO production, and actin reorganization in bEnd3 cells exposed to mechanical stress[229], as aforementioned.

TRPA1 channels

TRPA1 is the sole member of the TRPA family, whose structural hallmark is represented by the 14 ankyrin repeats at its NH_2 terminus[200,323]. These repeats might be required for the channel to interact with cytoskeletal components or to bind to specific agonists[323]. In addition, the NH_2 terminus possesses a Ca^{2+}-binding EF-domain[323]. TRPA1 homotetramers assemble to form a nonselective cation channel displaying a relatively high Ca^{2+} permeability when compared with other TRP channels, including TRPV1 (Table 5). TRPA1 may be activated by electrophilic compounds such as acrolein (an active component of tear gas), allicin (found in garlic), and allyl isothiocyanate (AITC) (derived from mustard oil). α, β-Unsaturated aldehydes produced endogenously in response to oxidative stress, such as 4-hydroxy-2-nonenal, 4-oxo-nonenal, and 4-hydroxyhexenal, also activate the channel[323]. Most identified TRPA1 agonists act *via* covalent modification of cysteines in the NH_2 termini, with specific binding residues identified[323]. In addition, TRPA1 might open in response to noxious cold (< 17 ℃), but the thermosensitivity of the channel is still debated[323]. TRPA1 has been detected in native ECs, localized to endothelial cell membrane projections through the internal elastic lamina in the direction of VSMCs. TRPA1-mediated Ca^{2+} influx causes endothelium-dependent SMC hyperpolarization and relaxation of cerebral arteries by activating IK_{Ca} and SK_{Ca}[304]. IK_{Ca}- and SK_{Ca}-gated K^+ efflux, in turn, activates K_{IR} in SMCs, thus amplifying myocyte relaxation and dilation[304].

Stim1 and Orai1

The physical interaction between the ER Ca^{2+}-sensor, Stim1, and the plasmalemmal, Ca^{2+}-permeable channel, Orai1, has recently been shown to mediate SOCE in a number of vascular ECs[205,206,243,334]. Stim1 is an ER-resident type I single transmembrane protein with an NH_2 terminus located either in the ER lumen or facing the extracellular space. It contains two NH_2-terminal EF hand domains (a canonical and a hidden one) followed by a sterile α-motif (SAM) domain, the transmembrane region, three predicted coiled-coil domains comprising an ezrin-radixin-moesin (ERM) motif and at the COOH terminus, a proline-rich and a lysine-rich domain[335,336]. The negatively charged NH_2-terminal EF hand domain is the Ca^{2+} sensing subunit of the protein. In the resting state, Stim1 seems to be present as a dimer in the ER membrane. The dimerization happens through the COOH-terminal coiled-coil domains. In case of store release, and subsequent Ca^{2+} dissociation from the canonical EF hand domain, further oligomerization of Stim1 through the SAM domain occurs. These Stim1 oligomers migrate towards sites of the ER that are in close vicinity to the plasma membrane, where they assembly into punctuate clusters and activate SOCE by directly binding to Orai1[335,336]. Accordingly, the latter has been found to be an essential component of SOCE by forming the pore unit of the channel in a variety of cell types[335,337], including vascular ECs[205,206,338]. Orai1 is a four-transmembrane protein with intracellular NH_2 and COOH termini. In the resting state, it is present as a dimer in the plasma mem-

Table 5 Pattern of expression of transient receptor potential ankyrin and polycystic channels in vascular endothelial cells as detected by reverse transcription/polymerase chain reaction, immunohistochemistry, and Western blotting

	TRPA1	TRPP1	TRPP2
PCa/PNa and conductance (pS)[202]	0.8-1.4, NM	1-5, 40-177	4, 137
Human renal artery ECs[332]			+(RT-PCR, WB, IC)
Human umbilical vein ECs[332]			+(RT-PCR, WB)
Mouse aortic ECs[332,333]		+(RT-PCR, WB, IC)	+(RT-PCR, WB)
Mouse femoral artery ECs[332]			+(RT-PCR, WB)
Rat cerebral arteries[287,304,311]	+(RT-PCR, IC)		+(RT-PCR)

RT-PCR: Reverse transcription/polymerase chain reaction; WB: Western blotting; IC: Immunostaining for cultured cells; NM: Not measured; ECs: Endothelial cells.

brane, however, the functional SOC channel seems to be formed by Orai1 tetramers. The interaction between Stim1 and Orai1 is mediated by the CRAC activation domain (CAD) located at the COOH terminus of Stim1 and the cytosolic NH_2 and COOH tails of Orai1[335,336]. Accordingly, the genetic suppression of either Orai1 or Stim1 suppresses SOCE induced by either pharmacological (CPA and thapsigargin) and physiological (VEGF, thrombin) stimulation in HUVECs, EA.hy296 cells, and porcine aortic ECs[205,206,218,243,334]. This, in turn, leads to the blockade of several key steps of the angiogenic process, such as proliferation and tubulogenesis[205,206,338]. These data are supported by the finding that, under resting conditions, the ER Ca^{2+} sensor is homogeneously distributed throughout the cytosol, whereas emptying of the $InsP_3$-sensitive Ca^{2+} reservoir in the absence of extracellular Ca^{2+} induces Stim1 homo-oligomerization and redistribution into sub-plasmalemmal puncta[206,218,339]. Both processes are recovered upon Ca^{2+} restoration to the bathing solution, as Ca^{2+} entry recharges the intracellular Ca^{2+} pool and inactivates Stim1[340]. Stim1 puncta in vascular EC reassembly at the same loci during repetitive cycles of ER Ca^{2+} depletion and refilling suggest that this process is driven by accessory proteins, such as the microtubule-plus-end tracking protein, EB1[335,336,340]. The engagement of Stim1 is, however, limited when ECs are stimulated in the presence of extracellular Ca^{2+}, which is consistent with modest reduction in the ER Ca^{2+} load observed under these conditions[40,41,340]. An important aspect of Stim1 dynamics in ECs regards its modulation by cytosolic Ca^{2+}: an increase in submembranal Ca^{2+} levels prevents Stim1 clustering, thereby impeding prolonged SOC activity and intracellular Ca^{2+} overload[340]. This feature might contribute to explain the long-known inhibition of SOCE by Ca^{2+} microdomains arising at the mouth of the channel[341]. SOCE mediated by Stim1 and Orai1 is gated by an inwardly rectifying, Ca^{2+}-selective current[205], whose amplitude may fall below the resolution limit of a whole-cell patch-clamp recording system[206]. Orai1 has two homologs in mammals, namely Orai2 and Orai3, which do not contribute to SOCE in ECs[205,218,338]. In contrast to Stim1, Stim2 has no major effect on SOCE, but might be involved in the regulation and stabilization of basal cytosolic and ER Ca^{2+} levels in ECs[342]. The involvement of Stim1 and Orai1 to endothelial SOCE is, however, still controversial and might depend on both the vascular bed and the cell type. For instance, recent work ruled out any detectable role for Orai1 in thrombin- and thapsigargin-induced Ca^{2+} inflow in mouse lung ECs[218], where SOCE activity requires functional TRPC1 and TRPC4[218]. The same study reported that, in these cells, Stim1 is mobilized into sub-plasmalemmal puncta in a TRPC4-dependent manner and, in turn, it signals TRPC1 and TRPC4 about the ER Ca^{2+} content.

Ca^{2+} EXTRUSION FROM THE CYTOSOL

A variety of pumps and exchangers fulfil the function to remove Ca^{2+} from the cytosol following EC stimulation, namely SERCA, NCX, PMCA, and the mitochondrial uniporter[36,40,41,139,343-349]. SERCA, NCX, PMCA, mitochondria may be aligned either in series or in parallel in ECs in order to remove Ca^{2+} from the cytosol properly, to achieve successful ER Ca^{2+} refilling, and facilitate SOCE by buffering sub-plasmalemmal Ca^{2+}. These functions are facilitated by their optimal coupling to either $InsP_3Rs$ or RyRs.

Expression of SERCA, NCX and PMCA in ECs

SERCA is encoded by three different genes (ATP2A1, ATP2A2, and ATP2A3) with numerous alternative splice isoforms at the 3' ends of the mRNA. More specifically, ATP2A1 encodes for SERCA1a-1b, ATP2A2 for SERCA2a-2c, and ATP2A3 for SERCA3a-3f[350]. The SERCA isoforms differ mainly by their affinity for Ca^{2+} (2b > 2a = 1 >> 3) and their Ca^{2+} transport turnover rates, SERCA2b having the lowest transport capacity of all SERCAs[350]. Both SERCA2a and SERCA3 have been found in isolated and *in situ* ECs[106,351-356], albeit SERCA3 transcript levels decrease during EC proliferation in culture and in hypertension[106,112]. SERCA3 expression is regulated by Ca^{2+} itself in a calcineurin/NFAT-dependent manner[357] and its genetic deletion leads to a decrease in agonist-induced elevation in $[Ca^{2+}]_i$ and NO synthesis[358]. The Ca^{2+}-ATPase activity in vascular endothelium may be enhanced by NO, which considerably increases Ca^{2+} loading into ER lumen[359]. Moreover, ECs may be endowed with phospholamban (PLB), a 24- 27-kDa phosphoprotein that is closely associated with the cardiac sarcoplasmic reticulum, where it modulates SERCA function[360]. More specifically, PLB inhibits Ca^{2+} uptake by SERCA in Ach-stimulated cells and dampens the endothelial-dependent vasorelaxation[360]. In addition, SERCA activity is more resistant to peroxynitrite and peroxide in ECs as compared to VSMCs[361,362]; a feature that may confer a protective effect during the massive production of free

radicals observed under pathological conditions, such as ischemia-reperfusion. Conversely, selective targeting of thiol groups (i.e., Cys residues) by myeloperoxidase-derived oxidants may inhibit the Ca^{2+}-ATPase activity and lead to an unbalanced increase in $[Ca^{2+}]_i$, which might contribute to the endothelial dysfunction occurring during several inflammatory diseases[363]. In this view, oxidized and glycated low-density lipoprotein causes SERCA oxidation, aberrant ER stress and atherosclerosis *in vivo*, although this detrimental chain of events is suppressed by AMPK[364,365].

The Na^+/Ca^{2+} exchanger family comprises three members, namely NCX1, NCX2, and NCX3, which act by expelling one Ca^{2+} ion for the uptake of three Na^+ ions (the so-called "forward-mode" of NCX). The electrochemical gradient for Na^+ provides the energy necessary to drive Ca^{2+} out of the cell against its large gradient and with a stoichiometric ratio of 3 Na^+ in: 1 Ca^{2+} out[366]. Cultured ECs may express all of the three NCX subtypes[355,367,368], while the only evidence about NCX presence *in situ* has been reported in rat brain[369]. NCX expression may be upregulated by an increase in $[Ca^{2+}]_i$[370]. In addition to extruding Ca^{2+} out of the cytosol, NCX may contribute to Ca^{2+} entry when operating in the so-called "reverse-mode" (3 Na^+ out: 1 Ca^{2+} in). More specifically, the close proximity between NCX and the TRPC channels, which may either physically interact[371] or be clustered in limited membrane nanodomains[372], causes NCX to switch into the reverse-mode upon Na^+ accumulation beneath the plasma membrane[371,372]. Intriguingly, NCX-mediated Ca^{2+} entry in ECs may be stimulated by Ach[373], histamine[43], and VEGF[374], but not by ATP[375]. In particular, NCX-driven Ca^{2+} influx underpins PKCα translocation to the plasma membrane, where it stimulates ERK1/2 to trigger a number of steps (proliferation, migration, and tubulogenesis) involved in VEGF-dependent angiogenesis[374]. It is, therefore, conceivable that NCX role in the regulation of Ca^{2+}-dependent processes in vascular endothelium may be governed by the incoming stimuli.

PMCA is encoded by four genes, termed PMCA1-4. Alternative splicing of their primary transcripts yields a large variety of PMCA proteins that differ in their kinetics and regulation[350]. The pump operates with high Ca^{2+} affinity and low transport capacity, with a 1:1 Ca^{2+}/ATP stoichiometry[350]. PMCA1 is the main isoform found in vascular endothelium, although lower levels of PMCA2 and PMCA4 may also be present[355,376]. PMCA-dependent Ca^{2+} clearance is triggered by local elevation in intracellular Ca^{2+}, which acts by promoting the association between CaM and a specific CaM-binding domain located at the COOH terminus of the pump[348,377]. PMCA activity and expression is negatively regulated by endothelin-1, which may thus lead to an increase in $[Ca^{2+}]_i$ by reducing Ca^{2+} clearance from the cytosol[378]. PMCA is preferentially located at caveolae on the luminal surface of ECs, and its activity is reduced by phosphatidylserine externalization[379,380]. In addition to extruding Ca^{2+} across the plasma membrane, PMCA suppresses both eNOS activity and the subsequent NO production by promoting Thr-495 phosphorylation in resting and activated ECs[381]. PMCA activity in vascular ECs is, in turn, inhibited by *S*-glutathionylation, which leads to a progressive raise in $[Ca^{2+}]_i$[124]. The elucidation of the role served by PMCA in endothelial signaling might benefit from the recent discovery of caloxin 1b3, a specific PMCA1 inhibitor[382].

Mitochondrial Ca^{2+} uptake

Mitochondria contribute to the dynamics of Ca^{2+} signaling by aiding SERCA, NCX, and PMCA in removing Ca^{2+} from the cytoplasm during the recovery phase. Mitochondrial Ca^{2+} release mechanisms, then, return Ca^{2+} back into the cytosol, where it can be sequestered by SERCA. Indeed, there is a close functional relationship between mitochondria and ER[10]. This interaction is reinforced by the chaperon glucose-regulated protein 75 (grp75), which tethers ER $InsP_3Rs$ to the voltage-dependent anion channels (VDACs) in the outer mitochondrial membrane. VDACs enable the transfer of cytosolic Ca^{2+} into the mitochondrial intermembrane space, from where the electrochemical gradient (the inner mitochondrial membrane potential is equal to about -180 mV) drives Ca^{2+} into the mitochondrial matrix mainly by two transport mechanisms[383]. The mitochondrial Ca^{2+} uniporter (MCU), which is located in the inner mitochondrial membrane, operates at the micromolar Ca^{2+} concentrations that are only transiently reached in cells near Ca^{2+} release channels, and might be contributed by uncoupling proteins 2 and 3 (UCP2 and UCP3) in ECs[384]. However, UCP2/3 buffer intracellularly mobilized Ca^{2+}, but not that entering the cells *via* SOCs[385]. Mitochondrial Ca^{2+} uptake from SOCE in ECs is accomplished by a second type of MCU, which mediates Ca^{2+} uptake into mitochondria at nanomolar cytosolic Ca^{2+} concentrations and has been termed Letm1[383]. This is a leucine-zipper-EF hand-containing a TM region which catalyzes the 1:1 electrogenic exchange of Ca^{2+} for H^+[386]. Conversely, the contribution of the so-called mitochondrial calcium uptake 1 (MICU1), which has recently been discovered by working on HeLa and HEK-293 cells[387], to endothelial Ca^{2+} signaling has not been ascertained yet The mitochondrial NCX (mNCX) is the main mechanism responsible for the outward transport of Ca^{2+} towards the cytosol with a stoichiometry of $3Na^+:1Ca^{2+}$ (as for the plasmalemmal NCX)[36,41]: the Na^+ that enters down the electrochemical gradient is exchanged for Ca^{2+}[383].

Microarchitecture of the Ca^{2+} transporting systems in ECs

The microstructural physical localization of Ca^{2+} pumps, transporters and channels in the ER and plasma membrane relative to each other determines the pattern of Ca^{2+} clearance from the endothelial cytosol[36,40,41,138,139,345,388]. This organization of the Ca^{2+} transporting systems creates Ca^{2+} gradients between the sub-plasmalemmal region and bulk cytoplasm, which, in turn, enables the same second

messenger, i.e., Ca^{2+}, to regulate diverse cellular functions depending on its proximity to specific Ca^{2+}-sensitive effectors[10]. The sequence of events leading to removal of cytosolic Ca^{2+} may be different in ECs from different vascular beds and three main Ca^{2+}-buffering pathways have hitherto been identified. (1) In-series arrangement of RyRs and NCX and of SERCA and RyRs[139,345]: according to this model, that has been described in rabbit aortic ECs, cytosolic Ca^{2+} is captured by SERCA, released from the ER *via* RyRs, and extruded across the plasma membrane *via* the NCX. PMCA acts in parallel to this pathway to bind to and deliver Ca^{2+} to the extracellular space; (2) Vectorial Ca^{2+} release from sub-plasmalemmal $InsP_3Rs$ to the extracellular space *via* NCX[389]: this system operates following either a moderate or a slow increase in $InsP_3$ levels in porcine coronary artery ECs, prevents sub-plasmalemmal Ca^{2+} from reaching the deeper cytosol by triggering CICR on RyRs, and has, therefore, been termed SCCU. The SCCU has also been found in EA.hy926 cells, where it subserves a signaling role by activating the endothelial Ca^{2+}-dependent large conductance K^+ channels (BK_{Ca}) after cell stimulation by low concentrations of histamine[140,141]. An increase in the agonist dose overcomes the moderate RyRs-dependent subplasmalemmal Ca^{2+} release due to the higher $InsP_3$-dependent Ca^{2+} mobilization; and (3) Mitochondrion-mediated ER refilling: this pathway redirects entering Ca^{2+} through SOCs to the ER in the presence of an $InsP_3$-generating agonist[36,41,388]. SOCE is buffered by sub-plasmalemmal mitochondria and vectorially transferred *via* mNCX to SERCA. When SOCE occurs in the absence of $InsP_3$, the superficial domains of the ER mimic mitochondrial Ca^{2+} buffering by immediately capturing entering Ca^{2+}. This Ca^{2+}-buffering mode has been mainly characterized in EA.hy926 cells, where it coexists with the SCCU system described above. The former is responsible for the extrusion of Ca^{2+} entering into the cells *via* SOCE, whereas the latter removes from the cytosol the intracellularly-released calcium ions. The mitochondria-mediated ER recharging might also be present in other vascular beds, but this mechanism has not been thoroughly investigated in mature endothelium.

The endothelial Ca^{2+}-buffering system may, however, operate in parallel, as depicted by a number of studies conducted on microvascular endothelial cells. In rat brain capillary ECs, for instance, the SERCA provides the major pathway responsible for clearing cytosolic Ca^{2+}, whereas NCX extrudes across the PM a minor percentage (around 30%) of the calcium ions mobilized from ER[343]. Conversely, in rat cardiac microvascular cells, PMCA and SERCA have been shown to restore endothelial Ca^{2+} to prestimulation levels during exposure to the agonist. However, upon interruption of the stimulation, SERCA quickly recaptures Ca^{2+} back into the ER lumen[349].

SPATIOTEMPORAL ORGANIZATION OF INTRACELLULAR Ca^{2+} SIGNALS IN ECS

The remarkable versatility of Ca^{2+} is due to the multitude of Ca^{2+} signals that can be generated by extracellular stimuli within a single cell. Such heterogeneity depends on the recruitment of specific components of the Ca^{2+} toolkit which lead to the generation of intracellular signals varying in amplitude, spatial dimension and frequency[10,390,391]. The opening of Ca^{2+}-permeable pathways either on the ER or plasma membrane may give rise to local or elementary Ca^{2+} signals, which are spatially restricted within hundreds of nanometers to micrometers from the mouth of the channel pore[392]. The local nature of these events is attributable to the concerted action of Ca^{2+} buffering and reuptake mechanisms that limit the diffusion of the Ca^{2+} signal[37]. The most simple Ca^{2+} release events are the Ca^{2+} blips and the Ca^{2+} quarks, which arise from the opening of single $InsP_3Rs$ and RyRs, respectively[10,392]. Endothelial Ca^{2+} blips have average amplitude of 23 nmol/L, are 1- to 3-μm wide, and last < 100 ms[393]. Conversely, there is no report of endothelial Ca^{2+} quarks. The concerted opening of a fixed cluster of $InsP_3Rs$ and RyRs give rise, respectively, to Ca^{2+} puffs and Ca^{2+} sparks[10,392]. Spontaneous Ca^{2+} puffs have been reported in native ECs[394-396], which can be excited by adjoining SMCs through gap junctional communication[396-398]. Isolated ECs display only agonist-induced Ca^{2+} puffs, which reach a peak of about 50 nmol/L and spread for no longer than 30 μm[393]. Elementary Ca^{2+} release events mediated by $InsP_3Rs$, but different from the classic Ca^{2+} puffs, may occur in restricted spaces of myoendothelial projections and have been termed Ca^{2+} pulsars[16,399]. The endothelial Ca^{2+} pulsars colocalize with the intermediate conductance, Ca^{2+}-sensitive K^+ channels ($K_{Ca}3.1$) so that local Ca^{2+} signals may be translated into a negative shift in V_M which is rapidly transmitted to the adjacent VSMCs in terms of EDHF[399]. Consistently, sympathetic nerve stimulation activates α-adrenergic receptors on SMCs to recruit new pulsar sites in the adjoining ECs in order to oppose vasoconstriction[400]. The spatiotemporal summation of adjacent Ca^{2+} puffs leads to a regenerative intracellular Ca^{2+} wave that can spread throughout the cytosol at an average speed of 5-60 μm/s[118,393,401]. The regenerative propagation of the Ca^{2+} elevation is, indeed, faster in the perinuclear region and at the initiation site[118]. The endothelial Ca^{2+} sweep tends to originate from multiple peripheral foci localized in the caveolae compartments of the plasma membrane[402]. These triggering sites remain constant during repetitive cell stimulation[393,401,402]. Indeed, when the information embedded within the $[Ca^{2+}]_i$ increase must be conveyed over a long time, both the elementary events and the global Ca^{2+} sweeps can adopt an oscillatory pattern[16,72,339,396]. However, the physiological outcome of local Ca^{2+} spikes depends on the Ca^{2+}-sensitive decoders located within a few nanometers from the channel pore[395], and the frequency of repetitive Ca^{2+} waves encodes for the selective engagement of specific downstream targets[403-405]. The interspike interval of the Ca^{2+} transients is inversely correlated to the strength of the stimulus until, at least at high agonist doses, they apparently fuse to generate a plateau level[16,72,406,407]. Endothelial Ca^{2+} oscillations may,

indeed, be elicited by mechanical forces, extracellular autacoids or oxidative stress. Shear stress and pressure may induce ECs to undergo repetitive Ca^{2+} transients by triggering Ca^{2+} entry through Gd^{3+}-sensitive MSCs followed by CICR from RyRs[22,24,25]. The opening of the MSC generates a transient local Ca^{2+} event that has been termed Ca^{2+} hotspot[24]. Unlike the oscillatory Ca^{2+} response to extracellular ligands[72,406,407], the amplitude of the Ca^{2+} spikes increases with the intensity of the mechanical stimulation[22-25]. Agonist-evoked Ca^{2+} oscillations require PLC activation by either GPCRs or TKRs[72,339,404,405,408]. The following $InsP_3$-dependent Ca^{2+} mobilization may be amplified by adjacent RyRs *via* CICR[44,134] and requires Ca^{2+} inflow to supply releasable Ca^{2+} over time and maintain $InsP_3R$ sensitivity to the ligand[72,407,409,410]. Ca^{2+} entering through either SOCE or the reverse-mode of NCX is sequestered by SERCA into ER lumen before being released again into the cytosol through the open $InsP_3Rs$[72,134,339,404,405]. Some[16], but not all[72], microvascular ECs may be independent on Ca^{2+} inflow. In addition to bringing about the downstroke of each Ca^{2+} spike, SERCA shapes both the rate of the initial Ca^{2+} release and the amplitude of the regenerative upstroke, whereas it does not affect the Ca^{2+} wave speed[339,411]. As a consequence of the rhythmic interplay between $InsP_3$-induced Ca^{2+} release and SERCA-mediated Ca^{2+} reuptake, SOCE is not constant during the Ca^{2+} train. Accordingly, store-sensitive Ca^{2+} inflow is activated by the partial depletion of the Ca^{2+} pool occurring during each Ca^{2+} spike and is then turn off by store replenishment[410,412]. Finally, ECs may display repetitive Ca^{2+} oscillations in response to the NAD(P)H oxidase-derived H_2O_2 that is endogenously produced during the ischemia/reperfusion injury[56,413]. H_2O_2-elicited Ca^{2+} spikes are supported by the periodic opening of $InsP_3Rs$, but rapidly run down in absence of extracellular Ca^{2+}[56,413]. It would, therefore, be interesting to assess whether the H_2O_2-sensitive plasmalemmal channel, TRPM2, is involved in the signal transduction pathways recruited under these conditions[320].

CONCLUSION

Once regarded as an inert barrier between circulation and vascular tissue, ECs constitute the largest signal transduction platform of the organism, which is essential for the proper functioning of the cardiovascular system. Any alteration in the endothelial capability to detect and decode both physical and humoural stimuli into the most suitable vessel wall phenotype may lead to an astonishing variety of severe diseases, including myocardial infarction, brain stroke, atherosclerosis, peripheral artery disease, and tumor. As recently outlined by Munaron and Florio Pla[11], it is imperative to understand the molecular mechanisms that enable vascular ECs to fulfil their multifaceted role in order to either prevent or reduce the adverse consequences of so-called endothelial dysfunction. The Ca^{2+} signalling toolkit plays a key role in the control of a wide array of endothelial processes and, therefore, any change in the pattern of expression of each of its components is somehow involved in the pathogenesis of an increasing number of pathologies. Despite the most recent advances in the comprehension of the molecular underpinnings of the Ca^{2+} responses to both physical and chemical stimulation, a huge amount of work is standing in front of us. First, the blend of endothelial ion channels, transporters and receptors may vary with species, age, sex, hormonal status, vascular bed, and blood vessel diameter. This issue has been further stressed by the recent finding that Stim1 and Orai1 control *in vitro* tubulogenesis in HUVECs[206], but not in the HUV-derived EC line, EA.hy926[224]. As a consequence, the information on the Ca^{2+} machinery acquired on animal or cell line models cannot undergo a straightforward clinical translation, but must be confirmed in humans and in the vascular region implicated in the disease under investigation. This task may not be easy due to the difficulties of obtaining human samples from either healthy or pathological vessels. Second, the controversy on the molecular nature of SOCs, which are regarded as the most important pathway for Ca^{2+} influx into vascular endothelium, is far from being fully resolved. As described above, a recent study suggested that Orai1 and Stim1, whose physical interaction mediates SOCE in the immune system, underlie this Ca^{2+} entry route only in HUVECs, whereas TRPC1 and TRPC4 are activated by the emptying of the Ca^{2+} pool in other vascular beds. The same study, however, contributed to boost such controversy by demonstrating that the genetic suppression of TRPC1 dampens SOCE in HUVECs, a finding which is not consistent with those previously reported on the same preparation. This conflict is not surprising when considering the abundance of discrepant data about TRPC1 modulation by Stim1. The possibility that the molecular structure of SOCE (i.e., either the ER Ca^{2+} sensor and the Ca^{2+}-permeable pore) might differ in distinct species and vascular beds, as put forward by Sundivakkam *et al*[218], should be also considered. Moreover, the possibility that the components of SOCE may be different in distinct phases of the cell cycle should be taken in account. Yet, it is evident that further studies will have to be devoted to clarify this issue in vascular ECs. A recent study attempted to solve this controversy by investigating the association between Orai1 and TRPC1, which has been described in a growing number of nonexcitable cells, in rat pulmonary artery ECs. In these cells, SOCE has long been associated with TRPC1 and TRPC4, as widely described above[217]. Surprisingly, Cioffi *et al*[414] have found that Orai1 constitutively interacts with TRPC4 and drives the activation of the TRPC1-TRPC complex upon intracellular Ca^{2+} release. It is not clear, however, whether and how protein 4.1 and Stim1 independently confer store-sensitivity to the ternary complex on the plasma membrane. Third, whereas the intracellular target of $InsP_3$ has long been recognized, the selective receptor of the two newly discovered second messengers, cADPr and NAADP, has recently been questioned. Indeed, a couple of recent investigations demonstrated that the genetic suppression of TPC1-2 does not prevent cell labeling by NAADP[415,416]. Conversely, the same studies suggested

that a 22- and 23-kDa pair of accessory proteins might serve as NAADP receptors and regulate the activity of its target channels, including TPC1-2 in ECs and RyRs in the immune system. The same controversy holds true also for the molecular effector of cADPr-dependent signalling in mature ECs. As mentioned already, the mechanistic link between cADPr and RyRs-mediated Ca^{2+} is yet to be elucidated. Fourth, ECs establish either transient or permanent intimate contacts with a variety of cell types, such as circulating blood cells or tumoral cells, fibroblasts, and SMCs. Only a few studies have hitherto addressed whether and how these tight relationships may affect endothelial Ca^{2+} homeostasis. In addition, ECs may undergo a rearrangement of their Ca^{2+} signaling proteins when detached from the vessel walls and cultured *in vitro*[7,153]. It follows that future studies should be conducted both *in vitro* and *in vivo*, by exploiting the genetic Ca^{2+}-sensors that have been described in the literature, to explore this fascinating aspect of Ca^{2+} signaling. Finally, intracellular Ca^{2+} waves have recently been shown to drive EPC proliferation, homing, and tubulogenesis. Only a few investigations have so far been devoted to unmask the players of the Ca^{2+} machinery involved in these tasks. In the light of the promising therapeutic potential of the Ca^{2+}-permeable channels in these cells, either as a target or facilitators of clinical interventions, we are confident that this field will gain the interest of researchers worldwide. Therefore, it appears that the way towards full comprehension of the intricate network of membrane receptors, ion channels, transporters, pumps, and cytosolic decoders that convert an extracellular stimulus into a meaningful Ca^{2+} signal still has a long way to go and overcome many obstacles, but a multidisciplinary approach will certainly be helpful to reach this goal.

ACKNOWLEDGMENTS

The authors are grateful to all their collaborators for the ongoing and fruitful discussions on Ca^{2+} signalling in endothelial cells and endothelial progenitor cells. They are also delighted to acknowledge Mrs Simona Laudadia for her excellent bibliographic assistance.

REFERENCES

1 **Tran QK**, Watanabe H. Calcium signalling in the endothelium. *Handb Exp Pharmacol* 2006; 145-187
2 **Félétou M**, Vanhoutte PM. Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). *Am J Physiol Heart Circ Physiol* 2006; **291**: H985-1002
3 **Khazaei M**, Moien-Afshari F, Laher I. Vascular endothelial function in health and diseases. *Pathophysiology* 2008; **15**: 49-67
4 **Di A**, Malik AB. TRP channels and the control of vascular function. *Curr Opin Pharmacol* 2010; **10**: 127-132
5 **Fiorio Pla A**, Avanzato D, Munaron L, Ambudkar IS. Ion channels and transporters in cancer. 6. Vascularizing the tumor: TRP channels as molecular targets. *Am J Physiol Cell Physiol* 2012; **302**: C9-C15
6 **Santella L**, Lim D, Moccia F. Calcium and fertilization: the beginning of life. *Trends Biochem Sci* 2004; **29**: 400-408
7 **Nilius B**, Droogmans G. Ion channels and their functional role in vascular endothelium. *Physiol Rev* 2001; **81**: 1415-1459
8 **Yao X**, Garland CJ. Recent developments in vascular endothelial cell transient receptor potential channels. *Circ Res* 2005; **97**: 853-863
9 **Mancardi D**, Pla AF, Moccia F, Tanzi F, Munaron L. Old and new gasotransmitters in the cardiovascular system: focus on the role of nitric oxide and hydrogen sulfide in endothelial cells and cardiomyocytes. *Curr Pharm Biotechnol* 2011; **12**: 1406-1415
10 **Berridge MJ**, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 2003; **4**: 517-529
11 **Munaron L**, Fiorio Pla A. Endothelial calcium machinery and angiogenesis: understanding physiology to interfere with pathology. *Curr Med Chem* 2009; **16**: 4691-4703
12 **Tiruppathi C**, Ahmmed GU, Vogel SM, Malik AB. Ca2+ signaling, TRP channels, and endothelial permeability. *Microcirculation* 2006; **13**: 693-708
13 **Nilius B**, Viana F, Droogmans G. Ion channels in vascular endothelium. *Annu Rev Physiol* 1997; **59**: 145-170
14 **Chetham PM**, Babál P, Bridges JP, Moore TM, Stevens T. Segmental regulation of pulmonary vascular permeability by store-operated Ca2+ entry. *Am J Physiol* 1999; **276**: L41-L50
15 **Moccia F**, Baruffi S, Spaggiari S, Coltrini D, Berra-Romani R, Signorelli S, Castelli L, Taglietti V, Tanzi F. P2y1 and P2y2 receptor-operated Ca2+ signals in primary cultures of cardiac microvascular endothelial cells. *Microvasc Res* 2001; **61**: 240-252
16 **Borisova L**, Wray S, Eisner DA, Burdyga T. How structure, Ca signals, and cellular communications underlie function in precapillary arterioles. *Circ Res* 2009; **105**: 803-810
17 **Pupo E**, Pla AF, Avanzato D, Moccia F, Cruz JE, Tanzi F, Merlino A, Mancardi D, Munaron L. Hydrogen sulfide promotes calcium signals and migration in tumor-derived endothelial cells. *Free Radic Biol Med* 2011; **51**: 1765-1773
18 **Hamdollah Zadeh MA**, Glass CA, Magnussen A, Hancox JC, Bates DO. VEGF-mediated elevated intracellular calcium and angiogenesis in human microvascular endothelial cells in vitro are inhibited by dominant negative TRPC6. *Microcirculation* 2008; **15**: 605-614
19 **Yin J**, Kuebler WM. Mechanotransduction by TRP channels: general concepts and specific role in the vasculature. *Cell Biochem Biophys* 2010; **56**: 1-18
20 **Schwarz G**, Callewaert G, Droogmans G, Nilius B. Shear stress-induced calcium transients in endothelial cells from human umbilical cord veins. *J Physiol* 1992; **458**: 527-538
21 **Shen J**, Luscinskas FW, Connolly A, Dewey CF, Gimbrone MA. Fluid shear stress modulates cytosolic free calcium in vascular endothelial cells. *Am J Physiol* 1992; **262**: C384-C390
22 **Kuebler WM**, Ying X, Bhattacharya J. Pressure-induced endothelial Ca(2+) oscillations in lung capillaries. *Am J Physiol Lung Cell Mol Physiol* 2002; **282**: L917-L923
23 **Ohata H**, Ikeuchi T, Kamada A, Yamamoto M, Momose K. Lysophosphatidic acid positively regulates the fluid flow-induced local Ca(2+) influx in bovine aortic endothelial cells. *Circ Res* 2001; **88**: 925-932
24 **Ohata H**, Yamada H, Momose K. Lysophosphatidic acid induces shear stress-dependent Ca2+ influx in mouse aortic endothelial cells in situ. *Exp Physiol* 2011; **96**: 468-475
25 **Hoyer J**, Köhler R, Distler A. Mechanosensitive Ca2+ oscillations and STOC activation in endothelial cells. *FASEB J* 1998; **12**: 359-366
26 **Ando J**, Yamamoto K. Vascular mechanobiology: endothelial cell responses to fluid shear stress. *Circ J* 2009; **73**: 1983-1992
27 **Mendoza SA**, Fang J, Gutterman DD, Wilcox DA, Bubolz AH, Li R, Suzuki M, Zhang DX. TRPV4-mediated endothelial Ca2+ influx and vasodilation in response to shear stress. *Am J Physiol Heart Circ Physiol* 2010; **298**: H466-H476
28 **Sobolewski P**, Kandel J, Klinger AL, Eckmann DM. Air

bubble contact with endothelial cells in vitro induces calcium influx and IP3-dependent release of calcium stores. *Am J Physiol Cell Physiol* 2011; **301**: C679-C686
29 **Hawkins BJ**, Solt LA, Chowdhury I, Kazi AS, Abid MR, Aird WC, May MJ, Foskett JK, Madesh M. G protein-coupled receptor Ca2+-linked mitochondrial reactive oxygen species are essential for endothelial/leukocyte adherence. *Mol Cell Biol* 2007; **27**: 7582-7593
30 **Lewalle JM**, Cataldo D, Bajou K, Lambert CA, Foidart JM. Endothelial cell intracellular Ca2+ concentration is increased upon breast tumor cell contact and mediates tumor cell transendothelial migration. *Clin Exp Metastasis* 1998; **16**: 21-29
31 **Nejime N**, Tanaka N, Yoshihara R, Kagota S, Yoshikawa N, Nakamura K, Kunitomo M, Hashimoto M, Shinozuka K. Effect of P2 receptor on the intracellular calcium increase by cancer cells in human umbilical vein endothelial cells. *Naunyn Schmiedebergs Arch Pharmacol* 2008; **377**: 429-436
32 **Wood PG**, Gillespie JI. Evidence for mitochondrial Ca(2+)-induced Ca2+ release in permeabilised endothelial cells. *Biochem Biophys Res Commun* 1998; **246**: 543-548
33 **King J**, Hamil T, Creighton J, Wu S, Bhat P, McDonald F, Stevens T. Structural and functional characteristics of lung macro- and microvascular endothelial cell phenotypes. *Microvasc Res* 2004; **67**: 139-151
34 **Uehara K**, Onoue H, Jeyakumar LH, Fleischer S, Uehara A. Localization of ryanodine receptor 3 in the sinus endothelial cells of the rat spleen. *Cell Tissue Res* 2004; **317**: 137-145
35 **Uehara K**. Localization of TRPC1 channel in the sinus endothelial cells of rat spleen. *Histochem Cell Biol* 2005; **123**: 347-356
36 **Malli R**, Frieden M, Osibow K, Zoratti C, Mayer M, Demaurex N, Graier WF. Sustained Ca2+ transfer across mitochondria is Essential for mitochondrial Ca2+ buffering, sore-operated Ca2+ entry, and Ca2+ store refilling. *J Biol Chem* 2003; **278**: 44769-44779
37 **Laude AJ**, Simpson AW. Compartmentalized signalling: Ca2+ compartments, microdomains and the many facets of Ca2+ signalling. *FEBS J* 2009; **276**: 1800-1816
38 **Berridge MJ**. The endoplasmic reticulum: a multifunctional signaling organelle. *Cell Calcium* 2002; **32**: 235-249
39 **Jousset H**, Malli R, Girardin N, Graier WF, Demaurex N, Frieden M. Evidence for a receptor-activated Ca2+ entry pathway independent from Ca2) store depletion in endothelial cells. *Cell Calcium* 2008; **43**: 83-94
40 **Malli R**, Frieden M, Osibow K, Graier WF. Mitochondria efficiently buffer subplasmalemmal Ca2+ elevation during agonist stimulation. *J Biol Chem* 2003; **278**: 10807-10815
41 **Malli R**, Frieden M, Trenker M, Graier WF. The role of mitochondria for Ca2+ refilling of the endoplasmic reticulum. *J Biol Chem* 2005; **280**: 12114-12122
42 **Osibow K**, Frank S, Malli R, Zechner R, Graier WF. Mitochondria maintain maturation and secretion of lipoprotein lipase in the endoplasmic reticulum. *Biochem J* 2006; **396**: 173-182
43 **Girardin NC**, Antigny F, Frieden M. Electrophysiological characterization of store-operated and agonist-induced Ca2+ entry pathways in endothelial cells. *Pflugers Arch* 2010; **460**: 109-120
44 **Mozhayeva MG**, Mozhayeva GN. Evidence for the existence of inositol (1,4,5)-trisphosphate- and ryanodine-sensitive pools in bovine endothelial cells. Ca2+ releases in cells with different basal level of intracellular Ca2+. *Pflugers Arch* 1996; **432**: 614-622
45 **Otun H**, Aidulis DM, Yang JM, Gillespie JI. Interactions between inositol trisphosphate and Ca2+ dependent Ca2+ release mechanisms on the endoplasmic reticulum of permeabilised bovine aortic endothelial cells. *Cell Calcium* 1996; **19**: 315-325
46 **Rusko J**, Wang X, van Breemen C. Regenerative caffeine-induced responses in native rabbit aortic endothelial cells. *Br J Pharmacol* 1995; **115**: 811-821
47 **Zhang G**, Teggatz EG, Zhang AY, Koeberl MJ, Yi F, Chen L, Li PL. Cyclic ADP ribose-mediated Ca2+ signaling in mediating endothelial nitric oxide production in bovine coronary arteries. *Am J Physiol Heart Circ Physiol* 2006; **290**: H1172-H1181
48 **Zhang AY**, Li PL. Vascular physiology of a Ca2+ mobilizing second messenger - cyclic ADP-ribose. *J Cell Mol Med* 2006; **10**: 407-422
49 **Lawrie AM**, Rizzuto R, Pozzan T, Simpson AW. A role for calcium influx in the regulation of mitochondrial calcium in endothelial cells. *J Biol Chem* 1996; **271**: 10753-10759
50 **Brailoiu GC**, Gurzu B, Gao X, Parkesh R, Aley PK, Trifa DI, Galione A, Dun NJ, Madesh M, Patel S, Churchill GC, Brailoiu E. Acidic NAADP-sensitive calcium stores in the endothelium: agonist-specific recruitment and role in regulating blood pressure. *J Biol Chem* 2010; **285**: 37133-37137
51 **Esposito B**, Gambara G, Lewis AM, Palombi F, D'Alessio A, Taylor LX, Genazzani AA, Ziparo E, Galione A, Churchill GC, Filippini A. NAADP links histamine H1 receptors to secretion of von Willebrand factor in human endothelial cells. *Blood* 2011; **117**: 4968-4977
52 **Srinivas SP**, Ong A, Goon L, Goon L, Bonanno JA. Lysosomal Ca(2+) stores in bovine corneal endothelium. *Invest Ophthalmol Vis Sci* 2002; **43**: 2341-2350
53 **Zhu MX**, Ma J, Parrington J, Galione A, Evans AM. TPCs: Endolysosomal channels for Ca2+ mobilization from acidic organelles triggered by NAADP. *FEBS Lett* 2010; **584**: 1966-1974
54 **Fiorio Pla A**, Munaron L. Calcium influx, arachidonic acid,and control of endothelial cell proliferation. *Cell Calcium* 2001; **30**: 235-244
55 **Edwards DH**, Li Y, Griffith TM. Hydrogen peroxide potentiates the EDHF phenomenon by promoting endothelial Ca2+ mobilization. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1774-1781
56 **Hu Q**, Corda S, Zweier JL, Capogrossi MC, Ziegelstein RC. Hydrogen peroxide induces intracellular calcium oscillations in human aortic endothelial cells. *Circulation* 1998; **97**: 268-275
57 **Zheng Y**, Shen X. H2O2 directly activates inositol 1,4,5-trisphosphate receptors in endothelial cells. *Redox Rep* 2005; **10**: 29-36
58 **Itagaki K**, Yun JK, Hengst JA, Yatani A, Hauser CJ, Spolarics Z, Deitch EA. Sphingosine 1-phosphate has dual functions in the regulation of endothelial cell permeability and Ca2+ metabolism. *J Pharmacol Exp Ther* 2007; **323**: 186-191
59 **Lucke S**, Levkau B. Endothelial functions of sphingosine-1-phosphate. *Cell Physiol Biochem* 2010; **26**: 87-96
60 **Mehta D**, Konstantoulaki M, Ahmmed GU, Malik AB. Sphingosine 1-phosphate-induced mobilization of intracellular Ca2+ mediates rac activation and adherens junction assembly in endothelial cells. *J Biol Chem* 2005; **280**: 17320-17328
61 **Seol GH**, Kim MY, Liang GH, Kim JA, Kim YJ, Oh S, Suh SH. Sphingosine-1-phosphate-induced intracellular Ca2+ mobilization in human endothelial cells. *Endothelium* 2005; **12**: 263-269
62 **Munaron L**. Intracellular calcium, endothelial cells and angiogenesis. *Recent Pat Anticancer Drug Discov* 2006; **1**: 105-119
63 **Rhee SG**. Regulation of phosphoinositide-specific phospholipase C. *Annu Rev Biochem* 2001; **70**: 281-312
64 **Suh PG**, Park JI, Manzoli L, Cocco L, Peak JC, Katan M, Fukami K, Kataoka T, Yun S, Ryu SH. Multiple roles of phosphoinositide-specific phospholipase C isozymes. *BMB Rep* 2008; **41**: 415-434
65 **Zoratti C**, Kipmen-Korgun D, Osibow K, Malli R, Graier WF. Anandamide initiates Ca(2+) signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. *Br J Pharmacol* 2003; **140**: 1351-1362
66 **Gilchrist A**, Vanhauwe JF, Li A, Thomas TO, Voyno-Yasenetskaya T, Hamm HE. G alpha minigenes expressing

C-terminal peptides serve as specific inhibitors of thrombin-mediated endothelial activation. *J Biol Chem* 2001; **276**: 25672-25679

67 **Hirano K**, Nomoto N, Hirano M, Momota F, Hanada A, Kanaide H. Distinct Ca2+ requirement for NO production between proteinase-activated receptor 1 and 4 (PAR1 and PAR4) in vascular endothelial cells. *J Pharmacol Exp Ther* 2007; **322**: 668-677

68 **Vanhauwe JF**, Thomas TO, Minshall RD, Tiruppathi C, Li A, Gilchrist A, Yoon EJ, Malik AB, Hamm HE. Thrombin receptors activate G(o) proteins in endothelial cells to regulate intracellular calcium and cell shape changes. *J Biol Chem* 2002; **277**: 34143-34149

69 **Liao JK**, Homcy CJ. The G proteins of the G alpha i and G alpha q family couple the bradykinin receptor to the release of endothelium-derived relaxing factor. *J Clin Invest* 1993; **92**: 2168-2172

70 **Lambert TL**, Kent RS, Whorton AR. Bradykinin stimulation of inositol polyphosphate production in porcine aortic endothelial cells. *J Biol Chem* 1986; **261**: 15288-15293

71 **Wilde JI**, Watson SP. Regulation of phospholipase C gamma isoforms in haematopoietic cells: why one, not the other? *Cell Signal* 2001; **13**: 691-701

72 **Moccia F**, Berra-Romani R, Tritto S, Signorelli S, Taglietti V, Tanzi F. Epidermal growth factor induces intracellular Ca2+ oscillations in microvascular endothelial cells. *J Cell Physiol* 2003; **194**: 139-150

73 **McLaughlin AP**, De Vries GW. Role of PLCgamma and Ca(2+) in VEGF- and FGF-induced choroidal endothelial cell proliferation. *Am J Physiol Cell Physiol* 2001; **281**: C1448-C1456

74 **Meyer RD**, Latz C, Rahimi N. Recruitment and activation of phospholipase Cgamma1 by vascular endothelial growth factor receptor-2 are required for tubulogenesis and differentiation of endothelial cells. *J Biol Chem* 2003; **278**: 16347-16355

75 **Xiong Y**, Huo Y, Chen C, Zeng H, Lu X, Wei C, Ruan C, Zhang X, Hu Z, Shibuya M, Luo J. Vascular endothelial growth factor (VEGF) receptor-2 tyrosine 1175 signaling controls VEGF-induced von Willebrand factor release from endothelial cells via phospholipase C-gamma 1- and protein kinase A-dependent pathways. *J Biol Chem* 2009; **284**: 23217-23224

76 **Ridefelt P**, Yokote K, Claesson-Welsh L, Siegbahn A. PDGF-BB triggered cytoplasmic calcium responses in cells with endogenous or stably transfected PDGF beta-receptors. *Growth Factors* 1995; **12**: 191-201

77 **Maffucci T**, Raimondi C, Abu-Hayyeh S, Dominguez V, Sala G, Zachary I, Falasca M. A phosphoinositide 3-kinase/phospholipase Cgamma1 pathway regulates fibroblast growth factor-induced capillary tube formation. *PLoS One* 2009; **4**: e8285

78 **Fleming I**, Busse R. Tyrosine phosphorylation and bradykinin-induced signaling in endothelial cells. *Am J Cardiol* 1997; **80**: 102A-109A

79 **Venema VJ**, Ju H, Sun J, Eaton DC, Marrero MB, Venema RC. Bradykinin stimulates the tyrosine phosphorylation and bradykinin B2 receptor association of phospholipase C gamma 1 in vascular endothelial cells. *Biochem Biophys Res Commun* 1998; **246**: 70-75

80 **Zeng H**, Zhao D, Yang S, Datta K, Mukhopadhyay D. Heterotrimeric G alpha q/G alpha 11 proteins function upstream of vascular endothelial growth factor (VEGF) receptor-2 (KDR) phosphorylation in vascular permeability factor/VEGF signaling. *J Biol Chem* 2003; **278**: 20738-20745

81 **Bhattacharya R**, Kwon J, Li X, Wang E, Patra S, Bida JP, Bajzer Z, Claesson-Welsh L, Mukhopadhyay D. Distinct role of PLCbeta3 in VEGF-mediated directional migration and vascular sprouting. *J Cell Sci* 2009; **122**: 1025-1034

82 **Mi LY**, Ettenson DS, Edelman ER. Phospholipase C-delta extends intercellular signalling range and responses to injury-released growth factors in non-excitable cells. *Cell Prolif* 2008; **41**: 671-690

83 **Li M**, Edamatsu H, Kitazawa R, Kitazawa S, Kataoka T. Phospholipase Cepsilon promotes intestinal tumorigenesis of Apc(Min/+) mice through augmentation of inflammation and angiogenesis. *Carcinogenesis* 2009; **30**: 1424-1432

84 **Mayati A**, Levoin N, Paris H, N'Diaye M, Courtois A, Uriac P, Lagadic-Gossmann D, Fardel O, Le Ferrec E. Induction of intracellular calcium concentration by environmental benzo(a)pyrene involves a β2-adrenergic receptor/adenylyl cyclase/Epac-1/inositol 1,4,5-trisphosphate pathway in endothelial cells. *J Biol Chem* 2012; **287**: 4041-4052

85 **Jaffe EA**, Grulich J, Weksler BB, Hampel G, Watanabe K. Correlation between thrombin-induced prostacyclin production and inositol trisphosphate and cytosolic free calcium levels in cultured human endothelial cells. *J Biol Chem* 1987; **262**: 8557-8565

86 **Moscat J**, Moreno F, Herrero C, López C, García-Barreno P. Endothelial cell growth factor and ionophore A23187 stimulation of production of inositol phosphates in porcine aorta endothelial cells. *Proc Natl Acad Sci USA* 1988; **85**: 659-663

87 **Pollock WK**, Wreggett KA, Irvine RF. Inositol phosphate production and Ca2+ mobilization in human umbilical-vein endothelial cells stimulated by thrombin and histamine. *Biochem J* 1988; **256**: 371-376

88 **Brock TA**, Dennis PA, Griendling KK, Diehl TS, Davies PF. GTP gamma S loading of endothelial cells stimulates phospholipase C and uncouples ATP receptors. *Am J Physiol* 1988; **255**: C667-C673

89 **Pirotton S**, Verjans B, Boeynaems JM, Erneux C. Metabolism of inositol phosphates in ATP-stimulated vascular endothelial cells. *Biochem J* 1991; **277** (Pt 1): 103-110

90 **Lin AY**, Rui YC. Platelet-activating factor induced calcium mobilization and phosphoinositide metabolism in cultured bovine cerebral microvascular endothelial cells. *Biochim Biophys Acta* 1994; **1224**: 323-328

91 **Faehling M**, Kroll J, Föhr KJ, Fellbrich G, Mayr U, Trischler G, Waltenberger J. Essential role of calcium in vascular endothelial growth factor A-induced signaling: mechanism of the antiangiogenic effect of carboxyamidotriazole. *FASEB J* 2002; **16**: 1805-1807

92 **Buxton IL**, Anzinger JJ. Agonist-specific regulation of inositol phosphate metabolism in cardiac endothelial cells. *Proc West Pharmacol Soc* 2008; **51**: 23-26

93 **Graier WF**, Hoebel BG, Paltauf-Doburzynska J, Kostner GM. Effects of superoxide anions on endothelial Ca2+ signaling pathways. *Arterioscler Thromb Vasc Biol* 1998; **18**: 1470-1479

94 **Sun L**, Yau HY, Lau OC, Huang Y, Yao X. Effect of hydrogen peroxide and superoxide anions on cytosolic Ca2+: comparison of endothelial cells from large-sized and small-sized arteries. *PLoS One* 2011; **6**: e25432

95 **Rosales OR**, Sumpio BE. Changes in cyclic strain increase inositol trisphosphate and diacylglycerol in endothelial cells. *Am J Physiol* 1992; **262**: C956-C962

96 **Prasad AR**, Logan SA, Nerem RM, Schwartz CJ, Sprague EA. Flow-related responses of intracellular inositol phosphate levels in cultured aortic endothelial cells. *Circ Res* 1993; **72**: 827-836

97 **Patel A**, Sharif-Naeini R, Folgering JR, Bichet D, Duprat F, Honoré E. Canonical TRP channels and mechanotransduction: from physiology to disease states. *Pflugers Arch* 2010; **460**: 571-581

98 **Chachisvilis M**, Zhang YL, Frangos JA. G protein-coupled receptors sense fluid shear stress in endothelial cells. *Proc Natl Acad Sci USA* 2006; **103**: 15463-15468

99 **Carter TD**, Ogden D. Kinetics of Ca2+ release by InsP3 in pig single aortic endothelial cells: evidence for an inhibitory role of cytosolic Ca2+ in regulating hormonally evoked Ca2+ spikes. *J Physiol* 1997; **504** (Pt 1): 17-33

100 **Braet K**, Mabilde C, Cabooter L, Rapp G, Leybaert L. Electroporation loading and photoactivation of caged InsP3: tools

to investigate the relation between cellular ATP release in response to intracellular InsP3 elevation. *J Neurosci Methods* 2004; **132**: 81-89

101 **Freay A**, Johns A, Adams DJ, Ryan US, Van Breemen C. Bradykinin and inositol 1,4,5-trisphosphate-stimulated calcium release from intracellular stores in cultured bovine endothelial cells. *Pflugers Arch* 1989; **414**: 377-384

102 **Foskett JK**, White C, Cheung KH, Mak DO. Inositol trisphosphate receptor Ca2+ release channels. *Physiol Rev* 2007; **87**: 593-658

103 **Bourguignon LY**, Iida N, Sobrin L, Bourguignon GJ. Identification of an IP3 receptor in endothelial cells. *J Cell Physiol* 1994; **159**: 29-34

104 **Laflamme K**, Domingue O, Guillemette BI, Guillemette G. Immunohistochemical localization of type 2 inositol 1,4,5-trisphosphate receptor to the nucleus of different mammalian cells. *J Cell Biochem* 2002; **85**: 219-228

105 **Gifford SM**, Yi FX, Bird IM. Pregnancy-enhanced Ca2+ responses to ATP in uterine artery endothelial cells is due to greater capacitative Ca2+ entry rather than altered receptor coupling. *J Endocrinol* 2006; **190**: 373-384

106 **Mountian I**, Manolopoulos VG, De Smedt H, Parys JB, Missiaen L, Wuytack F. Expression patterns of sarco/endoplasmic reticulum Ca(2+)-ATPase and inositol 1,4,5-trisphosphate receptor isoforms in vascular endothelial cells. *Cell Calcium* 1999; **25**: 371-380

107 **Grayson TH**, Haddock RE, Murray TP, Wojcikiewicz RJ, Hill CE. Inositol 1,4,5-trisphosphate receptor subtypes are differentially distributed between smooth muscle and endothelial layers of rat arteries. *Cell Calcium* 2004; **36**: 447-458

108 **Isakson BE**. Localized expression of an Ins(1,4,5)P3 receptor at the myoendothelial junction selectively regulates heterocellular Ca2+ communication. *J Cell Sci* 2008; **121**: 3664-3673

109 **Béliveau E**, Guillemette G. Microfilament and microtubule assembly is required for the propagation of inositol trisphosphate receptor-induced Ca2+ waves in bovine aortic endothelial cells. *J Cell Biochem* 2009; **106**: 344-352

110 **Fujimoto T**, Nakade S, Miyawaki A, Mikoshiba K, Ogawa K. Localization of inositol 1,4,5-trisphosphate receptor-like protein in plasmalemmal caveolae. *J Cell Biol* 1992; **119**: 1507-1513

111 **Vaca L**, Kunze DL. IP3-activated Ca2+ channels in the plasma membrane of cultured vascular endothelial cells. *Am J Physiol* 1995; **269**: C733-C738

112 **Mountian F**, Jonas JC, Humbert De Smedt F, Parys JB. Expression of Ca(2+) Transport Genes in Platelets and Endothelial Cells in Hypertension. *Hypertension* 2001; **37**: 135-141

113 **Haorah J**, Knipe B, Gorantla S, Zheng J, Persidsky Y. Alcohol-induced blood-brain barrier dysfunction is mediated via inositol 1,4,5-triphosphate receptor (IP3R)-gated intracellular calcium release. *J Neurochem* 2007; **100**: 324-336

114 **Taylor CW**, Tovey SC. IP(3) receptors: toward understanding their activation. *Cold Spring Harb Perspect Biol* 2010; **2**: a004010

115 **Adams DJ**, Barakeh J, Laskey R, Van Breemen C. Ion channels and regulation of intracellular calcium in vascular endothelial cells. *FASEB J* 1989; **3**: 2389-2400

116 **Aromolaran AA**, Blatter LA. Modulation of intracellular Ca2+ release and capacitative Ca2+ entry by CaMKII inhibitors in bovine vascular endothelial cells. *Am J Physiol Cell Physiol* 2005; **289**: C1426-C1436

117 **Aromolaran AS**, Zima AV, Blatter LA. Role of glycolytically generated ATP for CaMKII-mediated regulation of intracellular Ca2+ signaling in bovine vascular endothelial cells. *Am J Physiol Cell Physiol* 2007; **293**: C106-C118

118 **Béliveau È**, Lapointe F, Guillemette G. The activation state of the inositol 1,4,5-trisphosphate receptor regulates the velocity of intracellular Ca2+ waves in bovine aortic endothelial cells. *J Cell Biochem* 2011; **112**: 3722-3731

119 **Bourguignon LY**, Singleton PA, Diedrich F. Hyaluronan-CD44 interaction with Rac1-dependent protein kinase N-gamma promotes phospholipase Cgamma1 activation, Ca(2+) signaling, and cortactin-cytoskeleton function leading to keratinocyte adhesion and differentiation. *J Biol Chem* 2004; **279**: 29654-29669

120 **Pan L**, Wu X, Zhao D, Hessari NM, Lee I, Zhang X, Xu J. Sulfhydryl modification induces calcium entry through IP_3-sensitive store-operated pathway in activation-dependent human neutrophils. *PLoS One* 2011; **6**: e25262

121 **Volk T**, Mäding K, Hensel M, Kox WJ. Nitric oxide induces transient Ca2+ changes in endothelial cells independent of cGMP. *J Cell Physiol* 1997; **172**: 296-305

122 **Berra-Romani R**, Raqeeb A, Avelino-Cruz JE, Moccia F, Oldani A, Speroni F, Taglietti V, Tanzi F. Ca2+ signaling in injured in situ endothelium of rat aorta. *Cell Calcium* 2008; **44**: 298-309

123 **Moccia F**, Bertoni G, Pla AF, Dragoni S, Pupo E, Merlino A, Mancardi D, Munaron L, Tanzi F. Hydrogen sulfide regulates intracellular Ca2+ concentration in endothelial cells from excised rat aorta. *Curr Pharm Biotechnol* 2011; **12**: 1416-1426

124 **Lock JT**, Sinkins WG, Schilling WP. Effect of protein S-glutathionylation on Ca2+ homeostasis in cultured aortic endothelial cells. *Am J Physiol Heart Circ Physiol* 2011; **300**: H493-H506

125 **Kushnir A**, Marks AR. The ryanodine receptor in cardiac physiology and disease. *Adv Pharmacol* 2010; **59**: 1-30

126 **Fill M**, Copello JA. Ryanodine receptor calcium release channels. *Physiol Rev* 2002; **82**: 893-922

127 **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. *Cardiovasc Res* 2001; **51**: 160-168

128 **West DJ**, Williams AJ. Pharmacological regulators of intracellular calcium release channels. *Curr Pharm Des* 2007; **13**: 2428-2442

129 **Fransen P**, Katnik C, Adams DJ. ACh- and caffeine-induced Ca2+ mobilization and current activation in rabbit arterial endothelial cells. *Am J Physiol* 1998; **275**: H1748-H1758

130 **Graier WF**, Simecek S, Bowles DK, Sturek M. Heterogeneity of caffeine- and bradykinin-sensitive Ca2+ stores in vascular endothelial cells. *Biochem J* 1994; **300** (Pt 3): 637-641

131 **Wang X**, Lau F, Li L, Yoshikawa A, van Breemen C. Acetylcholine-sensitive intracellular Ca2+ store in fresh endothelial cells and evidence for ryanodine receptors. *Circ Res* 1995; **77**: 37-42

132 **Ziegelstein RC**, Spurgeon HA, Pili R, Passaniti A, Cheng L, Corda S, Lakatta EG, Capogrossi MC. A functional ryanodine-sensitive intracellular Ca2+ store is present in vascular endothelial cells. *Circ Res* 1994; **74**: 151-156

133 **Paltauf-Doburzynska J**, Posch K, Paltauf G, Graier WF. Stealth ryanodine-sensitive Ca2+ release contributes to activity of capacitative Ca2+ entry and nitric oxide synthase in bovine endothelial cells. *J Physiol* 1998; **513** (Pt 2): 369-379

134 **Paltauf-Doburzynska J**, Frieden M, Spitaler M, Graier WF. Histamine-induced Ca2+ oscillations in a human endothelial cell line depend on transmembrane ion flux, ryanodine receptors and endoplasmic reticulum Ca2+-ATPase. *J Physiol* 2000; **524 Pt 3**: 701-713

135 **Ullmer C**, Boddeke HG, Schmuck K, Lübbert H. 5-HT2B receptor-mediated calcium release from ryanodine-sensitive intracellular stores in human pulmonary artery endothelial cells. *Br J Pharmacol* 1996; **117**: 1081-1088

136 **Mauban JR**, Wilkinson K, Schach C, Yuan JX. Histamine-mediated increases in cytosolic [Ca2+] involve different mechanisms in human pulmonary artery smooth muscle and endothelial cells. *Am J Physiol Cell Physiol* 2006; **290**: C325-C336

137 **Sacks RS**, Firth AL, Remillard CV, Agange N, Yau J, Ko EA,

Yuan JX. Thrombin-mediated increases in cytosolic [Ca2+] involve different mechanisms in human pulmonary artery smooth muscle and endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2008; **295**: L1048-L1055
138 **Graier WF**, Paltauf-Doburzynska J, Posch K. Stealth Ca2-induced Ca2 release in the subplasmalemmal area contributes to activation of nitric oxide synthase upon autacoids in human endothelial cells. *Circulation* 1998; **98**: 667-667
139 **Liang W**, Buluc M, van Breemen C, Wang X. Vectorial Ca2+ release via ryanodine receptors contributes to Ca2+ extrusion from freshly isolated rabbit aortic endothelial cells. *Cell Calcium* 2004; **36**: 431-443
140 **Frieden M**, Graier WF. Subplasmalemmal ryanodine-sensitive Ca2+ release contributes to Ca2+-dependent K+ channel activation in a human umbilical vein endothelial cell line. *J Physiol* 2000; **524** Pt 3: 715-724
141 **Frieden M**, Malli R, Samardzija M, Demaurex N, Graier WF. Subplasmalemmal endoplasmic reticulum controls K(Ca) channel activity upon stimulation with a moderate histamine concentration in a human umbilical vein endothelial cell line. *J Physiol* 2002; **540**: 73-84
142 **Fliegert R**, Gasser A, Guse AH. Regulation of calcium signalling by adenine-based second messengers. *Biochem Soc Trans* 2007; **35**: 109-114
143 **Aley PK**, Porter KE, Boyle JP, Kemp PJ, Peers C. Hypoxic modulation of Ca2+ signaling in human venous endothelial cells. Multiple roles for reactive oxygen species. *J Biol Chem* 2005; **280**: 13349-13354
144 **Hecquet CM**, Malik AB. Role of H(2)O(2)-activated TRPM2 calcium channel in oxidant-induced endothelial injury. *Thromb Haemost* 2009; **101**: 619-625
145 **Long C**, Cook LG, Hamilton SL, Wu GY, Mitchell BM. FK506 binding protein 12/12.6 depletion increases endothelial nitric oxide synthase threonine 495 phosphorylation and blood pressure. *Hypertension* 2007; **49**: 569-576
146 **Long C**, Cook LG, Wu GY, Mitchell BM. Removal of FKBP12/12.6 from endothelial ryanodine receptors leads to an intracellular calcium leak and endothelial dysfunction. *Arterioscler Thromb Vasc Biol* 2007; **27**: 1580-1586
147 **Patel S**, Marchant JS, Brailoiu E. Two-pore channels: Regulation by NAADP and customized roles in triggering calcium signals. *Cell Calcium* 2010; **47**: 480-490
148 **Dietrich A**, Kalwa H, Gudermann T. TRPC channels in vascular cell function. *Thromb Haemost* 2010; **103**: 262-270
149 **Earley S**, Brayden JE. Transient receptor potential channels and vascular function. *Clin Sci* (*Lond*) 2010; **119**: 19-36
150 **Kwan HY**, Huang Y, Yao X. TRP channels in endothelial function and dysfunction. *Biochim Biophys Acta* 2007; **1772**: 907-914
151 **Beech DJ**. Harmony and discord in endothelial calcium entry. *Circ Res* 2009; **104**: e22-e23
152 **Trebak M**. STIM1/Orai1, ICRAC, and endothelial SOC. *Circ Res* 2009; **104**: e56-e57
153 **Adams DJ**, Hill MA. Potassium channels and membrane potential in the modulation of intracellular calcium in vascular endothelial cells. *J Cardiovasc Electrophysiol* 2004; **15**: 598-610
154 **Bossu JL**, Feltz A, Rodeau JL, Tanzi F. Voltage-dependent transient calcium currents in freshly dissociated capillary endothelial cells. *FEBS Lett* 1989; **255**: 377-380
155 **Bossu JL**, Elhamdani A, Feltz A. Voltage-dependent calcium entry in confluent bovine capillary endothelial cells. *FEBS Lett* 1992; **299**: 239-242
156 **Vinet R**, Vargas FF. L- and T-type voltage-gated Ca2+ currents in adrenal medulla endothelial cells. *Am J Physiol* 1999; **276**: H1313-H1322
157 **Wei Z**, Manevich Y, Al-Mehdi AB, Chatterjee S, Fisher AB. Ca2+ flux through voltage-gated channels with flow cessation in pulmonary microvascular endothelial cells. *Microcirculation* 2004; **11**: 517-526
158 **Zhou C**, Wu S. T-type calcium channels in pulmonary vascular endothelium. *Microcirculation* 2006; **13**: 645-656
159 **Townsley MI**, King JA, Alvarez DF. Ca2+ channels and pulmonary endothelial permeability: insights from study of intact lung and chronic pulmonary hypertension. *Microcirculation* 2006; **13**: 725-739
160 **Delpiano MA**, Altura BM. Modulatory effect of extracellular Mg2+ ions on K+ and Ca2+ currents of capillary endothelial cells from rat brain. *FEBS Lett* 1996; **394**: 335-339
161 **Yakubu MA**, Leffler CW. L-type voltage-dependent Ca2+ channels in cerebral microvascular endothelial cells and ET-1 biosynthesis. *Am J Physiol Cell Physiol* 2002; **283**: C1687-C1695
162 **Wu S**, Haynes J, Taylor JT, Obiako BO, Stubbs JR, Li M, Stevens T. Cav3.1 (alpha1G) T-type Ca2+ channels mediate vaso-occlusion of sickled erythrocytes in lung microcirculation. *Circ Res* 2003; **93**: 346-353
163 **Wu S**, Jian MY, Xu YC, Zhou C, Al-Mehdi AB, Liedtke W, Shin HS, Townsley MI. Ca2+ entry via alpha1G and TRPV4 channels differentially regulates surface expression of P-selectin and barrier integrity in pulmonary capillary endothelium. *Am J Physiol Lung Cell Mol Physiol* 2009; **297**: L650-L657
164 **Zhou C**, Chen H, Lu F, Sellak H, Daigle JA, Alexeyev MF, Xi Y, Ju J, van Mourik JA, Wu S. Cav3.1 (alpha1G) controls von Willebrand factor secretion in rat pulmonary microvascular endothelial cells. *Am J Physiol Lung Cell Mol Physiol* 2007; **292**: L833-L844
165 **Zhou C**, Chen H, King JA, Sellak H, Kuebler WM, Yin J, Townsley MI, Shin HS, Wu S. Alpha1G T-type calcium channel selectively regulates P-selectin surface expression in pulmonary capillary endothelium. *Am J Physiol Lung Cell Mol Physiol* 2010; **299**: L86-L97
166 **Vinet R**, Cortés M, Luxoro M, Delpiano MA. Nicotine-evoked cytosolic Ca(2+) increase and cell depolarization in capillary endothelial cells of the bovine adrenal medulla. *Biol Res* 2009; **42**: 111-119
167 **Bkaily G**, d'Orléans-Juste P, Naik R, Pérodin J, Stankova J, Abdulnour E, Rola-Pleszczynski M. PAF activation of a voltage-gated R-type Ca2+ channel in human and canine aortic endothelial cells. *Br J Pharmacol* 1993; **110**: 519-520
168 **Wang D**, Hirase T, Inoue T, Node K. Atorvastatin inhibits angiotensin II-induced T-type Ca2+ channel expression in endothelial cells. *Biochem Biophys Res Commun* 2006; **347**: 394-400
169 **Cooke JP**, Ghebremariam YT. Endothelial nicotinic acetylcholine receptors and angiogenesis. *Trends Cardiovasc Med* 2008; **18**: 247-253
170 **Gahring LC**, Rogers SW. Neuronal nicotinic acetylcholine receptor expression and function on nonneuronal cells. *AAPS J* 2005; **7**: E885-E894
171 **Cooke JP**. NO and angiogenesis. *Atheroscler Suppl* 2003; **4**: 53-60
172 **Fucile S**. Ca2+ permeability of nicotinic acetylcholine receptors. *Cell Calcium* 2004; **35**: 1-8
173 **Arias HR**, Richards VE, Ng D, Ghafoori ME, Le V, Mousa SA. Role of non-neuronal nicotinic acetylcholine receptors in angiogenesis. *Int J Biochem Cell Biol* 2009; **41**: 1441-1451
174 **Moccia F**, Frost C, Berra-Romani R, Tanzi F, Adams DJ. Expression and function of neuronal nicotinic ACh receptors in rat microvascular endothelial cells. *Am J Physiol Heart Circ Physiol* 2004; **286**: H486-H491
175 **Li XW**, Wang H. Non-neuronal nicotinic alpha 7 receptor, a new endothelial target for revascularization. *Life Sci* 2006; **78**: 1863-1870
176 **Roberts JA**, Vial C, Digby HR, Agboh KC, Wen H, Atterbury-Thomas A, Evans RJ. Molecular properties of P2X receptors. *Pflugers Arch* 2006; **452**: 486-500
177 **Egan TM**, Samways DS, Li Z. Biophysics of P2X receptors. *Pflugers Arch* 2006; **452**: 501-512
178 **Pulvirenti TJ**, Yin JL, Chaufour X, McLachlan C, Hambly BD, Bennett MR, Barden JA. P2X (purinergic) receptor redistribution in rabbit aorta following injury to endothelial cells

and cholesterol feeding. *J Neurocytol* 2000; **29**: 623-631

179 **Ray FR**, Huang W, Slater M, Barden JA. Purinergic receptor distribution in endothelial cells in blood vessels: a basis for selection of coronary artery grafts. *Atherosclerosis* 2002; **162**: 55-61

180 **Ramirez AN**, Kunze DL. P2X purinergic receptor channel expression and function in bovine aortic endothelium. *Am J Physiol Heart Circ Physiol* 2002; **282**: H2106-H2116

181 **Yamamoto K**, Korenaga R, Kamiya A, Ando J. Fluid shear stress activates Ca(2+) influx into human endothelial cells via P2X4 purinoceptors. *Circ Res* 2000; **87**: 385-391

182 **Yamamoto K**, Korenaga R, Kamiya A, Qi Z, Sokabe M, Ando J. P2X(4) receptors mediate ATP-induced calcium influx in human vascular endothelial cells. *Am J Physiol Heart Circ Physiol* 2000; **279**: H285-H292

183 **Bodin P**, Burnstock G. Purinergic signalling: ATP release. *Neurochem Res* 2001; **26**: 959-969

184 **Reigada D**, Mitchell CH. Release of ATP from retinal pigment epithelial cells involves both CFTR and vesicular transport. *Am J Physiol Cell Physiol* 2005; **288**: C132-C140

185 **Yamamoto K**, Shimizu N, Obi S, Kumagaya S, Taketani Y, Kamiya A, Ando J. Involvement of cell surface ATP synthase in flow-induced ATP release by vascular endothelial cells. *Am J Physiol Heart Circ Physiol* 2007; **293**: H1646-H1653

186 **Yoshida T**, Inoue R, Morii T, Takahashi N, Yamamoto S, Hara Y, Tominaga M, Shimizu S, Sato Y, Mori Y. Nitric oxide activates TRP channels by cysteine S-nitrosylation. *Nat Chem Biol* 2006; **2**: 596-607

187 **Yamamoto K**, Sokabe T, Ohura N, Nakatsuka H, Kamiya A, Ando J. Endogenously released ATP mediates shear stress-induced Ca2+ influx into pulmonary artery endothelial cells. *Am J Physiol Heart Circ Physiol* 2003; **285**: H793-H803

188 **Bradley J**, Reisert J, Frings S. Regulation of cyclic nucleotide-gated channels. *Curr Opin Neurobiol* 2005; **15**: 343-349

189 **Kaupp UB**, Seifert R. Cyclic nucleotide-gated ion channels. *Physiol Rev* 2002; **82**: 769-824

190 **Cheng KT**, Chan FL, Huang Y, Chan WY, Yao X. Expression of olfactory-type cyclic nucleotide-gated channel (CNGA2) in vascular tissues. *Histochem Cell Biol* 2003; **120**: 475-481

191 **Yao X**, Leung PS, Kwan HY, Wong TP, Fong MW. Rod-type cyclic nucleotide-gated cation channel is expressed in vascular endothelium and vascular smooth muscle cells. *Cardiovasc Res* 1999; **41**: 282-290

192 **Kwan HY**, Huang Y, Yao XQ, Leung FP. Role of cyclic nucleotides in the control of cytosolic Ca levels in vascular endothelial cells. *Clin Exp Pharmacol Physiol* 2009; Epub ahead of print

193 **Maurice DH**. Subcellular signaling in the endothelium: cyclic nucleotides take their place. *Curr Opin Pharmacol* 2011; **11**: 656-664

194 **Cheng KT**, Leung YK, Shen B, Kwok YC, Wong CO, Kwan HY, Man YB, Ma X, Huang Y, Yao X. CNGA2 channels mediate adenosine-induced Ca2+ influx in vascular endothelial cells. *Arterioscler Thromb Vasc Biol* 2008; **28**: 913-918

195 **Kwan HY**, Cheng KT, Ma Y, Huang Y, Tang NL, Yu S, Yao X. CNGA2 contributes to ATP-induced noncapacitative Ca2+ influx in vascular endothelial cells. *J Vasc Res* 2010; **47**: 148-156

196 **Yamamizu K**, Yamashita JK. Roles of cyclic adenosine monophosphate signaling in endothelial cell differentiation and arterial-venous specification during vascular development. *Circ J* 2011; **75**: 253-260

197 **Wu S**, Moore TM, Brough GH, Whitt SR, Chinkers M, Li M, Stevens T. Cyclic nucleotide-gated channels mediate membrane depolarization following activation of store-operated calcium entry in endothelial cells. *J Biol Chem* 2000; **275**: 18887-18896

198 **Shen B**, Cheng KT, Leung YK, Kwok YC, Kwan HY, Wong CO, Chen ZY, Huang Y, Yao X. Epinephrine-induced Ca2+ influx in vascular endothelial cells is mediated by CNGA2 channels. *J Mol Cell Cardiol* 2008; **45**: 437-445

199 **Zhang J**, Xia SL, Block ER, Patel JM. NO upregulation of a cyclic nucleotide-gated channel contributes to calcium elevation in endothelial cells. *Am J Physiol Cell Physiol* 2002; **283**: C1080-C1089

200 **Gees M**, Colsoul B, Nilius B. The role of transient receptor potential cation channels in Ca2+ signaling. *Cold Spring Harb Perspect Biol* 2010; **2**: a003962

201 **Ramsey IS**, Delling M, Clapham DE. An introduction to TRP channels. *Annu Rev Physiol* 2006; **68**: 619-647

202 **Owsianik G**, Talavera K, Voets T, Nilius B. Permeation and selectivity of TRP channels. *Annu Rev Physiol* 2006; **68**: 685-717

203 **Cioffi DL**, Lowe K, Alvarez DF, Barry C, Stevens T. TRPing on the lung endothelium: calcium channels that regulate barrier function. *Antioxid Redox Signal* 2009; **11**: 765-776

204 **Vandenbroucke E**, Mehta D, Minshall R, Malik AB. Regulation of endothelial junctional permeability. *Ann N Y Acad Sci* 2008; **1123**: 134-145

205 **Abdullaev IF**, Bisaillon JM, Potier M, Gonzalez JC, Motiani RK, Trebak M. Stim1 and Orai1 mediate CRAC currents and store-operated calcium entry important for endothelial cell proliferation. *Circ Res* 2008; **103**: 1289-1299

206 **Li J**, Cubbon RM, Wilson LA, Amer MS, McKeown L, Hou B, Majeed Y, Tumova S, Seymour VA, Taylor H, Stacey M, O' Regan D, Foster R, Porter KE, Kearney MT, Beech DJ. Orai1 and CRAC channel dependence of VEGF-activated Ca2+ entry and endothelial tube formation. *Circ Res* 2011; **108**: 1190-1198

207 **Antoniotti S**, Lovisolo D, Fiorio Pla A, Munaron L. Expression and functional role of bTRPC1 channels in native endothelial cells. *FEBS Lett* 2002; **510**: 189-195

208 **Antoniotti S**, Fiorio Pla A, Barral S, Scalabrino O, Munaron L, Lovisolo D. Interaction between TRPC channel subunits in endothelial cells. *J Recept Signal Transduct Res* 2006; **26**: 225-240

209 **Bishara NB**, Ding H. Glucose enhances expression of TRPC1 and calcium entry in endothelial cells. *Am J Physiol Heart Circ Physiol* 2010; **298**: H171-H178

210 **Mehta D**, Ahmmed GU, Paria BC, Holinstat M, Voyno-Yasenetskaya T, Tiruppathi C, Minshall RD, Malik AB. RhoA interaction with inositol 1,4,5-trisphosphate receptor and transient receptor potential channel-1 regulates Ca2+ entry. Role in signaling increased endothelial permeability. *J Biol Chem* 2003; **278**: 33492-33500

211 **Singh I**, Knezevic N, Ahmmed GU, Kini V, Malik AB, Mehta D. Galphaq-TRPC6-mediated Ca2+ entry induces RhoA activation and resultant endothelial cell shape change in response to thrombin. *J Biol Chem* 2007; **282**: 7833-7843

212 **Ahmmed GU**, Mehta D, Vogel S, Holinstat M, Paria BC, Tiruppathi C, Malik AB. Protein kinase Calpha phosphorylates the TRPC1 channel and regulates store-operated Ca2+ entry in endothelial cells. *J Biol Chem* 2004; **279**: 20941-20949

213 **Sundivakkam PC**, Kwiatek AM, Sharma TT, Minshall RD, Malik AB, Tiruppathi C. Caveolin-1 scaffold domain interacts with TRPC1 and IP3R3 to regulate Ca2+ store release-induced Ca2+ entry in endothelial cells. *Am J Physiol Cell Physiol* 2009; **296**: C403-C413

214 **Ye S**, Tan L, Ma J, Shi Q, Li J. Polyunsaturated docosahexaenoic acid suppresses oxidative stress induced endothelial cell calcium influx by altering lipid composition in membrane caveolar rafts. *Prostaglandins Leukot Essent Fatty Acids* 2010; **83**: 37-43

215 **Jho D**, Mehta D, Ahmmed G, Gao XP, Tiruppathi C, Broman M, Malik AB. Angiopoietin-1 opposes VEGF-induced increase in endothelial permeability by inhibiting TRPC1-dependent Ca2 influx. *Circ Res* 2005; **96**: 1282-1290

216 **Kusaba T**, Okigaki M, Matui A, Murakami M, Ishikawa K, Kimura T, Sonomura K, Adachi Y, Shibuya M, Shirayama T, Tanda S, Hatta T, Sasaki S, Mori Y, Matsubara H. Klotho is

associated with VEGF receptor-2 and the transient receptor potential canonical-1 Ca2+ channel to maintain endothelial integrity. *Proc Natl Acad Sci USA* 2010; **107**: 19308-19313
217 **Cioffi DL**, Barry C, Stevens T. Store-operated calcium entry channels in pulmonary endothelium: the emerging story of TRPCS and Orai1. *Adv Exp Med Biol* 2010; **661**: 137-154
218 **Sundivakkam PC**, Freichel M, Singh V, Yuan JP, Vogel SM, Flockerzi V, Malik AB, Tiruppathi C. The Ca(2+) sensor stromal interaction molecule 1 (STIM1) is necessary and sufficient for the store-operated Ca(2+) entry function of transient receptor potential canonical (TRPC) 1 and 4 channels in endothelial cells. *Mol Pharmacol* 2012; **81**: 510-526
219 **Bair AM**, Thippegowda PB, Freichel M, Cheng N, Ye RD, Vogel SM, Yu Y, Flockerzi V, Malik AB, Tiruppathi C. Ca2+ entry via TRPC channels is necessary for thrombin-induced NF-kappaB activation in endothelial cells through AMP-activated protein kinase and protein kinase Cdelta. *J Biol Chem* 2009; **284**: 563-574
220 **Thippegowda PB**, Singh V, Sundivakkam PC, Xue J, Malik AB, Tiruppathi C. Ca2+ influx via TRPC channels induces NF-kappaB-dependent A20 expression to prevent thrombin-induced apoptosis in endothelial cells. *Am J Physiol Cell Physiol* 2010; **298**: C656-C664
221 **Paria BC**, Malik AB, Kwiatek AM, Rahman A, May MJ, Ghosh S, Tiruppathi C. Tumor necrosis factor-alpha induces nuclear factor-kappaB-dependent TRPC1 expression in endothelial cells. *J Biol Chem* 2003; **278**: 37195-37203
222 **Paria BC**, Vogel SM, Ahmmed GU, Alamgir S, Shroff J, Malik AB, Tiruppathi C. Tumor necrosis factor-alpha-induced TRPC1 expression amplifies store-operated Ca2+ influx and endothelial permeability. *Am J Physiol Lung Cell Mol Physiol* 2004; **287**: L1303-L1313
223 **Yang LX**, Guo RW, Liu B, Wang XM, Qi F, Guo CM, Shi YK, Wang H. Role of TRPC1 and NF-kappaB in mediating angiotensin II-induced Ca2+ entry and endothelial hyperpermeability. *Peptides* 2009; **30**: 1368-1373
224 **Antigny F**, Girardin N, Frieden M. Transient receptor potential canonical channels are required for in vitro endothelial tube formation. *J Biol Chem* 2012; **287**: 5917-5927
225 **Yu PC**, Gu SY, Bu JW, Du JL. TRPC1 is essential for in vivo angiogenesis in zebrafish. *Circ Res* 2010; **106**: 1221-1232
226 **Ma X**, Qiu S, Luo J, Ma Y, Ngai CY, Shen B, Wong CO, Huang Y, Yao X. Functional role of vanilloid transient receptor potential 4-canonical transient receptor potential 1 complex in flow-induced Ca2+ influx. *Arterioscler Thromb Vasc Biol* 2010; **30**: 851-858
227 **Ma X**, Cao J, Luo J, Nilius B, Huang Y, Ambudkar IS, Yao X. Depletion of intracellular Ca2+ stores stimulates the translocation of vanilloid transient receptor potential 4-c1 heteromeric channels to the plasma membrane. *Arterioscler Thromb Vasc Biol* 2010; **30**: 2249-2255
228 **Ma X**, Cheng KT, Wong CO, O'Neil RG, Birnbaumer L, Ambudkar IS, Yao X. Heteromeric TRPV4-C1 channels contribute to store-operated Ca(2+) entry in vascular endothelial cells. *Cell Calcium* 2011; **50**: 502-509
229 **Berrout J**, Jin M, O'Neil RG. Critical role of TRPP2 and TRPC1 channels in stretch-induced injury of blood-brain barrier endothelial cells. *Brain Res* 2012; **1436**: 1-12
230 **Cheng HW**, James AF, Foster RR, Hancox JC, Bates DO. VEGF activates receptor-operated cation channels in human microvascular endothelial cells. *Arterioscler Thromb Vasc Biol* 2006; **26**: 1768-1776
231 **Ge R**, Tai Y, Sun Y, Zhou K, Yang S, Cheng T, Zou Q, Shen F, Wang Y. Critical role of TRPC6 channels in VEGF-mediated angiogenesis. *Cancer Lett* 2009; **283**: 43-51
232 **Kamouchi M**, Philipp S, Flockerzi V, Wissenbach U, Mamin A, Raeymaekers L, Eggermont J, Droogmans G, Nilius B. Properties of heterologously expressed hTRP3 channels in bovine pulmonary artery endothelial cells. *J Physiol* 1999; **518** Pt 2: 345-358
233 **Leung PC**, Cheng KT, Liu C, Cheung WT, Kwan HY, Lau KL, Huang Y, Yao X. Mechanism of non-capacitative Ca2+ influx in response to bradykinin in vascular endothelial cells. *J Vasc Res* 2006; **43**: 367-376
234 **Smedlund K**, Vazquez G. Involvement of native TRPC3 proteins in ATP-dependent expression of VCAM-1 and monocyte adherence in coronary artery endothelial cells. *Arterioscler Thromb Vasc Biol* 2008; **28**: 2049-2055
235 **Huang JH**, He GW, Xue HM, Yao XQ, Liu XC, Underwood MJ, Yang Q. TRPC3 channel contributes to nitric oxide release: significance during normoxia and hypoxia-reoxygenation. *Cardiovasc Res* 2011; **91**: 472-482
236 **Pocock TM**, Foster RR, Bates DO. Evidence of a role for TRPC channels in VEGF-mediated increased vascular permeability in vivo. *Am J Physiol Heart Circ Physiol* 2004; **286**: H1015-H1026
237 **Smedlund K**, Tano JY, Vazquez G. The constitutive function of native TRPC3 channels modulates vascular cell adhesion molecule-1 expression in coronary endothelial cells through nuclear factor kappaB signaling. *Circ Res* 2010; **106**: 1479-1488
238 **Liu CL**, Huang Y, Ngai CY, Leung YK, Yao XQ. TRPC3 is involved in flow- and bradykinin-induced vasodilation in rat small mesenteric arteries. *Acta Pharmacol Sin* 2006; **27**: 981-990
239 **Kwan HY**, Huang Y, Yao X. Protein kinase C can inhibit TRPC3 channels indirectly via stimulating protein kinase G. *J Cell Physiol* 2006; **207**: 315-321
240 **Takahashi S**, Lin H, Geshi N, Mori Y, Kawarabayashi Y, Takami N, Mori MX, Honda A, Inoue R. Nitric oxide-cGMP-protein kinase G pathway negatively regulates vascular transient receptor potential channel TRPC6. *J Physiol* 2008; **586**: 4209-4223
241 **Balzer M**, Lintschinger B, Groschner K. Evidence for a role of Trp proteins in the oxidative stress-induced membrane conductances of porcine aortic endothelial cells. *Cardiovasc Res* 1999; **42**: 543-549
242 **Poteser M**, Graziani A, Rosker C, Eder P, Derler I, Kahr H, Zhu MX, Romanin C, Groschner K. TRPC3 and TRPC4 associate to form a redox-sensitive cation channel. Evidence for expression of native TRPC3-TRPC4 heteromeric channels in endothelial cells. *J Biol Chem* 2006; **281**: 13588-13595
243 **Antigny F**, Jousset H, König S, Frieden M. Thapsigargin activates Ca^{2+} entry both by store-dependent, STIM1/Orai1-mediated, and store-independent, TRPC3/PLC/PKC-mediated pathways in human endothelial cells. *Cell Calcium* 2011; **49**: 115-127
244 **Thilo F**, Loddenkemper C, Berg E, Zidek W, Tepel M. Increased TRPC3 expression in vascular endothelium of patients with malignant hypertension. *Mod Pathol* 2009; **22**: 426-430
245 **Thilo F**, Baumunk D, Krause H, Schrader M, Miller K, Loddenkemper C, Zakrzewicz A, Krueger K, Zidek W, Tepel M. Transient receptor potential canonical type 3 channels and blood pressure in humans. *J Hypertens* 2009; **27**: 1217-1223
246 **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 Ca2+ signaling and hyperpolarization in endothelial cells. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2612-2618
247 **Dora KA**. Coordination of vasomotor responses by the endothelium. *Circ J* 2010; **74**: 226-232
248 **Chaudhuri P**, Colles SM, Bhat M, Van Wagoner DR, Birnbaumer L, Graham LM. Elucidation of a TRPC6-TRPC5 channel cascade that restricts endothelial cell movement. *Mol Biol Cell* 2008; **19**: 3203-3211
249 **Kini V**, Chavez A, Mehta D. A new role for PTEN in regulating transient receptor potential canonical channel 6-mediated Ca2+ entry, endothelial permeability, and angiogenesis. *J Biol Chem* 2010; **285**: 33082-33091

250 **Weissmann N**, Sydykov A, Kalwa H, Storch U, Fuchs B, Mederos y Schnitzler M, Brandes RP, Grimminger F, Meissner M, Freichel M, Offermanns S, Veit F, Pak O, Krause KH, Schermuly RT, Brewer AC, Schmidt HH, Seeger W, Shah AM, Gudermann T, Ghofrani HA, Dietrich A. Activation of TRPC6 channels is essential for lung ischaemia-reperfusion induced oedema in mice. *Nat Commun* 2012; **3**: 649
251 **Samapati R**, Yang Y, Yin J, Stoerger C, Arenz C, Dietrich A, Gudermann T, Adam D, Wu S, Freichel M, Flockerzi V, Uhlig S, Kuebler WM. Lung endothelial Ca2+ and permeability response to platelet-activating factor is mediated by acid sphingomyelinase and transient receptor potential classical 6. *Am J Respir Crit Care Med* 2012; **185**: 160-170
252 **Plant TD**, Schaefer M. Receptor-operated cation channels formed by TRPC4 and TRPC5. *Naunyn Schmiedebergs Arch Pharmacol* 2005; **371**: 266-276
253 **Otsuguro K**, Tang J, Tang Y, Xiao R, Freichel M, Tsvilovskyy V, Ito S, Flockerzi V, Zhu MX, Zholos AV. Isoform-specific inhibition of TRPC4 channel by phosphatidylinositol 4,5-bisphosphate. *J Biol Chem* 2008; **283**: 10026-10036
254 **Lee-Kwon W**, Wade JB, Zhang Z, Pallone TL, Weinman EJ. Expression of TRPC4 channel protein that interacts with NHERF-2 in rat descending vasa recta. *Am J Physiol Cell Physiol* 2005; **288**: C942-C949
255 **Blair NT**, Kaczmarek JS, Clapham DE. Intracellular calcium strongly potentiates agonist-activated TRPC5 channels. *J Gen Physiol* 2009; **133**: 525-546
256 **Obukhov AG**, Nowycky MC. TRPC5 activation kinetics are modulated by the scaffolding protein ezrin/radixin/moesin-binding phosphoprotein-50 (EBP50). *J Cell Physiol* 2004; **201**: 227-235
257 **Schindl R**, Frischauf I, Kahr H, Fritsch R, Krenn M, Derndl A, Vales E, Muik M, Derler I, Groschner K, Romanin C. The first ankyrin-like repeat is the minimum indispensable key structure for functional assembly of homo- and heteromeric TRPC4/TRPC5 channels. *Cell Calcium* 2008; **43**: 260-269
258 **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 Ca2+ current impairs agonist-dependent vasorelaxation in TRP4-/- mice. *Nat Cell Biol* 2001; **3**: 121-127
259 **Tiruppathi C**, Freichel M, Vogel SM, Paria BC, Mehta D, Flockerzi V, Malik AB. Impairment of store-operated Ca2+ entry in TRPC4(-/-) mice interferes with increase in lung microvascular permeability. *Circ Res* 2002; **91**: 70-76
260 **Cioffi DL**, Wu S, Alexeyev M, Goodman SR, Zhu MX, Stevens T. Activation of the endothelial store-operated ISOC Ca2+ channel requires interaction of protein 4.1 with TRPC4. *Circ Res* 2005; **97**: 1164-1172
261 **Brough GH**, Wu S, Cioffi D, Moore TM, Li M, Dean N, Stevens T. Contribution of endogenously expressed Trp1 to a Ca2+-selective, store-operated Ca2+ entry pathway. *FASEB J* 2001; **15**: 1727-1738
262 **Murata T**, Lin MI, Stan RV, Bauer PM, Yu J, Sessa WC. Genetic evidence supporting caveolae microdomain regulation of calcium entry in endothelial cells. *J Biol Chem* 2007; **282**: 16631-16643
263 **Cioffi DL**, Stevens T. Regulation of endothelial cell barrier function by store-operated calcium entry. *Microcirculation* 2006; **13**: 709-723
264 **Graziani A**, Poteser M, Heupel WM, Schleifer H, Krenn M, Drenckhahn D, Romanin C, Baumgartner W, Groschner K. Cell-cell contact formation governs Ca2+ signaling by TRPC4 in the vascular endothelium: evidence for a regulatory TRPC4-beta-catenin interaction. *J Biol Chem* 2010; **285**: 4213-4223
265 **Groschner K**. Polymodal TRPC signaling: Emerging role in phenotype switching and tissue remodeling. *Commun Integr Biol* 2010; **3**: 393-395
266 **Jung C**, Gené GG, Tomás M, Plata C, Selent J, Pastor M, Fandos C, Senti M, Lucas G, Elosua R, Valverde MA. A gain-of-function SNP in TRPC4 cation channel protects against myocardial infarction. *Cardiovasc Res* 2011; **91**: 465-471
267 **Wong CO**, Sukumar P, Beech DJ, Yao X. Nitric oxide lacks direct effect on TRPC5 channels but suppresses endogenous TRPC5-containing channels in endothelial cells. *Pflugers Arch* 2010; **460**: 121-130
268 **Wong CO**, Huang Y, Yao X. Genistein potentiates activity of the cation channel TRPC5 independently of tyrosine kinases. *Br J Pharmacol* 2010; **159**: 1486-1496
269 **Liu Y**, Xu Y, Thilo F, Friis UG, Jensen BL, Scholze A, Zheng J, Tepel M. Erythropoietin increases expression and function of transient receptor potential canonical 5 channels. *Hypertension* 2011; **58**: 317-324
270 **Baylie RL**, Brayden JE. TRPV channels and vascular function. *Acta Physiol* (*Oxf*) 2011; **203**: 99-116
271 **Ching LC**, Kou YR, Shyue SK, Su KH, Wei J, Cheng LC, Yu YB, Pan CC, Lee TS. Molecular mechanisms of activation of endothelial nitric oxide synthase mediated by transient receptor potential vanilloid type 1. *Cardiovasc Res* 2011; **91**: 492-501
272 **Golech SA**, McCarron RM, Chen Y, Bembry J, Lenz F, Mechoulam R, Shohami E, Spatz M. Human brain endothelium: coexpression and function of vanilloid and endocannabinoid receptors. *Brain Res Mol Brain Res* 2004; **132**: 87-92
273 **Bratz IN**, Dick GM, Tune JD, Edwards JM, Neeb ZP, Dincer UD, Sturek M. Impaired capsaicin-induced relaxation of coronary arteries in a porcine model of the metabolic syndrome. *Am J Physiol Heart Circ Physiol* 2008; **294**: H2489-H2496
274 **Kark T**, Bagi Z, Lizanecz E, Pásztor ET, Erdei N, Czikora A, Papp Z, Edes I, Pórszász R, Tóth A. Tissue-specific regulation of microvascular diameter: opposite functional roles of neuronal and smooth muscle located vanilloid receptor-1. *Mol Pharmacol* 2008; **73**: 1405-1412
275 **Poblete IM**, Orliac ML, Briones R, Adler-Graschinsky E, Huidobro-Toro JP. Anandamide elicits an acute release of nitric oxide through endothelial TRPV1 receptor activation in the rat arterial mesenteric bed. *J Physiol* 2005; **568**: 539-551
276 **Ye F**, Deng PY, Li D, Luo D, Li NS, Deng S, Deng HW, Li YJ. Involvement of endothelial cell-derived CGRP in heat stress-induced protection of endothelial function. *Vascul Pharmacol* 2007; **46**: 238-246
277 **Luo D**, Zhang YW, Peng WJ, Peng J, Chen QQ, Li D, Deng HW, Li YJ. Transient receptor potential vanilloid 1-mediated expression and secretion of endothelial cell-derived calcitonin gene-related peptide. *Regul Pept* 2008; **150**: 66-72
278 **Hoi PM**, Visintin C, Okuyama M, Gardiner SM, Kaup SS, Bennett T, Baker D, Selwood DL, Hiley CR. Vascular pharmacology of a novel cannabinoid-like compound, 3-(5-dimethylcarbamoyl-pent-1-enyl)-N-(2-hydroxy-1-methylethyl)benzamide (VSN16) in the rat. *Br J Pharmacol* 2007; **152**: 751-764
279 **Ching LC**, Chen CY, Su KH, Hou HH, Shyue SK, Kou YR, Lee TS. Implication of AMP-activated protein kinase in transient receptor potential vanilloid type 1-mediated activation of endothelial nitric oxide synthase. *Mol Med* 2012; Epub ahead of print
280 **Yang D**, Luo Z, Ma S, Wong WT, Ma L, Zhong J, He H, Zhao Z, Cao T, Yan Z, Liu D, Arendshorst WJ, Huang Y, Tepel M, Zhu Z. Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension. *Cell Metab* 2010; **12**: 130-141
281 **Hao X**, Chen J, Luo Z, He H, Yu H, Ma L, Ma S, Zhu T, Liu D, Zhu Z. TRPV1 activation prevents high-salt diet-induced nocturnal hypertension in mice. *Pflugers Arch* 2011; **461**: 345-353
282 **Pacher P**, Bátkai S, Kunos G. Haemodynamic profile and responsiveness to anandamide of TRPV1 receptor knock-out mice. *J Physiol* 2004; **558**: 647-657
283 **Wang Y**, Wang DH. Aggravated renal inflammatory re-

sponses in TRPV1 gene knockout mice subjected to DOCA-salt hypertension. *Am J Physiol Renal Physiol* 2009; **297**: F1550-F1559
284 **Mergler S**, Valtink M, Coulson-Thomas VJ, Lindemann D, Reinach PS, Engelmann K, Pleyer U. TRPV channels mediate temperature-sensing in human corneal endothelial cells. *Exp Eye Res* 2010; **90**: 758-770
285 **Ohanyan VA**, Guarini G, Thodeti CK, Talasila PK, Raman P, Haney RM, Meszaros JG, Damron DS, Bratz IN. Endothelin-mediated in vivo pressor responses following TRPV1 activation. *Am J Physiol Heart Circ Physiol* 2011; **301**: H1135-H1142
286 **Min JK**, Han KY, Kim EC, Kim YM, Lee SW, Kim OH, Kim KW, Gho YS, Kwon YG. Capsaicin inhibits in vitro and in vivo angiogenesis. *Cancer Res* 2004; **64**: 644-651
287 **Earley S**, Gonzales AL, Garcia ZI. A dietary agonist of transient receptor potential cation channel V3 elicits endothelium-dependent vasodilation. *Mol Pharmacol* 2010; **77**: 612-620
288 **Watanabe H**, Vriens J, Suh SH, Benham CD, Droogmans G, Nilius B. Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. *J Biol Chem* 2002; **277**: 47044-47051
289 **Vriens J**, Watanabe H, Janssens A, Droogmans G, Voets T, Nilius B. Cell swelling, heat, and chemical agonists use distinct pathways for the activation of the cation channel TRPV4. *Proc Natl Acad Sci USA* 2004; **101**: 396-401
290 **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**: e827
291 **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. *Arterioscler Thromb Vasc Biol* 2006; **26**: 1495-1502
292 **Thodeti CK**, Matthews B, Ravi A, Mammoto A, Ghosh K, Bracha AL, Ingber DE. TRPV4 channels mediate cyclic strain-induced endothelial cell reorientation through integrin-to-integrin signaling. *Circ Res* 2009; **104**: 1123-1130
293 **Matthews BD**, Thodeti CK, Tytell JD, Mammoto A, Overby DR, Ingber DE. Ultra-rapid activation of TRPV4 ion channels by mechanical forces applied to cell surface beta1 integrins. *Integr Biol* (*Camb*) 2010; **2**: 435-442
294 **Watanabe H**, Davis JB, Smart D, Jerman JC, Smith GD, Hayes P, Vriens J, Cairns W, Wissenbach U, Prenen J, Flockerzi V, Droogmans G, Benham CD, Nilius B. Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. *J Biol Chem* 2002; **277**: 13569-13577
295 **Ma X**, He D, Ru X, Chen Y, Cai Y, Bruce IC, Xia Q, Yao X, Jin J. Apigenin, a plant-derived flavone, activates transient receptor potential vanilloid 4 cation channel. *Br J Pharmacol* 2012; **166**: 349-358
296 **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. *Cardiovasc Res* 2008; **80**: 445-452
297 **Marrelli SP**, O'neil RG, Brown RC, Bryan RM. PLA2 and TRPV4 channels regulate endothelial calcium in cerebral arteries. *Am J Physiol Heart Circ Physiol* 2007; **292**: H1390-H1397
298 **Watanabe H**, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature* 2003; **424**: 434-438
299 **Vriens J**, Owsianik G, Fisslthaler B, Suzuki M, Janssens A, Voets T, Morisseau C, Hammock BD, Fleming I, Busse R, Nilius B. Modulation of the Ca2 permeable cation channel TRPV4 by cytochrome P450 epoxygenases in vascular endothelium. *Circ Res* 2005; **97**: 908-915
300 **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 Ca2+ entry and mitochondrial ROS signaling. *Am J Physiol Heart Circ Physiol* 2012; **302**: H634-H642
301 **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: Ca2+ signals and gap junction function are regulated by caveolin in endothelial cells. *Circulation* 2008; **117**: 1065-1074
302 **Gao F**, Wang DH. Hypotension induced by activation of the transient receptor potential vanilloid 4 channels: role of Ca2+-activated K+ channels and sensory nerves. *J Hypertens* 2010; **28**: 102-110
303 **Willette RN**, Bao W, Nerurkar S, Yue TL, Doe CP, Stankus G, Turner GH, Ju H, Thomas H, Fishman CE, Sulpizio A, Behm DJ, Hoffman S, Lin Z, Lozinskaya I, Casillas LN, Lin M, Trout RE, Votta BJ, Thorneloe K, Lashinger ES, Figueroa DJ, Marquis R, Xu X. Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. *J Pharmacol Exp Ther* 2008; **326**: 443-452
304 **Earley S**, Gonzales AL, Crnich R. Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca2+-Activated K+ channels. *Circ Res* 2009; **104**: 987-994
305 **Earley S**, Pauyo T, Drapp R, Tavares MJ, Liedtke W, Brayden JE. TRPV4-dependent dilation of peripheral resistance arteries influences arterial pressure. *Am J Physiol Heart Circ Physiol* 2009; **297**: H1096-H1102
306 **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**: 532-538
307 **Adapala RK**, Talasila PK, Bratz IN, Zhang DX, Suzuki M, Meszaros JG, Thodeti CK. PKCα mediates acetylcholine-induced activation of TRPV4-dependent calcium influx in endothelial cells. *Am J Physiol Heart Circ Physiol* 2011; **301**: H757-H765
308 **Gao F**, Wang DH. Impairment in function and expression of transient receptor potential vanilloid type 4 in Dahl salt-sensitive rats: significance and mechanism. *Hypertension* 2010; **55**: 1018-1025
309 **Gao F**, Sui D, Garavito RM, Worden RM, Wang DH. Salt intake augments hypotensive effects of transient receptor potential vanilloid 4: functional significance and implication. *Hypertension* 2009; **53**: 228-235
310 **Troidl C**, Nef H, Voss S, Schilp A, Kostin S, Troidl K, Szardien S, Rolf A, Schmitz-Rixen T, Schaper W, Hamm CW, Elsässer A, Möllmann H. Calcium-dependent signalling is essential during collateral growth in the pig hind limb-ischemia model. *J Mol Cell Cardiol* 2010; **49**: 142-151
311 **Schierling W**, Troidl K, Apfelbeck H, Troidl C, Kasprzak PM, Schaper W, Schmitz-Rixen T. Cerebral arteriogenesis is enhanced by pharmacological as well as fluid-shear-stress activation of the Trpv4 calcium channel. *Eur J Vasc Endovasc Surg* 2011; **41**: 589-596
312 **Troidl C**, Troidl K, Schierling W, Cai WJ, Nef H, Möllmann H, Kostin S, Schimanski S, Hammer L, Elsässer A, Schmitz-Rixen T, Schaper W. Trpv4 induces collateral vessel growth during regeneration of the arterial circulation. *J Cell Mol Med* 2009; **13**: 2613-2621
313 **Alvarez DF**, King JA, Weber D, Addison E, Liedtke W, Townsley MI. Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. *Circ Res* 2006; **99**: 988-995
314 **Hamanaka K**, Jian MY, Weber DS, Alvarez DF, Townsley MI, Al-Mehdi AB, King JA, Liedtke W, Parker JC. TRPV4 initiates the acute calcium-dependent permeability increase during ventilator-induced lung injury in isolated mouse lungs. *Am J Physiol Lung Cell Mol Physiol* 2007; **293**: L923-L932
315 **Jian MY**, King JA, Al-Mehdi AB, Liedtke W, Townsley MI.

High vascular pressure-induced lung injury requires P450 epoxygenase-dependent activation of TRPV4. *Am J Respir Cell Mol Biol* 2008; **38**: 386-392
316 **Yin J**, Hoffmann J, Kaestle SM, Neye N, Wang L, Baeurle J, Liedtke W, Wu S, Kuppe H, Pries AR, Kuebler WM. Negative-feedback loop attenuates hydrostatic lung edema via a cGMP-dependent regulation of transient receptor potential vanilloid 4. *Circ Res* 2008; **102**: 966-974
317 **Fian R**, Grasser E, Treiber F, Schmidt R, Niederl P, Rosker C. The contribution of TRPV4-mediated calcium signaling to calcium homeostasis in endothelial cells. *J Recept Signal Transduct Res* 2007; **27**: 113-124
318 **Mergler S**, Valtink M, Taetz K, Sahlmüller M, Fels G, Reinach PS, Engelmann K, Pleyer U. Characterization of transient receptor potential vanilloid channel 4 (TRPV4) in human corneal endothelial cells. *Exp Eye Res* 2011; **93**: 710-719
319 **Fiorio Pla A**, Ong HL, Cheng KT, Brossa A, Bussolati B, Lockwich T, Paria B, Munaron L, Ambudkar IS. TRPV4 mediates tumor-derived endothelial cell migration via arachidonic acid-activated actin remodeling. *Oncogene* 2012; **31**: 200-212
320 **Hecquet CM**, Ahmmed GU, Vogel SM, Malik AB. Role of TRPM2 channel in mediating H2O2-induced Ca2+ entry and endothelial hyperpermeability. *Circ Res* 2008; **102**: 347-355
321 **Hecquet CM**, Ahmmed GU, Malik AB. TRPM2 channel regulates endothelial barrier function. *Adv Exp Med Biol* 2010; **661**: 155-167
322 **Guinamard R**, Demion M, Launay P. Physiological roles of the TRPM4 channel extracted from background currents. *Physiology* (*Bethesda*) 2010; **25**: 155-164
323 **Wu LJ**, Sweet TB, Clapham DE. International Union of Basic and Clinical Pharmacology. LXXVI. Current progress in the mammalian TRP ion channel family. *Pharmacol Rev* 2010; **62**: 381-404
324 **Gerzanich V**, Woo SK, Vennekens R, Tsymbalyuk O, Ivanova S, Ivanov A, Geng Z, Chen Z, Nilius B, Flockerzi V, Freichel M, Simard JM. De novo expression of Trpm4 initiates secondary hemorrhage in spinal cord injury. *Nat Med* 2009; **15**: 185-191
325 **Becerra A**, Echeverría C, Varela D, Sarmiento D, Armisén R, Nuñez-Villena F, Montecinos M, Simon F. Transient receptor potential melastatin 4 inhibition prevents lipopolysaccharide-induced endothelial cell death. *Cardiovasc Res* 2011; **91**: 677-684
326 **Yogi A**, Callera GE, Antunes TT, Tostes RC, Touyz RM. Vascular biology of magnesium and its transporters in hypertension. *Magnes Res* 2010; **23**: S207-S215
327 **Baldoli E**, Maier JA. Silencing TRPM7 mimics the effects of magnesium deficiency in human microvascular endothelial cells. *Angiogenesis* 2012; **15**: 47-57
328 **Inoue K**, Xiong ZG. Silencing TRPM7 promotes growth/proliferation and nitric oxide production of vascular endothelial cells via the ERK pathway. *Cardiovasc Res* 2009; **83**: 547-557
329 **Paravicini TM**, Yogi A, Mazur A, Touyz RM. Dysregulation of vascular TRPM7 and annexin-1 is associated with endothelial dysfunction in inherited hypomagnesemia. *Hypertension* 2009; **53**: 423-429
330 **Oancea E**, Wolfe JT, Clapham DE. Functional TRPM7 channels accumulate at the plasma membrane in response to fluid flow. *Circ Res* 2006; **98**: 245-253
331 **Wilson PD**. Polycystic kidney disease: new understanding in the pathogenesis. *Int J Biochem Cell Biol* 2004; **36**: 1868-1873
332 **AbouAlaiwi WA**, Takahashi M, Mell BR, Jones TJ, Ratnam S, Kolb RJ, Nauli SM. Ciliary polycystin-2 is a mechanosensitive calcium channel involved in nitric oxide signaling cascades. *Circ Res* 2009; **104**: 860-869
333 **Nauli SM**, Kawanabe Y, Kaminski JJ, Pearce WJ, Ingber DE, Zhou J. Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. *Circulation* 2008; **117**: 1161-1171
334 **Hirano K**, Hirano M, Hanada A. Involvement of STIM1 in the proteinase-activated receptor 1-mediated Ca2+ influx in vascular endothelial cells. *J Cell Biochem* 2009; **108**: 499-507
335 **Feske S**. ORAI1 and STIM1 deficiency in human and mice: roles of store-operated Ca2+ entry in the immune system and beyond. *Immunol Rev* 2009; **231**: 189-209
336 **Hogan PG**, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* 2010; **28**: 491-533
337 **Giachini FR**, Lima VV, Hannan JL, Carneiro FS, Webb RC, Tostes RC. STIM1/Orai1-mediated store-operated Ca2+ entry: the tip of the iceberg. *Braz J Med Biol Res* 2011; **44**: 1080-1087
338 **Beech DJ**. Orai1 calcium channels in the vasculature. *Pflugers Arch* 2012; **463**: 635-647
339 **Mumtaz S**, Burdyga G, Borisova L, Wray S, Burdyga T. The mechanism of agonist induced Ca2+ signalling in intact endothelial cells studied confocally in in situ arteries. *Cell Calcium* 2011; **49**: 66-77
340 **Malli R**, Naghdi S, Romanin C, Graier WF. Cytosolic Ca2+ prevents the subplasmalemmal clustering of STIM1: an intrinsic mechanism to avoid Ca2+ overload. *J Cell Sci* 2008; **121**: 3133-3139
341 **Madge L**, Marshall IC, Taylor CW. Delayed autoregulation of the Ca2+ signals resulting from capacitative Ca2+ entry in bovine pulmonary artery endothelial cells. *J Physiol* 1997; **498** (Pt 2): 351-369
342 **Brandman O**, Liou J, Park WS, Meyer T. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca2+ levels. *Cell* 2007; **131**: 1327-1339
343 **Dömötör E**, Abbott NJ, Adam-Vizi V. Na+-Ca2+ exchange and its implications for calcium homeostasis in primary cultured rat brain microvascular endothelial cells. *J Physiol* 1999; **515** (Pt 1): 147-155
344 **Goto Y**, Miura M, Iijima T. Extrusion mechanisms of intracellular Ca2+ in human aortic endothelial cells. *Eur J Pharmacol* 1996; **314**: 185-192
345 **Wang X**, Reznick S, Li P, Liang W, van Breemen C. Ca(2+) removal mechanisms in freshly isolated rabbit aortic endothelial cells. *Cell Calcium* 2002; **31**: 265-277
346 **Li L**, van Breemen C. Na(+)-Ca2+ exchange in intact endothelium of rabbit cardiac valve. *Circ Res* 1995; **76**: 396-404
347 **Paltauf-Doburzynska J**, Frieden M, Graier WF. Mechanisms of Ca2+ store depletion in single endothelial cells in a Ca(2+)-free environment. *Cell Calcium* 1999; **25**: 345-353
348 **Sedova M**, Blatter LA. Dynamic regulation of [Ca2+]i by plasma membrane Ca(2+)-ATPase and Na+/Ca2+ exchange during capacitative Ca2+ entry in bovine vascular endothelial cells. *Cell Calcium* 1999; **25**: 333-343
349 **Moccia F**, Berra-Romani R, Baruffi S, Spaggiari S, Signorelli S, Castelli L, Magistretti J, Taglietti V, Tanzi F. Ca2+ uptake by the endoplasmic reticulum Ca2+-ATPase in rat microvascular endothelial cells. *Biochem J* 2002; **364**: 235-244
350 **Brini M**, Carafoli E. Calcium pumps in health and disease. *Physiol Rev* 2009; **89**: 1341-1378
351 **Anger M**, Samuel JL, Marotte F, Wuytack F, Rappaport L, Lompré AM. The sarco(endo)plasmic reticulum Ca(2+)-ATPase mRNA isoform, SERCA 3, is expressed in endothelial and epithelial cells in various organs. *FEBS Lett* 1993; **334**: 45-48
352 **Hadri L**, Ozog A, Soncin F, Lompré AM. Basal transcription of the mouse sarco(endo)plasmic reticulum Ca2+-ATPase type 3 gene in endothelial cells is controlled by Ets-1 and Sp1. *J Biol Chem* 2002; **277**: 36471-36478
353 **Khan I**, Sandhu V, Misquitta CM, Grover AK. SERCA pump isoform expression in endothelium of veins and arteries: every endothelium is not the same. *Mol Cell Biochem* 2000; **203**: 11-15
354 **Ozog A**, Pouzet B, Bobe R, Lompré AM. Characterization of

the 3′ end of the mouse SERCA 3 gene and tissue distribution of mRNA spliced variants. *FEBS Lett* 1998; **427**: 349-352
355 **Szewczyk MM**, Davis KA, Samson SE, Simpson F, Rangachari PK, Grover AK. Ca2+-pumps and Na2+-Ca2+-exchangers in coronary artery endothelium versus smooth muscle. *J Cell Mol Med* 2007; **11**: 129-138
356 **Wu KD**, Lee WS, Wey J, Bungard D, Lytton J. Localization and quantification of endoplasmic reticulum Ca(2+)-ATPase isoform transcripts. *Am J Physiol* 1995; **269**: C775-C784
357 **Hadri L**, Pavoine C, Lipskaia L, Yacoubi S, Lompré AM. Transcription of the sarcoplasmic/endoplasmic reticulum Ca2+-ATPase type 3 gene, ATP2A3, is regulated by the calcineurin/NFAT pathway in endothelial cells. *Biochem J* 2006; **394**: 27-33
358 **Liu LH**, Paul RJ, Sutliff RL, Miller ML, Lorenz JN, Pun RY, Duffy JJ, Doetschman T, Kimura Y, MacLennan DH, Hoying JB, Shull GE. Defective endothelium-dependent relaxation of vascular smooth muscle and endothelial cell Ca2+ signaling in mice lacking sarco(endo)plasmic reticulum Ca2+-ATPase isoform 3. *J Biol Chem* 1997; **272**: 30538-30545
359 **Dedkova EN**, Blatter LA. Nitric oxide inhibits capacitative Ca2+ entry and enhances endoplasmic reticulum Ca2+ uptake in bovine vascular endothelial cells. *J Physiol* 2002; **539**: 77-91
360 **Sutliff RL**, Hoying JB, Kadambi VJ, Kranias EG, Paul RJ. Phospholamban is present in endothelial cells and modulates endothelium-dependent relaxation. Evidence from phospholamban gene-ablated mice. *Circ Res* 1999; **84**: 360-364
361 **Grover AK**, Samson SE. Peroxide resistance of ER Ca2+ pump in endothelium: implications to coronary artery function. *Am J Physiol* 1997; **273**: C1250-C1258
362 **Schmidt T**, Zaib F, Samson SE, Kwan CY, Grover AK. Peroxynitrite resistance of sarco/endoplasmic reticulum Ca2+ pump in pig coronary artery endothelium and smooth muscle. *Cell Calcium* 2004; **36**: 77-82
363 **Cook NL**, Viola HM, Sharov VS, Hool LC, Schöneich C, Davies MJ. Myeloperoxidase-derived oxidants inhibit sarco/endoplasmic reticulum Ca2+-ATPase activity and perturb Ca2+ homeostasis in human coronary artery endothelial cells. *Free Radic Biol Med* 2012; **52**: 951-961
364 **Dong Y**, Zhang M, Wang S, Liang B, Zhao Z, Liu C, Wu M, Choi HC, Lyons TJ, Zou MH. Activation of AMP-activated protein kinase inhibits oxidized LDL-triggered endoplasmic reticulum stress in vivo. *Diabetes* 2010; **59**: 1386-1396
365 **Dong Y**, Zhang M, Liang B, Xie Z, Zhao Z, Asfa S, Choi HC, Zou MH. Reduction of AMP-activated protein kinase alpha2 increases endoplasmic reticulum stress and atherosclerosis in vivo. *Circulation* 2010; **121**: 792-803
366 **Lytton J**. Na+/Ca2+ exchangers: three mammalian gene families control Ca2+ transport. *Biochem J* 2007; **406**: 365-382
367 **Minelli A**, Castaldo P, Gobbi P, Salucci S, Magi S, Amoroso S. Cellular and subcellular localization of Na+-Ca2+ exchanger protein isoforms, NCX1, NCX2, and NCX3 in cerebral cortex and hippocampus of adult rat. *Cell Calcium* 2007; **41**: 221-234
368 **Yamaji R**, Fujita K, Takahashi S, Yoneda H, Nagao K, Masuda W, Naito M, Tsuruo T, Miyatake K, Inui H, Nakano Y. Hypoxia up-regulates glyceraldehyde-3-phosphate dehydrogenase in mouse brain capillary endothelial cells: involvement of Na+/Ca2+ exchanger. *Biochim Biophys Acta* 2003; **1593**: 269-276
369 **Gobbi P**, Castaldo P, Minelli A, Salucci S, Magi S, Corcione E, Amoroso S. Mitochondrial localization of Na+/Ca2+ exchangers NCX1-3 in neurons and astrocytes of adult rat brain in situ. *Pharmacol Res* 2007; **56**: 556-565
370 **Kaye DM**, Kelly RA. Expression and regulation of the sodium-calcium exchanger in cardiac microvascular endothelial cells. *Clin Exp Pharmacol Physiol* 1999; **26**: 651-655
371 **Eder P**, Poteser M, Romanin C, Groschner K. Na(+) entry and modulation of Na(+)/Ca(2+) exchange as a key mechanism of TRPC signaling. *Pflugers Arch* 2005; **451**: 99-104
372 **Poburko D**, Fameli N, Kuo KH, van Breemen C. Ca2+ signaling in smooth muscle: TRPC6, NCX and LNats in nanodomains. *Channels* (*Austin*) 2008; **2**: 10-12
373 **Bondarenko A**. Sodium-calcium exchanger contributes to membrane hyperpolarization of intact endothelial cells from rat aorta during acetylcholine stimulation. *Br J Pharmacol* 2004; **143**: 9-18
374 **Andrikopoulos P**, Baba A, Matsuda T, Djamgoz MB, Yaqoob MM, Eccles SA. Ca2+ influx through reverse mode Na+/Ca2+ exchange is critical for vascular endothelial growth factor-mediated extracellular signal-regulated kinase (ERK) 1/2 activation and angiogenic functions of human endothelial cells. *J Biol Chem* 2011; **286**: 37919-37931
375 **Berra-Romani R**, Raqeeb A, Guzman-Silva A, Torres-Jácome J, Tanzi F, Moccia F. Na+-Ca2+ exchanger contributes to Ca2+ extrusion in ATP-stimulated endothelium of intact rat aorta. *Biochem Biophys Res Commun* 2010; **395**: 126-130
376 **Pande J**, Mallhi KK, Sawh A, Szewczyk MM, Simpson F, Grover AK. Aortic smooth muscle and endothelial plasma membrane Ca2+ pump isoforms are inhibited differently by the extracellular inhibitor caloxin 1b1. *Am J Physiol Cell Physiol* 2006; **290**: C1341-C1349
377 **Di Leva F**, Domi T, Fedrizzi L, Lim D, Carafoli E. The plasma membrane Ca2+ ATPase of animal cells: structure, function and regulation. *Arch Biochem Biophys* 2008; **476**: 65-74
378 **Yokomori H**, Oda M, Ogi M, Yoshimura K, Nomura M, Fujimaki K, Kamegaya Y, Tsukada N, Ishii H. Endothelin-1 suppresses plasma membrane Ca++-ATPase, concomitant with contraction of hepatic sinusoidal endothelial fenestrae. *Am J Pathol* 2003; **162**: 557-566
379 **Manoonkitiwongsa PS**, Whitter EF, Wareesangtip W, McMillan PJ, Nava PB, Schultz RL. Calcium-dependent ATPase unlike ecto-ATPase is located primarily on the luminal surface of brain endothelial cells. *Histochem J* 2000; **32**: 313-324
380 **Zhang J**, Xiao P, Zhang X. Phosphatidylserine externalization in caveolae inhibits Ca2+ efflux through plasma membrane Ca2+-ATPase in ECV304. *Cell Calcium* 2009; **45**: 177-184
381 **Holton M**, Mohamed TM, Oceandy D, Wang W, Lamas S, Emerson M, Neyses L, Armesilla AL. Endothelial nitric oxide synthase activity is inhibited by the plasma membrane calcium ATPase in human endothelial cells. *Cardiovasc Res* 2010; **87**: 440-448
382 **Szewczyk MM**, Pande J, Akolkar G, Grover AK. Caloxin 1b3: a novel plasma membrane Ca(2+)-pump isoform 1 selective inhibitor that increases cytosolic Ca(2+) in endothelial cells. *Cell Calcium* 2010; **48**: 352-357
383 **Malli R**, Graier WF. Mitochondrial Ca2+ channels: Great unknowns with important functions. *FEBS Lett* 2010; **584**: 1942-1947
384 **Trenker M**, Malli R, Fertschai I, Levak-Frank S, Graier WF. Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca2+ uniport. *Nat Cell Biol* 2007; **9**: 445-452
385 **Waldeck-Weiermair M**, Malli R, Naghdi S, Trenker M, Kahn MJ, Graier WF. The contribution of UCP2 and UCP3 to mitochondrial Ca(2+) uptake is differentially determined by the source of supplied Ca(2+). *Cell Calcium* 2010; **47**: 433-440
386 **Waldeck-Weiermair M**, Jean-Quartier C, Rost R, Khan MJ, Vishnu N, Bondarenko AI, Imamura H, Malli R, Graier WF. Leucine zipper EF hand-containing transmembrane protein 1 (Letm1) and uncoupling proteins 2 and 3 (UCP2/3) contribute to two distinct mitochondrial Ca2+ uptake pathways. *J Biol Chem* 2011; **286**: 28444-28455
387 **Drago I**, Pizzo P, Pozzan T. After half a century mitochondrial calcium in- and efflux machineries reveal themselves. *EMBO J* 2011; **30**: 4119-4125
388 **Malli R**, Frieden M, Hunkova M, Trenker M, Graier WF. Ca2+ refilling of the endoplasmic reticulum is largely preserved albeit reduced Ca2+ entry in endothelial cells. *Cell Calcium* 2007; **41**: 63-76

389 **Graier WF**, Paltauf-Doburzynska J, Hill BJ, Fleischhacker E, Hoebel BG, Kostner GM, Sturek M. Submaximal stimulation of porcine endothelial cells causes focal Ca2+ elevation beneath the cell membrane. *J Physiol* 1998; **506** (Pt 1): 109-125
390 **Bootman MD**, Berridge MJ, Lipp P. Cooking with calcium: the recipes for composing global signals from elementary events. *Cell* 1997; **91**: 367-373
391 **Clapham DE**. Calcium signaling. *Cell* 2007; **131**: 1047-1058
392 **Rizzuto R**, Pozzan T. Microdomains of intracellular Ca2+: molecular determinants and functional consequences. *Physiol Rev* 2006; **86**: 369-408
393 **Hüser J**, Blatter LA. Elementary events of agonist-induced Ca2+ release in vascular endothelial cells. *Am J Physiol* 1997; **273**: C1775-C1782
394 **Burdyga T**, Shmygol A, Eisner DA, Wray S. A new technique for simultaneous and in situ measurements of Ca2+ signals in arteriolar smooth muscle and endothelial cells. *Cell Calcium* 2003; **34**: 27-33
395 **Duza T**, Sarelius IH. Localized transient increases in endothelial cell Ca2+ in arterioles in situ: implications for coordination of vascular function. *Am J Physiol Heart Circ Physiol* 2004; **286**: H2322-H2331
396 **Kansui Y**, Garland CJ, Dora KA. Enhanced spontaneous Ca2+ events in endothelial cells reflect signalling through myoendothelial gap junctions in pressurized mesenteric arteries. *Cell Calcium* 2008; **44**: 135-146
397 **Duza T**, Sarelius IH. Increase in endothelial cell Ca(2+) in response to mouse cremaster muscle contraction. *J Physiol* 2004; **555**: 459-469
398 **Isakson BE**, Ramos SI, Duling BR. Ca2+ and inositol 1,4,5-trisphosphate-mediated signaling across the myoendothelial junction. *Circ Res* 2007; **100**: 246-254
399 **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. *Proc Natl Acad Sci USA* 2008; **105**: 9627-9632
400 **Nausch LW**, Bonev AD, Heppner TJ, Tallini Y, Kotlikoff MI, Nelson MT. Sympathetic nerve stimulation induces local endothelial Ca2+ signals to oppose vasoconstriction of mouse mesenteric arteries. *Am J Physiol Heart Circ Physiol* 2012; **302**: H594-H602
401 **Missiaen L**, Lemaire FX, Parys JB, De Smedt H, Sienaert I, Casteels R. Initiation sites for Ca2+ signals in endothelial cells. *Pflugers Arch* 1996; **431**: 318-324
402 **Isshiki M**, Ando J, Korenaga R, Kogo H, Fujimoto T, Fujita T, Kamiya A. Endothelial Ca2+ waves preferentially originate at specific loci in caveolin-rich cell edges. *Proc Natl Acad Sci USA* 1998; **95**: 5009-5014
403 **Hu Q**, Deshpande S, Irani K, Ziegelstein RC. [Ca(2+)](i) oscillation frequency regulates agonist-stimulated NF-kappaB transcriptional activity. *J Biol Chem* 1999; **274**: 33995-33998
404 **Scharbrodt W**, Abdallah Y, Kasseckert SA, Gligorievski D, Piper HM, Böker DK, Deinsberger W, Oertel MF. Cytosolic Ca2+ oscillations in human cerebrovascular endothelial cells after subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2009; **29**: 57-65
405 **Zhu L**, Luo Y, Chen T, Chen F, Wang T, Hu Q. Ca2+ oscillation frequency regulates agonist-stimulated gene expression in vascular endothelial cells. *J Cell Sci* 2008; **121**: 2511-2518
406 **Carter TD**, Bogle RG, Bjaaland T. Spiking of intracellular calcium ion concentration in single cultured pig aortic endothelial cells stimulated with ATP or bradykinin. *Biochem J* 1991; **278** (Pt 3): 697-704
407 **Hallam TJ**, Jacob R, Merritt JE, Rink TJ. cytoplasmic calcium oscillations in cultured human umbilical vein endothelial-cells stimulated by histamine. *J Physiol* 1988; **403**: 93
408 **Jacob R**, Merritt JE, Hallam TJ, Rink TJ. Repetitive spikes in cytoplasmic calcium evoked by histamine in human endothelial cells. *Nature* 1988; **335**: 40-45
409 **Morgan AJ**, Jacob R. Ca2+ influx does more than provide releasable Ca2+ to maintain repetitive spiking in human umbilical vein endothelial cells. *Biochem J* 1996; **320** (Pt 2): 505-517
410 **Sedova M**, Klishin A, Huser J, Blatter LA. Capacitative Ca2+ entry is graded with degree of intracellular Ca2+ store depletion in bovine vascular endothelial cells. *J Physiol* 2000; **523 Pt 3**: 549-559
411 **Morgan AJ**, Jacob R. Differential modulation of the phases of a Ca2+ spike by the store Ca2+-ATPase in human umbilical vein endothelial cells. *J Physiol* 1998; **513** (Pt 1): 83-101
412 **Koyama T**, Kimura C, Park SJ, Oike M, Ito Y. Functional implications of Ca2+ mobilizing properties for nitric oxide production in aortic endothelium. *Life Sci* 2002; **72**: 511-520
413 **Hu Q**, Zheng G, Zweier JL, Deshpande S, Irani K, Ziegelstein RC. NADPH oxidase activation increases the sensitivity of intracellular Ca2+ stores to inositol 1,4,5-trisphosphate in human endothelial cells. *J Biol Chem* 2000; **275**: 15749-15757
414 **Cioffi DL**, Wu S, Chen H, Alexeyev M, St Croix CM, Pitt BR, Uhlig S, Stevens T. Orai1 determines calcium selectivity of an endogenous TRPC heterotetramer channel. *Circ Res* 2012; **110**: 1435-1444
415 **Walseth TF**, Lin-Moshier Y, Jain P, Ruas M, Parrington J, Galione A, Marchant JS, Slama JT. Photoaffinity labeling of high affinity nicotinic acid adenine dinucleotide phosphate (NAADP)-binding proteins in sea urchin egg. *J Biol Chem* 2012; **287**: 2308-2315
416 **Lin-Moshier Y**, Walseth TF, Churamani D, Davidson SM, Slama JT, Hooper R, Brailoiu E, Patel S, Marchant JS. Photoaffinity labeling of nicotinic acid adenine dinucleotide phosphate (NAADP) targets in mammalian cells. *J Biol Chem* 2012; **287**: 2296-2307

S- Editor Cheng JX **L- Editor** Kerr C **E- Editor** Zhang DN