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Transient Receptor Potential Channels and Endothelial Cell Calcium Signaling

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Abstract

The vascular endothelium is a broadly distributed and highly specialized organ. The endothelium has a number of functions including the control of blood vessels diameter through the production and release of potent vasoactive substances or direct electrical communication with underlying smooth muscle cells, regulates the permeability of the vascular barrier, stimulates the formation of new blood vessels, and influences inflammatory and thrombotic processes. Endothelial cells that make up the endothelium express a variety of cell-surface receptors and ion channels on the plasma membrane that are capable of detecting circulating hormones, neurotransmitters, oxygen tension, and shear stress across the vascular wall. Changes in these stimuli activate signaling cascades that initiate an appropriate physiological response. Increases in the global intracellular Ca^{2+} concentration and localized Ca^{2+} signals that occur within specialized subcellular microdomains are fundamentally important components of many signaling pathways in the endothelium. The transient receptor potential (TRP) channels are a superfamily of cation-permeable ion channels that act as a primary means of increasing cytosolic Ca^{2+} in endothelial cells. Consequently, TRP channels are vitally important for the major functions of the endothelium. In this review, we provide an in-depth discussion of Ca^{2+}-permeable TRP channels in the endothelium and their role in vascular regulation.

Introduction

The vascular endothelium is a large, spatially distributed organ that is formed by a monolayer of specialized endothelial cells lining the lumen of all blood vessels. The term “endothelium” first appeared in print in 1865 in a report from the Swiss anatomist Wilhelm His (6). Historically, the endothelium was described as an inert protective barrier between the contents of the vascular lumen and the interstitium. This changed in 1976, when Moncada and colleagues proposed a potential role for the endothelium as a source of potent vasoactive substances based on the discovery of a novel endothelium-derived prostanoid, later identified as prostacyclin, with antithrombotic and vasodilatory properties (186). A subsequent landmark study by Furchgott and Zawadzki(93) led to the discovery of nitric oxide (NO) as a critically important endothelium-derived vasodilator (123, 125). In the

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decades following these ground-breaking investigations, the importance of the endothelium in regulating vascular function has become firmly established. Importantly, endothelial dysfunction is a hallmark of many common cardiovascular diseases, such as hypertension and atherosclerosis, as well as inflammatory diseases, including systemic lupus erythematosus and rheumatoid arthritis.

The endothelium regulates the contractility of the underlying smooth muscle cells (SMCs), the permeability of the vascular wall, promotes the formation of new blood vessels, and prevents coagulation of blood. Optimal function of the endothelium is dependent on the ability of endothelial cells to detect a vast array of chemical and physical stimuli and engage appropriate intracellular signal transduction cascades to elicit suitable physiological responses. The endothelium is the only tissue in the body that maintains constant contact with the circulating blood. Because of this unique anatomical position, endothelial cells are able to detect circulating hormones and neurotransmitters, oxygen tension, and shear stress resulting from the flow of blood. The ability to detect these diverse stimuli is enabled by a large number of G-protein-coupled and enzyme-linked cell surface receptors and ion channels on the plasma membrane of endothelial cells. Consequently, the endothelium can be viewed as an intricate sensory system that is capable of sensing and integrating numerous inputs to achieve vascular homeostasis and adaptability.

This review explores the current literature on the roles of the transient receptor potential (TRP) superfamily of cation channels in the regulation of endothelial cell function, focusing on signaling pathways that are regulated by Ca^{2+} ions.

Functions of the Endothelium

Endothelium-dependent vasodilation

Stimulation of the vascular endothelium with specific agonists that signal through G-protein-coupled receptors (GPCRs) results in the generation of vasodilator substances that diffuse to and relax the underlying SMCs (Fig. 1). In addition to agonist-induced stimulation, increases in blood flow velocity and relaxation of the smooth muscle layer can occur in response to the laminar shear stress experienced by the vascular wall, a vasodilator response known as “flow-induced dilation” (60). From an *in vivo* perspective, endothelial-derived vasodilators offset the effects of tonic vasoconstrictor influences and serve to maintain the optimal balance for the appropriate intermediate level of smooth muscle contractility and arterial diameter (“vascular tone”). Prostacyclin (PGI_2), produced by cyclooxygenase (COX) enzymes (209), and NO, produced by endothelial nitric oxide synthase (eNOS)(93), were the first endothelium-derived vasodilators to be identified at the molecular level. However, agonist-induced, endothelium-dependent vasodilation persists during pharmacological inhibition of NOS and COX, demonstrating the existence of additional pathways. This response was initially credited to an unidentified substance termed “endothelium-derived hyperpolarizing factor” (EDHF), reflecting the fact that NOS- and COX-independent relaxation of SMCs was associated with hyperpolarization of the SMC plasma membrane (44). The precise identity of EDHF has been the subject of countless investigation by numerous laboratories. These studies have implicated multiple diffusible factors as candidate vasodilators, including K^+ ions (81); epoxyeicosatrienoic acids (EETs) (40, 88), a family of

four regioisomers (5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET) generated from arachidonic acid by the action of cytochrome P450 (CYP) epoxygenase enzymes (40, 78, 83, 88); hydrogen peroxide (H_2O_2); hydrogen sulfide (H_2S); C-type natriuretic peptide; and carbon monoxide, among others (82). In addition to these diffusible substances, direct propagation of membrane hyperpolarization from endothelial cells to SMCs through myoendothelial gap junctions has been shown to induce vasodilation. This is often referred to as endothelium-dependent hyperpolarization (EDH) to differentiate it from EDHF-type vasodilation mediated by diffusible substances (95).

Vascular permeability and transfer of solutes and macromolecules

The vascular endothelium forms a semipermeable barrier between the blood and the surrounding tissue that regulates the passage of solutes and macromolecules across the vascular wall. This permeability takes two forms: basal permeability at the level of capillaries, which mediates exchange of nutrients and gases, and permeability associated with inflammation, which occurs primarily at the level of post-capillary venules. Transcellular transportation involves the shuttling of molecules with molecular radii greater than 3 nm through the endothelial cell by caveolae via receptor-dependent or -independent mechanisms (182). An important signaling event in this pathway is Src-dependent tyrosine phosphorylation of caveolin-1, which initiates fission of the membrane-bound vesicle, resulting in endocytosis at the apical/luminal membrane, transmigration of the vesicle across the cell, fusion with the basolateral/abluminal membrane, and exocytosis (121, 158, 237). Under basal conditions, tight and adherens junctions interact with the endothelial actin cytoskeleton, allowing mechanical coupling between adjacent endothelial cells and forming a restrictive barrier that prevents transport of macromolecules across the endothelial layer at cell-cell junctions (i.e., paracellular). Endothelial cell morphology also governs the adhesive force coupling adjacent cells. However, in response to certain receptor agonists, including histamine, serotonin, thrombin, and bradykinin, or under certain conditions, such as inflammation or angiogenesis, endothelial cells retract causing a reorganization of the cytoskeleton that allows passage of macromolecules via the paracellular route(54). Dysfunction of the endothelial barrier, for example during inflammation, leads to excessive plasma extravasation. When plasma extravasation exceeds both reabsorption into the blood stream and the ability of the lymphatic system to remove fluid, the result is edema formation in the surrounding tissue.

Formation of the vascular network

The endothelium plays a critical role in the development of the vascular network. The process by which a stable vasculature is created can be subdivided into three categories: vasculogenesis, angiogenesis, and arteriogenesis. Vasculogenesis refers to the process responsible for the formation of a primitive vascular network during embryonic development. In contrast, angiogenesis is the tightly regulated process by which new blood vessels form from pre-existing vessels. The turnover rate of quiescent endothelial cells is typically on the order of hundreds of days. However, in response to proangiogenic factors, such as vascular endothelial growth factor (VEGF), endothelial cells proliferate rapidly, exhibiting a turnover rate of less than 5 days (90). Destabilization of the basal lamina and surrounding extracellular matrix of the mature vessel is required for new endothelial cells to

emigrate. Following this destabilization process, growth factors and chemoattractants promote the proliferation and migration of endothelial cells toward a target location. Arteriogenesis is the process by which newly formed blood vessels stabilize to form a mature blood vessel through recruitment of SMCs or pericytes and generation of an extracellular matrix.

Leukocyte trafficking

Interactions between circulating leukocytes and endothelial cells contribute to the immune response and wound repair. Under basal conditions, leukocytes do not adhere to the endothelium. However, following stimulation of endothelial cells, leukocytes rolling on the luminal surface of the endothelium establish close contact, beginning the recruitment process. This process is dependent on membrane localization of selectin family of adhesion molecules on both cell types. Rolling facilitates leukocyte activation in response to endothelium-derived chemokines. Leukocyte activation results in a conformational change in leukocyte integrins, which interact with their respective adhesion molecule ligands on endothelial cells, resulting in firm adhesion and arrest of rolling. Transendothelial migration of leukocytes occurs primarily through inter-endothelial junctions following disruption of tight and adherence junctions, but transcellular migration has also been observed (313).

Thrombosis

Quiescent endothelial cells release and express certain factors on their luminal surface that confer an anticoagulant quality. In contrast, the basal lamina, which may become accessible to blood components under certain conditions (see later), is strongly thrombogenic, as are endothelial cells stimulated with procoagulant factors. Thus, endothelial cells are important in regulating the equilibrium of thrombus formation.

NO and prostacyclin, which inhibit platelet adhesion and aggregation, are constitutively released by endothelial cells (223). 3-hydroxyoctadecadienoic acid, synthesized via lipoxygenase pathways in resting endothelial cells, inhibit platelet aggregation by acting as a negative regulator for the adhesion receptor vitronectin (35, 36). Membrane-bound ectonucleotidases on cell surface of endothelial cells metabolize ATP and ADP to prevent platelet activation (191, 289). The thrombin receptor thrombomodulin is also expressed on the surface of endothelial cells (226). Binding to this receptor converts thrombin, a highly potent procoagulant, to an anticoagulant enzyme that serves to activate protein C, which halts the coagulation cascade. The cell surface of endothelial cells are enriched with heparin-like sulfated glycosaminoglycan molecules that bind and activate the thrombin inhibitor antithrombin (203).

Disruption of the continuity of the endothelium exposes the highly procoagulant basal lamina, allowing circulating platelets to adhere and initiate hemostasis. von Willebrand factor (vWf), a large, multimeric protein that is synthesized and stored within endothelial cells (129), plays a critical role in initial platelet recruitment and thrombus formation. Following release, vWf binds to exposed collagen, serving as a bridge between platelets and the exposed tissue, and a mediator of endothelial cell-platelet interactions (33, 289). Endothelial cells also synthesize platelet-activating factor (PAF), which further promotes

platelet activation and adherence. Following initiation of coagulation, degranulation of activated platelets is predominantly responsible for further recruitment and aggregation of platelets. However, platelet-derived ADP, ATP, or serotonin can facilitate endothelium production of the arachidonic acid metabolite thromboxane A2, whereas platelet-derived thrombin upregulates vWf and tissue factor to further promote thrombogenesis (210). Once the blood clot has served its purpose, the endothelium induces fibrinolysis by converting inactive plasminogen to active plasmin through tissue-type plasminogen activator or urokinase-type plasminogen activator.

Ca^{2+} as a Second Messenger in the Endothelium

Free cytosolic Ca^{2+} ions are integral components of the signaling cascades that regulate many of the primary functions of endothelial cells, including the synthesis and release of vasoactive factors, control of permeability, cellular proliferation, and angiogenesis. The effects of Ca^{2+} are mediated by factors that directly associate with free Ca^{2+}. Although some effector proteins contain intrinsic Ca^{2+} domains, such as the C2 domain or the EF-hand motif, in most cases, Ca^{2+} associated with the intermediate factor calmodulin, an EF-hand motif-containing Ca^{2+}-binding protein, underlies Ca^{2+}-dependent regulation. Binding of Ca^{2+}/calmodulin to such effectors elicits a number of physiological effects, including eNOS-dependent production of NO (39).

Endothelial cells do not express voltage-dependent K^+, Ca^{2+}, or Na^+ ion channels and thus are nonexcitable cells. In such cells, changes in intracellular Ca^{2+} concentration result from activation of GPCRs that signal through $G_{q/11}$-type G-proteins (G_qPCRs) or responses to mechanical stimuli, with shear stress being the prime example in the endothelium. G_qPCR-mediated increases in intracellular Ca^{2+} occur in a biphasic manner, reflecting an initial release of Ca^{2+} from intracellular stores, primarily from inositol 1,4,5-trisphosphate (IP_3) on the endoplasmic reticulum (ER) membrane, and subsequent influx of Ca^{2+} from the extracellular space through integral plasma membrane ion channels (38,298). The ER accounts for approximately 75% of the Ca^{2+} reserves of endothelial cells, while the remaining 25% is contained within mitochondria (283). Increases in intracellular Ca^{2+} are typically associated with membrane hyperpolarization due to activation of intermediate- and small-conductance Ca^{2+}-sensitive K^+ channels (IK and SK, respectively) (38, 175). GPCR activation may also facilitate entry of Ca^{2+} through receptor-operated channels. Depletion of Ca^{2+} reserves in the ER, either through G_qPCR stimulation or inhibition of the Ca^{2+} pumps responsible for Ca^{2+} re-uptake into the ER, promotes a rise in intracellular Ca^{2+} through store-operated channels, a process known as store-operated Ca^{2+} entry (SOCE).

Heterogeneity of the Endothelium

The endothelium exhibits differences in cellular morphology, protein expression, and function between tissues, segments of the vascular network within the same organ, and among adjacent endothelial cells in a single artery (153). For example, functional IK and SK channels are present endothelial cells from mouse cerebral arteries and arterioles (151) but are absent from cerebral capillary endothelial cells (168). Another illustration is provided by high-resolution Ca^{2+} imaging experiments demonstrating that individual endothelial cells

within the intact endothelium exhibit diffing sensitivity to carbachol and adenosine triphosphate (153). Phenotypic heterogeneity of the endothelium is thought to be a consequence of transcription factor-dependent gene expression in response to the local microenvironment as well as epigenetic modification (5). Transcriptional plasticity limits the utility of cell culture methodology for studying endothelial cell function, as illustrated by a study comparing the transcriptional profile of freshly isolated and cultured porcine coronary and iliac artery endothelial cells. Differences in gene expression between native endothelial cells isolated from the two vascular beds were largely absent from cell exposed to culture conditions (37,334). These findings are supported by a study from Lacorre et al. reporting a loss of 50% of vascular bed-specific genes of human tonsil endothelial cells when cultured *in vitro* (148). Adaptation to cell culture conditions could account for discrepancies between observations made *in vitro* compared with native endothelial cell and *in vivo* studies discussed later in this review.

The TRP Superfamily of Cation Channels

TRP channels were discovered during investigations of Drosophila phototransduction mutants that could see normally in dim light, but behaved as if blind under bright light(58). Subsequent bioinformatic studies revealed the presence of several conserved genes encoding TRP channel proteins in many other organisms, including mammals. Mammalian genomes were found to encode 27 or 28 (depending on species) individual genes encoding distinct TRP subunits, which together constitute the TRP superfamily. The TRP genes and their products have been assigned to six subfami-lies based on sequence homology: ankyrin (TRPA), canonical (TRPC), vanilloid (TRPV), melastatin (TRPM), mucolipin (TRPML), and polycystin (TRPP) (55). TRPC2, important for pheromone sensing in rodents (171), is a nonexpressed pseudogene in humans (326). Transcriptional splice variants have been described for almost all individual TRP proteins (295).

The terminology used for the TRPP subfamily is confusing and requires clarification. Polycystin-1 protein encoded by the *PKD1* gene is often referred to as TRPP1. However, *PKD1* encodes a protein with 11 transmembrane domains that does not form an ion-channel and is unrelated to TRP channels (308). Further, TRPP2, TRPP3, and TRPP5 have been described as products of the *PKD2* (polycystic kidney disease 2) gene, *PKD2L1* (polycystic kidney disease 2-like1) gene and *PKD2L2* (polycystic kidney disease 2-like 2). In this review, TRPP1 refers to *PKD2*, TRPP2 for *PKD2L1*, and TRPP3 for *PKD2L2*.

Additional reviews on TRP channels can be found elsewhere (74,89,197).

TRP Channel Structure

Electron cryo-microscopy (cryo-EM) (162) has been used to reveal the protein structures of many TRP channels at near-atomic resolution (3–4 Å), providing unique insights into channel function and regulation. These studies show that the transmembrane core region of all TRP channels are similar in overall structural organization to voltage-gated K^+ (K_V) channels (167). Fully assembled TRP channels are composed of four subunits symmetrically arranged around a central ion-permeable pore. Each TRP subunit contains six trans-

membrane α-helix domains (S1–S6) and a re-entrant pore loop between the S5 and S6 domains. Distinctive intracellular domains present on the N- and C-termini outside of this core pore-forming region characteristic of each TRP subfamily impact regulation and protein-protein interactions.

TRPV1, the first full-length TRP channel structure to be reported at high-resolution, typifies the TRPV subfamily (41, 163). Six ankyrin repeat domains are present at the intracellular N-terminus followed by a linker region that is close to an α-helix forming part of the intracellular pre-S1 domain. The TRP domain, a 23 to 25 amino acid region that is conserved in many members of the TRP superfamily, is present on the C-terminal side of the pore-forming region, immediately distal to the S6 domain. The function of the TRP domain has not been definitively established, but it has been proposed to be involved in subunit assembly or allosteric regulation of channel activity. The overall structure of TRPV2 channels determined by cryo-EM is nearly identical to that of TRPV1, with subtle differences in the selectivity filter (124, 341). X-ray crystallography and cryo-EM structures of TRPV4 channels from *Xenopus tropicalis* also reveal a shared structural organization with TRPV1 and TRPV2 (63). A unique structural feature of TRPV4 channels is the organization of the S1–S4 domain, which is rotated 90° compared with that in TRPV1 (63). In addition, the selectivity filter of TRPV4 is in an expanded configuration compared with that in TRPV1, possibly accounting for differences in the selectivity for monovalent compared with divalent cations between the two channels (63). The cryo-EM structures of the highly Ca^{2+}-selective channels, TRPV5 and TRPV6, demonstrate that these channels share the overall organization that is common to all TRPV subfamily members (122,231). Unique features of these channels include the α-helical linker region between the S4 and S5 transmembrane domains, which is unstructured in TRPV6 (231). In addition, differences in the cation binding sites of the selectivity filter account for the conspicuous Ca^{2+} selectivity of TRPV5 and TRPV6 compared with TRPV1 and TRPV2 channels (231).

A high-resolution cryo-EM structure of the human TRPA1 channel has also been reported (208). The defining structural feature of TRPA1 is an array of 16 ankyrin repeat domains at the N terminus. Structural analyses also show that specific cysteine and lysine residues (Cys 621, Cys 641, Cys 665, and Lys 710), which are necessary for channel activation by electrophilic compounds, are located in a pre-S1 α-helix located within a solvent-accessible pocket that lies just below the plasma membrane. The pore loop domain between S5 and S6 contains two α-helix domains, and a TRP domain immediately follows S6. A coiled-coil domain, which is proposed to stabilize subunit interactions (208), is present at the N-terminus. Compared with TRPV1, the intracellular region of assembled TRPA1 channels adopts a more compact configu-ration. In addition, the outer pore region of TRPA1 contains two pore helices in contrast with TRPV1, which contains a single helix in this region.

Several studies have reported the full-length structure of the monovalent-cation-selective TRPM4 channel from humans and mice at high resolution (16, 72, 103, 309). The N-terminus of this channel encodes four melastatin homology regions (MHRs)—unique domains that are highly conserved among TRPM subfamily members (16, 309). It is proposed that part of the MHRs in TRPM4 form a nucleotide-binding domain (NBD) that is responsible for ATP-dependent regulation of the channel (103). The MHRs are also

proposed to contain a binding pocket for decavanadate (309), a compound that potentiates TRPM4 channel activity (199). The MHRs contain two ankyrin repeat domains that separate the NBD from a region composed of 12 linker helical domains preceding a pre-S1 helix. The TRP domain adjacent to S6 is atypical and is composed of two helices, one of which forms part of a re-entrant loop. The intracellular C-terminus contains a stretcher helical domain and a coiled-coil helix. The structure of the cold- and menthol-sensing channel, TRPM8, from the collared flycatcher is remarkably similar to that of TRPM4, although the pore diameter is slightly larger (328). The difference in pore diameter compared with the TRPM4 channel may account for the differences in permeability of divalent cations between these channels. The structure of TRPM2 from *Nematostella vectensis* has also been reported and shown to have an organization similar to that of TRPM4 and TRPM8, with a few notable differences (337). The C-terminus of TRPM2 contains a NUDT9-homology domain that binds ADP-ribose and is involved in regulation of channel activity. In addition, the ionic pore of TRPM2 channels has a large diameter and a greater negative surface charge compared with that of TRPM4, possibly accounting for the larger unitary conductance and higher permeability of TRPM2 for divalent cations. TRPM7, important for Mg^{2+} homeostasis, shares its overall organization with that of other TRPM channels (71), but also contains a kinase domain at the C-terminus (323). Notably, a single amino acid difference in the selectivity filter of TRPM7 (^{1045}FGE^{1047}) compared with that of TRPM4 (^{975}FGQ^{977}) likely imparts the permeability of TRPM7 to divalent cations (71).

High-resolution structures of TRPC3, TRPC4, and TRPC6, members of the canonical TRP subfamily, have been reported (17, 70, 85, 272, 292). TRPC channels share a similar overall structural organization that includes four ankyrin repeat domains and several linker helical domains in the N-terminus immediately prior to the pre-S1 helix. A pre-S1 “elbow” domain embedded within the lipid bilayer is present in all TRPC channel subtypes and may contribute to lipid-dependent channel activation (85, 272, 292). The S3 domain of TRPC3 and TRPC6 is longer than that of TRPV, TRPA1, and TRPM, and may serve as a binding site for extracellular molecules (85). Intracellular rib and vertical helix domains, which form an inverted “L” shape, are present at the C-terminus immediately following the TRP domain region.

The TRPML and TRPP subfamilies form the group II TRP channels (287). Cryo-EM characterizations of the structures of these channels indicate an overall architecture similar to that of other TRP channels, but with the inclusion of a large extracellular polycystin-mucolipin domain (PMD; also referred to as the TOP domain) between the S1 and S2 trans-membrane domains (46,101,116,157,236,242,263,306,336, 338). It has been proposed that the PMD acts as a physical cover of the pore that can interact with extracellular factors to regulate channel activity (242). The structures of TRPP1 (*PKD2*) and TRPP2 (*PKD2L1*) channels are remarkably similar, with differences in the selectivity filter that account for differences in monovalent versus divalent ion permeability.

All TRP subtypes, with the possible exception of TRPC1 (256), appear to form functional homomeric cation channels. In addition, multiple TRP subtypes can form heteromeric channels composed of two (or even three) related types in various cell types *in vivo*. Formation of heteromeric channels is best characterized for TRPC channels. TRPC1 can

form heteromeric channels with TRPC3, TRPC4, TRPC5, TRPC6, and TRPC7; TRPC4 and TRPC5 can heteromultimerize with each other; and combinations of TRPC3, TRPC6, and TRPC7 can form heteromeric channels. Other arrangements include channels composed of the closely related sub-types TRPV5 and TRPV6, TRPM6 and TRPM7 (156), and TRPML1 and TRPML3. There is less evidence supporting interactions between channels from different subfamilies, although formation of TRPC1/TRPV4 heteromeric channels in the endothelium (172) and TRPV1/TRPA1 channels in sensory nerves, and other combinations, has been reported. Notably, heteromeric channels display conduction properties and regulatory mechanisms that are distinct from those of homomeric channels.

TRP Channel Currents

All TRP channels conduct cations and are impermeable to anions, but the relative permeability of specific superfamily members to different types of cations varies widely. TRPM4 and TRPM5 channels are selective for monovalent cations and are essentially impermeable to divalent cations (118, 149, 198, 219), whereas TRPV5 and TRPV6 channels are highly selective for Ca^{2+} ions compared with Na^+ ions (P_{Ca}:P_{Na} ~100:1) (200, 288, 332). The remaining TRP channels conduct mixed currents composed of monovalent (primarily Na^+ and K^+) and divalent (primarily Ca^{2+}) cations. The specific fractional composition of these mixed cationic currents establishes the physiological effects of channel activity on cellular function. For example, TRPA1, which conducts the highest fractional Ca^{2+} current of all the TRP channels (with the exception of TRPV5 and TRPV6) (97, 135), can increase global intracellular [Ca^{2+}] and/or generate a Ca^{2+} microdomain at the mouth of the channel to initiate Ca^{2+}-sensitive signaling pathways. In contrast, TRPC subfamily members conduct current with a small Ca^{2+} fraction. Under physiological conditions, the resting membrane potential of endothelial cells in intact blood vessels is hyperpolarized relative to the Na^+ equilibrium potential (E_{Na} = +60 mV) and depolarized relative to the K^+ equilibrium potential (E_K = −88 mV), and TRPC channels primarily conduct inward Na^+ currents. Interestingly, the relative permeability of K^+ ions can influence the fractional Ca^{2+} composition of TRP channel currents in physiological ionic gradients (114). For example, where the permeability of TRP channels to Na^+ and K^+ ions is similar ($P_K = P_{Na}$), the reversal potential of the monovalent portion of the current is ~0 mV because no concentration gradient exists between total monovalent cations; thus, the fractional Ca^{2+} current is determined by the P_{Ca} of the conducting channel. However, if $P_{Na} > P_K$, the reversal potential for the monovalent fraction is depolarized (compared with the case of $P_K = P_{Na}$), which reduces the driving force for Ca^{2+} influx and diminishes the Ca^{2+} fraction of the current. Conversely, if $P_K > P_{Na}$, the cationic reversal potential is more hyperpolarized and the driving force for Ca^{2+} influx is increased. Differential K^+ permeability may explain why the Ca^{2+} fraction of TRPV4 currents is greater compared with TRPC6 currents under physiological conditions, despite a similar P_{Ca}:P_{Na} ratio, established using ion-substitution methods (114,119,258).

Spatially distinct Ca^{2+} signals representing Ca^{2+} influx through single TRP channels or clusters of functionally coupled channels can be recorded from cells loaded with Ca^{2+} indicators dyes, such as Fluo-4-AM (96), or in cells from animals expressing genetically encoded Ca^{2+} biosensor proteins, such as GCaMP (130), using fluorescence microscopy

techniques. This approach has been used to record the activity of TRP channel subtypes that conduct mixed currents with the highest Ca^{2+} fraction: TRPV4, TRPV3, TRPV1, and TRPA1. These optically detected Ca^{2+} signals are generically referred to as “sparklets” (297), and are further defined by the name of the conducting channel (e.g., “TRPV4 sparklets”). TRP channel sparklets have been recorded from endothelial cells using spinning disc confocal microscopy and total internal reflection fluorescence (TIRF) microscopy (267). The ability of TIRF microscopy to detect fluorescent Ca^{2+} signals at the plasma membrane is based on the generation of a low-energy evanescent illumination field when excitation light is reflected away from a glass surface supporting the cells under investigation. The low-energy TIRF field illuminates to a depth of only ~100 nm, producing an excellent signal-to-noise ratio. TRPV3, TRPV4, and TRPA1 sparklets have been recorded from the endothelium using TIRF microscopy (215,266,267). The thin morphology of endothelial cells (~0.3 μm) facilitates recording of transient subcellular Ca^{2+} signals using confocal imaging. This method has been used to record TRPV4 sparklets in intact arteries isolated from mice expressing GCaMP Ca^{2+} biosensors exclusively in the endothelium (253,254). In these studies, TRPV4 sparklet amplitudes were shown to display a quantal distribution and exhibit cooperative gating, suggesting that the endothelial TRPV4 channel is present as a four-channel metastructure (253). This coupled gating arrangement amplifies the initial Ca^{2+} signal created by TRPV4 channel activation and, combined with the biophysical properties of the channel, which favor Ca^{2+} influx, produces large-amplitude Ca^{2+} signals in the endothelium. An elegant study from the Santana laboratory that used a combination of TIRF microscopy and patch-clamp electrophysiology to quantify these signals demonstrated that, although the basal channel activity was low, the Ca^{2+} signal mass of a single TRPV4 sparklet was ~100-fold greater than that of a Ca_V 1.2 L-type Ca^{2+} channel sparklet (184). The modal amplitudes of TRPA1 and TRPV3 sparklets are twofold to threefold greater than those of TRPV4 sparklets (215), suggesting that all of these channels can act as a significant point source of Ca^{2+} influx capable of generating high local intra-cellular Ca^{2+} concentrations, an essential feature underlying the concept of Ca^{2+} microdomains. Although the amplitude of individual TRP channel sparklets is large, the total number of active sites per cell is surprisingly low. For example, both Sonkusare et al. (253) and Sullivan et al. (266) showed that only a few TRPV4 sparklets (approximately 3–8 sites/cell) are active in individual endothelial cells during maximal agonist stimulation. The biophysical and pharmacological properties of endothelial TRP channels are summarized in Table 2.

Functional Roles of TRP Channels in the Vascular Endothelium

Multiple TRP channel subunits have been reported to be expressed in endothelial cells (265). However, only 11 of these have been reported to have a functional role (summarized in Table 1).

TRP channels and endothelial cell store-operated Ca^{2+} entry

SOCE is a specialized Ca^{2+} influx pathway initiated by the depletion of intracellular Ca^{2+} from stores within the ER (250). This mechanism is thought to provide a means to rapidly replenish the empty Ca^{2+} stores and maintain elevated levels of free cytosolic Ca^{2+}. A

landmark study using unbiased high-throughput siRNA screens discovered that two proteins, stromal interacting protein 1 (STIM1) and the Ca^{2+}- selective plasma membrane ion channel Orai1, are required for SOCE (290). Extensive work characterizing the properties of STIM1 has demonstrated that STIM1 is a transmembrane protein with a Ca^{2+}-binding EF-hand domain exposed within the ER and an Orai1-interacting domain within the cytosol (62,251). Upon Ca^{2+} store depletion, the interaction between the EF hand domain and free Ca^{2+} within the ER is reduced, resulting in the redistribution of STIM1 dimers into clusters. These clusters form peripheral coupling sites between the ER and plasma membrane that engage Ora1 channels, which in turn mediate extracellular Ca^{2+} influx (300).

Many early studies supported the concept that TRPC channels are necessary for SOCE in endothelial cells. For example, SOCE involving TRPC1 (32) and TRPC4 (91) channels has been implicated in endothelium-dependent vasodilation (91) and increases in microvascular permeability (53, 280, 314). A study by Cioffi et al. provided evidence that heteromeric channels containing TRPC4 and TRPC1 subunits are present in cultured rat pulmonary artery endothelial cells and directly interact with Orai1, and further showed that Oria1 activity is necessary for the formation of inter-endothelial cell breaches (53). Investigations by Sundivakkam et al. demonstrated that STIM1 interacts with TRPC1 and TRPC4 to form a store-operated channel in cultured endothelial cells (268). However, the potential involvement of TRP channels in SOCE has become a matter of controversy (217, 249). The original shotgun siRNA screening study that identified STIM1 and Orai1 as obligatory components of SOCE also demonstrated that SOCE was not affected by down-regulation of TRP channels (290). Strong evidence from several laboratories further supports the concept that endothelial cell SOCE can occur without TRPC1, TRPC4, or TRPC6 expression (1,243,284). One report indicates that Ca^{2+} release from internal stores persists in Purkinje neurons from TRPC1/C4/C6 triple-knockout mice, suggesting that these channels are not required for the SOCE-dependent maintenance of intracellular stores (110). Abdullaev et al. reported that knockdown of either TRPC1 or TRPC4 in cultured human umbilical vein endothelial cells (HUVECs) had no effect on SOCE or the Ca^{2+} release-activated Ca^{2+} current, and speculated that evidence for TRPC channel involvement in SOCE may be an artifact of the patch-clamp conditions employed in some studies, which may have resulted in phospholipase C (PLC)-mediated activation of TRPC channels downstream of store depletion (1).

Thus, these discrepancies may reflect methodological issues. The majority of studies supporting a role for TRPC channels in SOCE-mediated Ca^{2+} influx were performed using cultured endothelial cells or overexpression systems. Although the effects of culture conditions on the phenotypic properties of endothelial cells, notably including TRPC1 and TRPC4 expression, were not investigated in these studies, it is possible that such phenotypic transformations might have influenced SOCE and permeability regulation. This possibility is supported by a report from Bergdahl et al. demonstrating that culture conditions significantly increase TRPC1 and TRPC6 expression and SOCE in SMCs (26). A study by DeHaven et al. questioned the ability of TRPC channels to interact with either STIM1 or Orai1, and concluded that STIM1/Orai1 and TRPC signaling occur independently of each another and are not localized to the same microdomains (61). It should be noted that this

latter study was conducted using cultured HEK cells, thus the direct applicability of these findings to endothelial cells is unclear and will require additional investigation.

TRP channels and endothelium-dependent vasodilation

The specific Ca^{2+}-influx mechanisms associated with endothelium-dependent vasodilation were unknown until the discovery of TRP channels (196). Here, we review evidence supporting the involvement of TRPA1, TRPV4, TRPV3, TRPV1, TRPC1, TRPC3, TRPC4, TRPC5, and TRPP1 channel activity in endothelium-dependent vasodilation.

TRPA1—TRPA1 channels have a large unitary conductance (~98 pS) (192), are more permeable to Ca^{2+} than Na^{+} (P_{Ca}:P_{Na} = 7.9) (135), and conduct mixed cation currents with a high Ca^{2+} fraction. TRPA1 channels are activated by electrophilic compounds, including dietary molecules, such as allicin, a pungent compound found in garlic (173); allyl isothiocyanate (AITC), a derivative of mustard oil (19); and cinnamaldehyde from cinnamon (128). TRPA1 is present in sensory neurons, where it acts as a receptor for these pungent substances. This channel may also be activated by noxious cold (257), but this has been disputed (22,131).

Recent studies demonstrate that TRPA1 channel activity contributes to vascular regulation (for review, see (73)). Activation of TRPA1 in perivascular nerves was shown to evoke dilation of rat mesenteric arteries through a signaling pathway that ultimately promoted the release of the neuropeptide, calcitonin gene-related peptide (CGRP) (23). Noxious cold has been shown to modulate blood flow within the mouse hindpaw in a manner that is dependent on TRPA1 activity(15). Although the precise cellular location of TRPA1 was not determined in this latter study, the increase in blood flow was thought to occur through activation of TRPA1 in sensory neurons. An *in vivo* study demonstrated that systemic TRPA1 activation results in a profound hypotensive response that was attributed to stimulation of TRPA1 in sensory neurons (218). However, basal blood pressure did not differ between control and global $TRPA1^{-/-}$ mice (30), suggesting that TRPA1 activity is not involved in the regulation of systemic blood pressure.

Work from Earley and colleagues indicated that TRPA1 channels are present in endothelial cells of cerebral arteries of rodents and humans (75), but are not detectable in mesenteric, coronary, dermal, or renal arteries (267). TRPA1 activation with AITC was shown to induce endothelium-dependent dilation and SMC membrane hyperpolarization in pressurized mouse cerebral arteries, an effect that was blocked by the selective TRPA1 antagonist, HC-030031 (75). AITC-induced responses were unaffected by NOS and COX inhibition, but were diminished by inhibition of IK, SK and inwardly-rectifying K^{+} (K_{IR}) channels, suggesting that TRPA1 mediates dilation through an EDH mechanism (75). Furthermore, TRPA1 channels were found to colocalize with IK channels and were abundant at myoendothelial projections (MEPs), sites of close contact between endothelial cells and SMCs, in the cerebral endothelium (75). Qian et al. further characterized the TRPA1-dependent dilation of isolated rat cerebral arteries, demonstrating that activation of TRPA1 produced large, dynamic Ca^{2+} signals in the endothelium of cerebral arteries that were generated by IP_3 receptor (IP_3R)-mediated release of Ca^{2+} from the ER (221). The

amplitude, spatial spread, and duration of these Ca^{2+} signals were distinct from those of other types Ca^{2+} signals, including TRPA1 sparklets and Ca^{2+} pulsars. In addition, these large-amplitude Ca^{2+} signals were shown to be associated with a TRPA1 channel activity-dependent vasodilatory response (221).

It is unlikely that TRPA1 channel activity in the cerebral vasculature is regulated by AITC or allicin under physiological conditions. TRPA1 channels are activated by reactive oxygen species (ROS) such as H_2O_2, as well as by ROS-derived metabolites generated by peroxidation of ώ6 polyunsaturated fatty acids, including 4-hydroxy-2-nonenal (4-HNE), 4-oxononenal (4-ONE), and 4-hydroxylhexenal (4-HHE) (10,286). Sullivan et al. demonstrated that TRPA1 sparklets induced by AITC can be recorded from cerebral artery endothelial cells. The modal amplitude of unitary TRPA1 sparklets was approximately twice that of TRPV4 sparklets, due to the larger unitary conductance and Ca^{2+} fraction of currents conducted by TRPA1 (267). Moreover, TRPA1 was found to colocalize with the ROS-generating enzyme NOX2 (NADPH oxidase isoform 2) in the endothelium of isolated cerebral arteries; notably, stimulation of NOX2 was shown to increase the frequency of TRPA1 sparklets and induce dilation (267). These responses were dependent on the generation of extracellular H_2O_2 and hydroxyl radicals and the subsequent production of lipid peroxidation products, which activate TRPA1 channels. Thus, ROS and ROS metabolites appear to be endogenous agonists of TRPA1 in the cerebral endothelium. These results suggest TRPA1 channels are important for cerebral vascular regulation during pathophysiological conditions associated with increased ROS production and oxidative stress. Further support for this concept was provided by a report by Pires and Earley showing that activation of TRPA1 channels in the cerebral endothelium by mitochondrial ROS dilated mouse cerebral pial arteries and parenchymal arterioles in response to hypoxia (214). This study also demonstrated that activation of TRPA1 channels in the cerebral endothelium reduced infarct volume following ischemic stroke (214).

TRPV4—TRPV4 channels are more permeable to Ca^{2+} than Na^+ ions (P_{Ca}:P_{Na} = ~6:1) (258) and conduct a mixed cation current with a moderately high Ca^{2+} fraction. The channel is activated by cell swelling induced by hypotonic conditions (164) as well as by several chemical agonists (279,301). TRPV4-mediated currents exhibit slight outward rectification, and are reported to have a unitary conductance of ~30–60 pS at −60 mV and ~88–100 pS at +60 mV (258, 301, 303). The availability of the selective, small-molecule agonists 4α-PDD (301) and GSK1016790A (279), and inhibitors RN-1732 (293) and HC-067047 (84), have enabled extensive characterization of the role of TRPV4 channels in endothelium-dependent vasodilation.

Nilius and colleagues provided the first functional evidence of TRPV4 channels in the endothelium of systemic arteries, demonstrating that TRPV4 channels are present in freshly isolated mouse aortic endothelial cells and could be activated by the phorbol compound 4α-PDD (301), heat (25–43°C) (303), and epoxyeicosatrienoic acids (EETs) (302). A subsequent study by Vriens and colleagues established that EETs generated by the CYP2C9 epoxygenase isoform could activate TRPV4 in an autocrine manner in cultured mouse aortic endothelial cells (294). This finding suggested that endogenous production of EETs has autocrine and paracrine effects that facilitate Ca^{2+} entry through TRPV4 channels. A report

from Earley et al. demonstrated that exogenous application of 11,12-EET induced dilation of isolated pressurized mesenteric arteries from wild-type mice, but not TRPV4$^{-/-}$ mice (79). This study further demonstrated that TRPV4 activation induced hyperpolarization of the surrounding SMCs that was sensitive to inhibition of endothelial cell IK and SK channels and SMC large-conductance Ca^{2+}-activated K^+ (BK) channels, but was not affected by NOS or COX inhibition. Interestingly, approximately 50% of this response was retained following mechanical disruption of the endothelium, indicating that TRPV4 channels present on both endothelial cells and SMCs contribute to the dilation induced by the application of EETs (79). Consistent with this, several other studies have shown that activation of TRPV4 channels in cerebral artery SMCs causes dilation (77, 184, 270). It appears that TRPV4 channels stimulate dilation by functionally coupling with Ca^{2+}-activated IK and SK channels in rat cerebral endothelial cells and with ryanodine receptors in SMCs (77). In this latter study, activation of SMC TRPV4 channels by EETs was shown to induce dilation by stimulating ryanodine receptor activity through a Ca^{2+}-induced Ca^{2+}-release (CICR) mechanism, with subsequent activation of BK channels and hyperpolarization of the SMC membrane potential. Interestingly, endothelial BK channels have also been implicated in dilatory responses through an entirely different mechanism. Naik et al. reported that endothelium-dependent dilation of rat mesenteric arteries in response to the hydrogen sulfide H_2S donor NaHS was sensitive to blockade of TRPV4 and BK channels (193). A related study showed that acetylcholine (ACh)-induced dilation of mesenteric arteries isolated from rats subjected to hypoxia (0.5 ATM) for 48 h was diminished by block of TRPV4 channels (194). Further, dilation of arteries from hypoxic rats in response to the selective TRPV4 agonist GSK1016790A could only be inhibited by simultaneous block of IK, SK, and BK channels (194). However, the presence of functional BK channels in native endothelial cells has been disputed based on patch-clamp electrophysiological studies, which failed to find BK currents in native endothelial cells (150). This view is currently held by most investigators working in this area.

TRPV4 channels are also localized to the endothelium of pulmonary arteries. The TRPV4 agonist GSK1016790A was shown to induce an endothelium-dependent relaxation of rat pulmonary artery rings that was sensitive to inhibition of TRPV4 with the selective antagonist HC-067047 and blockade of IK and SK channels (264). A similar study reported that GSK1016790A-induced dilation of rat pulmonary arteries was dependent on NOS activity, as well as EDHF or EDH mechanisms (3). A recent report proposed that increased NO production following TRPV4 activation in pulmonary arteries not only dilates the underlying smooth muscle layer, but also is involved in a negative feedback loop that reduces TRPV4 activity through protein kinase G-mediated TRPV4 phosphorylation (178). Interestingly, this signaling cascade was not found to operate in mesenteric arteries, a vascular bed in which these authors proposed that TRPV4 activation is not associated with NO production (178). However, ACh-induced relaxation of rat pulmonary artery rings was not affected by TRPV4 inhibition with two different antagonists (HC-067047 and RN-1734), suggesting that TRPV4 channels are not involved in muscarinic agonist-induced relaxation in this vascular segment (264). In agreement with these findings, Pankey et al. showed that the reduction in pulmonary arterial pressure of rats in response to intravenous (i.v.) administration of ACh was unaffected by systemic administration of the TRPV4 antagonist

GSK2193874 (204). Interestingly, Ke et al. reported that endothelial TRPV4 stimulation with the agonist GSK1016790A significantly increased pulmonary vascular resistance in isolated perfused lungs (136). Activation of smooth muscle TRPV4 in the absence of endothelial signaling may also contribute to increased pulmonary artery pressure (204). Overall, these studies suggest that, although pharmacological stimulation of TRPV4 in the pulmonary artery endothelium can cause endothelium-dependent dilation, the physiological role of this channel in the regulation of the pulmonary vasculature remains unclear.

Multiple studies have implicated TRPV4 in endothelium-dependent relaxation of systemic arteries in response to muscarinic (59, 227, 333) and purinergic receptor (178) stimulation. Saliez et al. reported that EDHF/EDH- and NO-dependent relaxation of mesenteric arteries following carbachol application was attenuated in isolated mesenteric arteries from TRPV4$^{-/-}$ mice compared with those from wild-type animals (227). Interestingly, they further demonstrated that TRPV4 is localized in distinct subcellular microdomains with caveolin-1 and connexins. A study by Goedicke-Fritz and colleagues further suggested compartmentalization of TRPV4, and demonstrated that the channel is spatially coupled to caveolin-1 and SK channels, but interestingly not to IK channels (98). Gutterman and colleagues confirmed that ACh-induced dilation was blunted in isolated pressurized mesenteric arteries from TRPV4$^{-/-}$ mice and further demonstrated that Ca^{2+} influx and NO production induced by ACh in endothelial cells of freshly isolated mesenteric and carotid arteries from TRPV4$^{-/-}$ mice was diminished compared with that in wild-type controls (333). Receptor-dependent activation of TRPV4 has been noted in other vascular beds as well, including by Senadheera et al., who showed that blockade of TRPV4 with RN-1734 blunts ACh-induced dilation of rat uterine radial arteries (239). Working with freshly isolated rat cerebral pial arteries, Marrelli and colleagues found that activation of purinergic receptors with UTP stimulated Ca^{2+} influx in the endothelium and induced a subsequent vasodilation that was attenuated by inhibition of phospholipase A_2 (PLA_2), which liberates arachidonic acid from the plasma membrane, and by the non-selective TRPV antagonist ruthenium red (177). These data suggest that arachidonic acid generation activates TRPV4-mediated Ca^{2+} influx, most likely through the production of EETs, leading to dilation through EDHF or EDH mechanisms. The Nelson laboratory showed that muscarinic receptor activation increases TRPV4 sparklet activity and causes dilation of mouse mesenteric arteries, effects that are blunted by blocking IK and SK channels, providing evidence that muscarinic receptor signaling-induced TRPV4 sparklets activate an EDH vasodilator pathway (253). A recent study by Harraz and colleagues revealed a potentially novel mechanism for activation of TRPV4 channels in endothelial cells downstream of G_qPCRs (109). This study showed that TRPV4 channels in freshly isolated mouse brain capillary endothelial cells are tonically inhibited by phosphatidylinositol 4,5-bisphosphate (PIP_2) in the plasma membrane. Activation of G_qPCRs and subsequent hydrolysis of PIP_2 by phospholipase C relives this inhibition and activates TRPV4 channel activity. These finding provide a universal mechanism for activation of TRPV4 channels downstream of G_qPCRs. A recent study Hong et al. implicated TRPV4 in heterocellular communication between SMCs and endothelial cells of isolated small mouse mesenteric arteries (120). They proposed that stimulation of G_qPCR signaling pathways following adrenergic or thromboxane A2 receptor activation stimulates endothelial TRPV4 sparklets within MEPs to inhibit phenylephrine-

induced contraction (120). The authors hypothesized that, upon G_q activation, IP_3 translocates from SMCs to the endothelium via gap junctions to activate TRPV4, thereby serving as a negative feedback pathway that counteracts agonist-induced contraction. However, Senadheera et al. were unable to detect TRPV4 expression within gaps in the internal elastic lamina (IEL) of uterine arteries from control or pregnant rats (239), suggesting that signaling mechanisms and cellular localization patterns of TRPV4 may be specific to particular vascular beds.

Dora and colleagues proposed a mechanism in which Ca^{2+} signaling through TRPV4 triggers CICR from the ER(18). They demonstrated that TRPV4 channels form clusters within MEPs of rat cremaster and mesenteric arteries that are spatially coupled with Ca^{2+}-release sites on the ER. Interestingly, the frequency of spontaneous Ca^{2+} release from the ER was low at physiological pressures (80 mmHg), but was increased by reducing the intraluminal pressure to less than 50 mmHg, an effect that was blunted by TRPV4 inhibition. TRPV4 antagonism was also shown to produce a modest increase in spontaneous myogenic tone development at low levels of intraluminal pressure (20 and 40 mmHg) (18), suggesting that this channel mediates an ER Ca^{2+}-release-dependent mechanism that induces vasodilation at low levels of intraluminal pressure. The authors proposed that Ca^{2+} influx through TRPV4 channels at low intraluminal pressures is amplified by Ca^{2+} release from IP_3Rs on the ER and serves to generate large-amplitude Ca^{2+} signals, which in turn activate EDH to suppress myogenic constriction (18). Because this mechanism operates at subphysiological levels of intraluminal pressure, its relevance is not immediately apparent. It is plausible that activation of endothelial cell TRPV4 channels at low intraluminal pressure contributes to a precipitous drop in blood pressure, for example during hypovolemic shock.

Patch-clamp experiments in immortalized cell lines have shown that TRPV4 currents can be induced by exposing cells to hypotonic conditions, which cause swelling and stretching of the plasma membrane (164). These observations have led to the hypothesis that TRPV4 channels are mechanosensitive and may act as sensors of blood flow and shear stress in the endothelium and play a role in mediating flow-induced vasodilation. In support of this concept, Kohler and colleagues reported that dilation of rat gracilis arteries following exposure to the TRPV4 agonist 4α-PDD or application of shear stress was attenuated by the non-selective TRPV blocker ruthenium red (144). Interestingly, flow-induced dilation was attenuated by NOS inhibition, whereas 4α-PDD-induced dilation was unaffected by NOS inhibition, but was sensitive to IK and SK channel blockade (144). A follow-up study from this group provided additional support for this hypothesis, demonstrating that shear stress-induced dilation was absent in carotid arteries of $TRPV4^{-/-}$ mice, while vasodilation in response to agonist stimulation was unchanged (111). Additional studies have reported that flow-induced dilation of mouse mesenteric arteries is significantly attenuated in arteries from $TRPV4^{-/-}$ mice and that flow-dependent Ca^{2+} influx in cultured human coronary artery endothelial cells is dependent on TRPV4 (183). Flow-induced dilation of human coronary arteries is blunted by ruthenium red, the selective TRPV4 blocker RN-1732, and by small interfering RNA (siRNA)-mediated knockdown of TRPV4 expression (34). In cultured HUVECs, shear stress was reported to increase TRPV4 translocation to the plasma membrane (21) within distinct microdomains associated with caveolin and SK channels (170). However, a recent study by Darby et al. using an *ex vivo* preparation of rat cremaster

arterioles suggested that shear stress sensitizes TRPV4 channels independent of any effects on its cellular localization (59). Arterioles conditioned to shear stress are more sensitive to relaxation produced the TRPV4 agonist GSK1016790A (59). The authors of this latter study further demonstrated that TRPV4 antagonism significantly impaired ACh-induced dilation in shear-exposed arterioles, but not in arterioles that did not experience shear stress(59). Collectively, these observations provide evidence for a role of TRPV4 channels in shear stress-induced responses in *ex vivo* models.

Further investigations on the mechanosensitivity of TRPV4 demonstrated that these channels are not directly activated by suction applied to the plasma membrane of transfected HEK-293 cells in cell-attached patch-clamp experiments (258), suggesting that these channels lack intrinsic mechanosensitivity. In fact, several studies now support the concept that activation of TRPV4 channels during cell swelling occurs indirectly as the result of force-sensitive signaling pathways. Loot et al. proposed that EETs link TRPV4 activation to the flow-sensor function, as evidenced by the fact that inhibition of CYP epoxygenases, which are responsible for EETs production, attenuated flow-induced dilation of mouse carotid arteries (169). These findings are further supported by reports that cell-swelling induced activation and Ca^{2+} entry through TRPV4 channels are blocked by inhibition of CYP2C9 in cultured mouse aortic endothelial cells (294) and that flow-induced dilation of mouse carotid arteries is impaired by inhibition of PLA_2 (111). Collectively, these studies support a mechanism in which shear indirectly activates TRPV4 through PLA_2-mediated liberation of AA and its subsequent conversion to EETs by CYP2C9. However, the nature of the endothelial cell flow sensor responsible for initiation of this pathway has not yet been identified. A potential candidate is the angiotensin II (Ang II) type 1 receptor (AT_1R). AT_1R receptors are expressed in the endothelium and can be activated by mechanical stimuli independently of Ang II, but it is not known if AT1Rs are linked to CYP epoxygenase activity. Although AT_1Rs are functionally coupled to TRPV4 channels in vascular SMCs (184), this has yet to be shown in endothelial cells. Another potential candidate is GPR68, a class A rhodopsin-like G_qPCR that is required for shear stress responses and flow-induced dilation in third-order mouse mesenteric arteries (321). Interactions between GPR68 and TRPV4 channels in the endothelium have not been demonstrated.

Although many of the studies cited above used *ex vivo* preparations to demonstrate that endothelial TRPV4 channels contribute to vascular regulation in response to various stimuli, these data are at odds with several *in vivo* studies reporting little involvement of TRPV4 channels in cardiovascular control. *S*eparate reports demonstrated that systemic i.v. administration in mouse, rat and dog of the TRPV4 agonist GSK1016790A produces a significant reduction in mean arterial pressure (MAP) that is independent of changes in cardiac output (204,307), indicative of reduced total peripheral resistance (TPR). Administration of 4α-PDD (i.v.) in wild-type mice was also reported to reduce MAP, a response that is absent in $TRPV4^{-/-}$ mice (333). These findings suggest that a loss of TRPV4 function *in vivo* increases TPR and consequently increases MAP. However, basal MAP was reported to be unchanged in $TRPV4^{-/-}$ mice compared with wild-type mice (79, 333), or slightly decreased (201). Moreover, acute or chronic (8 days) administration of selective TRPV4 antagonists (GSK2193874 or GSK2263095) in rats had no effect on MAP or heart rate (278), suggesting the lack of involvement of TRPV4 on basal blood pressure

regulation. Although Zhang et al. reported that i.v. administration of ACh blunted the reduction in MAP observed in TRPV4$^{-/-}$ mice (333), Pankey et al. reported that i.v. ACh had no effect on the reduction in MAP induced by inhibition of TRPV4 channels with GSK2193974 (204). The reasons for these discrepancies are currently unknown. However, one study observed a blunted blood pressure reduction following bolus ACh administration(i.v.) in TRPV4$^{-/-}$ mice, suggesting the potential importance of TRPV4 signaling *in vivo* (333). Collectively, these *in vivo* studies suggest that that TRPV4 channels in the endothelium are minimally active under basal conditions and exert only modest, localized effects on cardiovascular hemodynamics. It is possible that that inconsistencies between *in vivo* and *ex vivo* findings may reflect restriction of the actions of TRPV4 to specific vascular beds and potential compensation for loss of channel activity by other regions in an *in vivo* setting, and may be addressed with the aid of cell-specific knockout animals.

TRPV3—TRPV3 channels have a large unitary conductance (~150–200 pS) (51) and are highly permeable to Ca^{2+} relative to Na^+ (P_{Ca}:P_{Na} = ~12:1) (320). TRPV3 is activated by innocuous heat (320) and dietary monoterpenes, including carvacrol (a derivative of oregano), eugenol (clove oil), and thymol (a compound found in thyme) (319). TRPV3 channels are expressed in the skin, and oral and nasal epithelium, and are involved in chemosensation in these tissues (319).

Earley et al. discovered that TRPV3 channels are present in the endothelium of rat cerebral arteries and further showed that carvacrol activated TRPV3-like cation currents and increased intracellular Ca^{2+} levels in freshly isolated endothelial cells of isolated rat cerebral arteries, effects that were sensitive to inhibition with the nonselective TRPV3 blocker ruthenium red (76). Carvacrol application was also shown to evoke endothelium-dependent dilation of isolated cerebral arteries through a mechanism that was independent of NOS and COX activity, but sensitive to block of SK, IK, and K_{IR} channels, suggesting the Ca^{2+} influx through TRPV3 evokes an EDH response (76). A follow-up study by Pires et al. demonstrated that carvacrol induced an increase the frequency of TRPV3 sparklets in native endothelial cells of mouse cerebral pial arteries and parenchymal arterioles that was sensitive to TRPV3 blockade with isopentenyl pyrophosphate (215). The unitary amplitude of TRPV3 sparklets was greater than that of TRPV4 and TRPA1 sparklets. Carvacrol produced a robust dilation of isolated pressurized parenchymal arterioles that was sensitive to endothelium disruption, TRPV3 inhibition with isopentenyl pyrophosphate, and IK and SK channel blockade (215). It should be noted that Murphy et al. reported that carvacrol induced an endothelium-independent dilatory effect in isolated rat uterine radial arteries that was sensitive to TRPV3 inhibition, suggesting that TRPV3 is expressed in SMCs in this vascular bed (190).

The endogenous mechanisms involved in the regulation of TRPV3 channels in the endothelium have not been identified.

TRPV1—The unitary conductance of TRPV1 is 35 pS at −60 mV and 77 pS at +60 mV, and these channels are more permeable to Ca^{2+} than Na^+ ions (P_{Ca}:P_{Na} = 9.6) (42). TRPV1 channels are activated by noxious heat and capsaicin, a substance found in hot peppers (42).

Several studies have attempted to demonstrate the involvement TRPV1 channels in endothelium-dependent vasodilation. Work from Yang et al. demonstrated that Ca^{2+} influx through TRPV1 channels induced by capsaicin resulted in increased phosphorylation of eNOS via protein kinase A and subsequent elevation in NO production in mouse aortic endothelial cells (325). This study also demonstrated that capsaicin caused endothelium-dependent relaxation of isolated mesenteric arteries from wild-type mice, a response that was absent in arteries from $TRPV1^{-/-}$ mice (325). Poblete et al. reported that the cannabinoid receptor agonist anandamide and capsaicin stimulated NO production and release in the rat mesenteric bed, an effect that was sensitive to TRPV1 inhibition (216). Surprisingly however, this response was not associated with any dilatory effects. Bratz et al. observed capsaicin-dependent dilation of coronary arteries from pigs, a response that was diminished by the TRPV1 antagonist capsazepine and by inhibition of NO production (31), supporting the concept that Ca^{2+} influx through TRPV1 channels stimulates eNOS activity. Similar observations have been reported for rat coronary arteries (281). Ching et al. reported that Ca^{2+} influx through TRPV1 channels in bovine aortic endothelial cells allows direct interaction between TRPV1 and eNOS, thereby facilitating NO production (50). The formation of this TRPV1-eNOS complex was dependent on Akt and calmodulin-dependent protein kinase II (CaMKII), such that inhibition of either prevented complex formation and subsequent NO production (50). From an *in vivo* perspective, capsaicin has been shown to increase myocardial blood flow in wild-type mice, an effect that was absent in $TRPV1^{-/-}$ animals (102). This change in blood flow was not associated with a change in heart rate, indicating a direct influence of TRPV1 on vascular resistance. Capsaicin treatment has also been shown to inhibit agonist-induced constriction of human skeletal feed arteries, a response that was significantly attenuated by disruption of the endothelium (127). This report also proposed that capsaicin sensitized cells to ACh-mediated dilation, suggesting the Ca^{2+} entry through TRPV1 potentiates muscarinic receptor signaling-dependent relaxation. Work from the Gollasch laboratory suggested that TRPV1-dependent dilation is restricted to specific vascular beds. They observed that capsaicin produced a robust endothelium-dependent relaxation in mouse mesenteric arteries, but caused relaxation in mouse renal arteries or rat descending vasa recta only a ~100-fold greater concentrations (45).

Interestingly, TRPV1 reporter mice created using a transgenic approach showed no evidence of TRPV1 expression in endothelial cells (43). However, TRPV1 expression was detected in a subset of arteriolar SMCs, in which channel activation was found to cause vasoconstriction. Thus, a role for TRPV1 channels in endothelium-dependent vasodilation is not universally accepted by the field.

TRPC1—While several research groups have demonstrated TRPC1 expression in the endothelium, it is unclear if the TRPC1 sub-type forms homomeric channels in native cells. TRPC1 subunits have been found in heteromeric channels containing TRPC3 (259), TRPC4 (260), TRPC5 (260), TRPP1 (*PKD2*) (141,335), TRPV4 (100,172), and/or TRPV6 (234) subunits. TRPC1 channels have a unitary conductance of ~5 pS and are not selective for Ca^{2+} versus Na^{+} in heterologous expression systems (260).

One study showed that TRPC1 was functionally coupled to the Ca^{2+}-sensing receptor (CaSR) in cultured HUVEC (222). siRNA-mediated TRPC1 knockdown attenuated the

increase in intracellular Ca^{2+} and NO production induced by CaSR stimulation with spermine (222). The authors proposed that CaSR-induced NO production was mediated by TRPC1-dependent SOCE (222). Further studies by Greenburg et al. demonstrated colocalization of TRPC1 with TRPV4 in freshly isolated endothelial cells from rabbit mesenteric arteries; in these cells, CaSR stimulation induced an increase in NO production that was blocked by inhibition of either TRPC1 or TRPV4 (100). Stimulating CaSRs by increasing extracellular Ca^{2+} concentration resulted in relaxation of isolated rabbit mesenteric arteries, an effect that was sensitive to TRPC1 and TRPV4 inhibition. Moreover, relaxation evoked by the TRPV4 agonist GSK1016790A was blunted by inhibition of TRPC1 and NOS, but not IK (100), suggesting that CaSR-mediated vasorelaxation occurs through the TRPC1/TRPV4-dependent production of NO. Ma et al. proposed that heteromeric TRPC1/TRPV4 channels form SOCE channels in cultured aortic endothelial cells (172). However, it unknown whether this mechanism underlies the observations of Greenburg et al.

TRPC3—TRPC3 channels have a unitary conductance of ~60 pS; they are equally permeable to Ca^{2+} and Na^{+} ions, but are more permeable to Na^{+} than K^{+} (134). This channel is activated by G_qPCR signaling, specifically through PLC activity and the secondary messenger diacylglycerol (DAG), and may also be stimulated by elevated intracellular [Ca^{2+}]. Several studies have suggested a potential role for TRPC3 in endothelium-dependent vasodilation. A study by Liu et al. reported that decreased TRPC3 expression following treatment of rats (tail vein injection) with antisense oligonucleotide resulted in diminished flow- and bradykinin-induced vasodilation and attenuated the rise in endothelial intracellular Ca^{2+} induced by these treatments (166). Gao et al. showed that TRPC3 is present in the endothelium and smooth muscle layers of human internal mammary artery (IMA) and demonstrated that TRPC3 channel blockade with Pyr3 modestly diminished ACh-induced relaxation of precontracted IMA rings(94). Carbachol-induced relaxation and increases in endothelial intracellular Ca^{2+} were also impaired in aortic rings from $TRPC3^{-/-}$ mice and wild-type rings treated with Pyr3 (143), further demonstrating that muscarinic-dependent relaxation may involve activation of TRPC3 channels. A subsequent study by the Sandow laboratory demonstrated that ~70% of TRPC3 channels in the endothelium of rat mesenteric arteries are localized to MEPs, and that inhibition of these channels with Pyr3 attenuated ACh-induced endothelial cell hyperpolarization and arterial relaxation (240). A report from Kirby et al. further supported localization of TRPC3 to MEPs in distinct microdomains that also contain SK and IK channels in rat popliteal and first-order skeletal muscle arteries from the gastrocnemius muscle (138). Collectively, these data suggest that TRPC3 channel stimulation produces EDH responses following muscarinic receptor activation. Marrelli and colleagues also demonstrated that TRPC3 channels contribute to EDH-mediated dilation of mouse posterior cerebral arteries in response to ATP, an effect that involves the activation of SK and IK channels (142). Using patch-clamp electrophysiology, they further characterized changes in freshly isolated cerebral endothelial cell membrane potential induced by TRPC3 activation and concluded that there are two components of the hyperpolarization response: an early, IK channel-dependent phase and a sustained phase that is dependent on rapid recruitment of TRPC3 channels to the plasma membrane by the SK channel (142). Interestingly, Wong et al. observed that inhibition of TRPC3 following bradykinin

application attenuated relaxation in isolated pig coronary arteries from males, but not females (312). The implications of this observation for other species is not clear, since many of the studies discussed here were performed in tissue collected from male rodents, while investigations on human IMAs used vessels collected from both males and females.

Collectively, the findings cited above support a mechanism whereby activation of muscarinic G_qPCRs stimulates TRPC3 activity through PLC-dependent generation of DAG. TRPC3 activity, in turn, leads to increases in intracellular Ca^{2+} that activate SK and IK channels to cause EDH. However, at the endothelial cell resting membrane potential under physiological conditions (~−50 mV), the Ca^{2+} fraction of TRPC3-mediated mixed cation currents is likely to be small, arguing against direct activation of SK and IK channels by TRPC3-mediated Ca^{2+} influx. One possible explanation for this apparent discrepancy is that the TRPC3 subtype could constitute part of a heteromeric Pyr3-sensitive channel with higher Ca^{2+} permeability. Heteromeric channels consisting of TRPC1 and TRPC3 subunits have been reported and may be involved in endothelium-dependent relaxation (143). However, the biophysical properties of such channels are unknown, and it is unclear whether these channels are sensitive to Pyr3. An alternative possibility is that Na^+ influx through TRPC3 channels promotes activation of the Na^+/Ca^{2+} exchanger (NCX) acting in reverse mode, thereby indirectly resulting in Ca^{2+} influx. This hypothetical mechanism requires colocalization of the NCX and TRPC3 to produce subcellular domains with locally elevated Na^+ concentrations sufficient to activate reverse-mode Ca^{2+} entry without causing membrane depolarization, which would diminish the electrical driving force for Ca^{2+} entry. Support for this hypothesis is provided by studies in HEK cell over-expression models (224) and cardiomyocytes (68, 80, 220), which used KB-R7943, a compound that inhibits reverse-mode NCX activity, to demonstrate functional coupling between TRPC3 and NCX. It should be noted that KBR7943 is known to have multiple off-target actions, including effects on other TRPC channels (147) and smooth muscle BK channels (160), raising questions about conclusions reached using this compound. However, a more recent study using cultured HUVEC demonstrated that NCX inhibition with either SEA0400 or SN-6 reduced Ca^{2+} influx mediated by a DAG analog (11), providing support for functional coupling between TRPC3 and NCX within the endothelium. TRPC3 channels functionally couple with IP_3Rs on the sarcoplasmic reticulum in SMCs (316). If this complex is present in endothelial cells, it is possible that activation of TRPC3 channels could stimulate Ca^{2+} release from the ER through IP_3Rs.

TRPC4—The unitary conductance of TRPC4 channels is approximately 42 pS (232). The selectivity of channels to Na^+ and Ca^{2+} ions is not clear as P_{Ca}:P_{Na} between 1.1 and 7.7 have been reported, which may be explained by possible heteromeric association with other TRPC subunits (25,232). A study by Freichel et al. reported that SOCE, ACh-induced Ca^{2+} influx of mouse aortic endothelial cells, and relaxation of aortic rings were impaired in TRPC4$^{-/-}$ mice compared with controls (91), suggesting that TRPC4 is involved in SOCE- and muscarinic receptor-dependent dilation of conduit arteries. The authors proposed that arterial relaxation is mediated through increased Ca^{2+}- dependent eNOS activity and NO production (92). However, the impact of this study has been diminished by subsequent reports that TRPC4 channels are not involved in endothelial SOCE (1,110,243).

TRPC5—TRPC5 channels have a unitary conductance ranging from 40 to 66 pS (133, 232) and a P_{Ca}:P_{Na} between 1.8 and 9.5 (132, 202, 232). Several studies have reported TRPC5 channel expression in bovine and mouse aortic endothelial cells (159, 311) as well as human saphenous veins, and coronary and cerebral arteries (9, 329). However, these studies did not evaluate the role of TRPC5 channel activity in endothelium function. TRPC5 is subject to S-nitrosylation by NO donors, a modification that facilitates Ca^{2+} entry through TRPC5 in cultured bovine aortic endothelial cells (330). Reports from this group further suggest that TRPC5 colocalizes with caveolin-1 and eNOS, leading these authors to propose that increased activity of TRPC5 following S-nitrosylation may act as a positive feedback mechanism that serves to increase production of NO by eNOS (271). However, others have reported that TRPC5 activity in cultured bovine aortic endothelial cells is not regulated by NO-mediated nitrosylation of serine residues (311). Li et al. demonstrated that ACh-induced relaxation was unchanged in aortic rings from 6-week-old TRPC5$^{-/-}$ mice (159), indicating that increased eNOS activity following agonist stimulation is not potentiated by TRPC5. However, this study reported that ACh-dependent relaxation of aortic segments from aged TRPC5$^{-/-}$ mice (60 weeks) was impaired, whereas the response to sodium nitroprusside was unaffected. The authors concluded that this differential response was attributable to increased endothelial cell senescence in the aged animals, rather than a direct effect of TRPC5 on dilatory pathways (159).

TRPP1—TRPP1 (*PKD2*) has a single-channel conductance of 97 pS (140). It is more permeable to Ca^{2+} than Na^{+}, but is approximately twofold or more selective for K^{+} than Ca^{2+} (140). Some reports have suggested that TRPP1 forms a Ca^{2+}-release channel in the ER membrane (146). Du et al. reported that TRPP1 is expressed in cultured rat mesenteric artery endothelial cells and forms a complex with TRPC1 and TRPV4 that responds to shear stress (69). These authors further demonstrated that transfection of primary cultured rat mesenteric artery endothelial cells with nonfunctional pore mutants of these channels prevented increases in endothelium Ca^{2+} in response to flow, and lentiviral transfection of small rat mesenteric arteries with a nonfunctional TRPP1 mutant prevented flow-mediated dilation (69). Further support was provided Berrout et al., who demonstrated that stretch-induced Ca^{2+} influx and NO production in cultured mouse brain microvascular endothelial cells was significantly reduced following pharmacological inhibition or siRNA-mediated knockdown of either TRPC1 or TRPP1 (27). These data suggest the TRPP1, in combination with TRPC1 and TRPV4, evokes relaxation in response to flow through production of NO. It is unclear whether these complexes are mechanosensitive or are activated indirectly by a component of a separate flow-sensing element. TRPP1 complexes with TRPP2 are known to be sensitive to flow in other tissues, but TRPP1 may lose its mechanosensitive properties when expressed alone (195,241). The study by Berrout et al. demonstrated that siRNA-mediated knockdown of TRPP1 and TRPC1 reduced Ca^{2+} influx, suggesting that both TRPP1 and TRPC1 subunits, and presumably TRPV4 subunits, in these complexes contribute to mechanosensation (27).

TRPM2—TRPM2 has a unitary conductance of 58 pS at negative membrane potentials and 76 pS at positive potentials (230), and is equally permeable to Ca^{2+} and Na^{2+} ions (P_{Ca}:P_{Na} = ~0.7–0.9) (230, 317). TRPM2 channels are activated by intracellular adenosine

diphosphate ribose (213,230), arachidonic acid (107), H_2O_2 (145), and nicotinic acid adenine dinucleotide phosphate (24).

A few studies have investigated the role of TRPM2 channels in endothelium-dependent vasodilation. Chidgey et al. reported that dilation and endothelial cell Ca^{2+} influx in rat cremaster arterioles evoked by H_2O_2 was diminished by blocking TRPM2 channels with antibodies, and by SK and IK channel blockade, but inhibition of TRPM2 had no effect on carbachol-induced responses (49). This would suggest that ROS, but not receptor stimulation, facilitates Ca^{2+} entry through TRPM2 channels and evokes EDH. Iadecola and colleagues tested the hypothesis that ROS activation of TRPM2 and subsequent dilation are involved in the vascular dysfunction associated with Alzheimer's dementia. They observed that amyloid-β, a key factor in the pathogenesis of Alzheimer's disease, generated an inward current and Ca^{2+} influx in cultured mouse brain endothelial cells, both of which were sensitive to siRNA-mediated TRPM2 knockdown or TRPM2 inhibition with antibodies (206). The inward current was also inhibited by poly (ADP-ribose) polymerase (PARP) and PARP antagonists, implicating this enzyme in TRPM2 activation. *In vivo*, TRPM2 inhibition was shown to prevent cerebrovascular dysfunction in a transgenic mouse model of Alzheimer's disease as well as that associated with amyloid-β treatment (206). Moreover, amyloid-β had no effect on neurovascular function in $TRPM2^{-/-}$ mice (206), further suggesting a pathological role for this channel in this disease.

TRP channels and permeability of the vascular endothelium

In this section, we review evidence supporting the influence of TRPV4, TRPV1, TRPC1, TRPC4, TRPC6, and TRPM2 channels on the permeability of the endothelial barrier.

TRPV4—Multiple studies have proposed an important role for endothelial TRPV4 channels in the regulation of pulmonary vascular permeability. A study from the Townsley laboratory showed that activation of TRPV4 with 4α-PDD, 5,6-EET, or 14,15-EET increased pulmonary microvascular permeability in isolated rat lungs, measured as an increase in the vascular filtration coefficient, a response that was blocked by the non-selective TRPV antagonist ruthenium red (8). Moreover, no response to 4α-PDD was observed in isolated lungs from $TRPV4^{-/-}$ mice, but increased permeability persisted in response to store depletion with thapsigargin and initiation of SOCE. A microscopic analysis suggested that increased permeability in response to initiation of SOCE and activation of TRPV4 differed in that TRPV4 agonists increased permeability within the alveolar septal wall by disrupting the space between endothelial and epithelial layers, whereas thapsigargin affected interendothelial junctions in extra-alveolar vessels (8). A subsequent study from this group demonstrated that TRPV4-dependent increases in microvascular permeability induced by 14,15-EET were sensitive to blockade of IK and SK channels (165), suggesting functional coupling between these components. Ca^{2+} influx through TRPV4 recruits matrix metalloproteinases-2 and −9, causing disruption of the endothelial barrier and promoting edema formation (291). A recent study has proposed that H_2O_2 facilitates Ca^{2+} entry through TRPV4 channels, reducing transmembrane electrical resistance and conferring increased barrier permeability in cultured mouse and human pulmonary microvascular endothelial cells (269).

Wu et al. further explored the concept of functional specificity of Ca^{2+} influx pathways in the pulmonary endothelium. Although stimulation of TRPV4-mediated Ca^{2+} entry using 4α-PDD increased permeability of isolated mouse lungs, this treatment had no effect on cell surface expression of the adhesion molecule P-selectin on pulmonary capillary endothelium (315). In contrast, Ca^{2+} entry through T-type Ca^{2+} channels promoted cell surface expression of P-selectin, but had no effect on permeability (315). Inhibition of myosin light chain kinase (MLCK) has been shown to reduce membrane-bound TRPV4 expression and intracellular Ca^{2+} entry following 4α-PDD application in rat pulmonary microvascular endothelial cells (207), suggesting that MLCK regulates plasma membrane trafficking of TRPV4 channels in the pulmonary endothelium. Yin et al. demonstrated that increases in vascular pressure facilitated endothelial Ca^{2+} entry and alveolar liquid accumulation in isolated mouse lungs, a response that was absent in lungs from TRPV4$^{-/-}$ mice (327). Increasing pressure was shown to produce a corresponding increase in endothelial NO production, where a cGMP analog attenuated Ca^{2+} influx in response to 4α-PDD. The authors proposed that this increase in NO production acts through cGMP to regulate Ca^{2+} entry via TRPV4 channels, providing a negative feedback mechanism to regulate TRPV4-mediated increases in permeability in response to increases in vascular pressure (327). Pulmonary edema induced in a rat model of heart failure was attenuated by TRPV4 blockade (278), further confirming a role for TRPV4 in increasing the permeability of the pulmonary vasculature *in vivo*.

TRPV1—The role of TRPV1 channels in the regulation of microvascular permeability is controversial. Thomas et al. reported that TRPV1 antagonism or genetic deletion in mice did not alter the increase in lung-wet weight induced by treatment with the endotoxin, lipopolysaccharide (LPS) (277). In support of this, Alvarez and colleagues showed that the TRPV1 agonist 4α-phorbol-12,13-didecanoate-20 homovanillate had no effect on lung permeability in isolated rat lung preparations (8). In contrast, Wang and colleagues reported that TRPV1 activation with capsaicin exerted a protective effect *in vivo* following lung ischemia-reperfusion injury in rabbits (296). This protection was associated with an increase in CGRP levels (296), suggesting that it is likely due to activation of TRPV1 on sensory neurons, rather than stimulation of endothelial Ca^{2+} influx. Moreover, although mRNA transcripts for TRPV1 channels are highly expressed in the mouse lung, expression in human lung microvascular endothelial cells is minimal (277). In addition, endothelial cells are not labeled in transgenic TRPV1 reporter mice, suggesting that the channel is not expressed in these cells (43).

TRPC1—TRPC1 channels, most likely in a heteromeric configuration, have been implicated in the control of endothelial permeability in the pulmonary vasculature. Moore et al. reported that increases in intracellular Ca^{2+} in cultured rat pulmonary arterial endothelial cells result in redistribution of peripheral filamentous actin and lead to changes in cell shape (189). They further proposed that TRPC1 channels may contribute to this process by acting through SOCE (189), which has been reported to increase pulmonary microvascular permeability (48). Consistent with this, Brough et al. demonstrated that SOCE is diminished in cultured human pulmonary artery endothelial cells following antisense-mediated knockdown of TRPC1 (32). Mehta el al. observed that treatment of cultured human

pulmonary arterial endothelial cells with thrombin resulted in translocation of TRPC1 and IP_3Rs, which directly associated with RhoA; notably, inhibition of RhoA attenuated SOCE (181). A separate study demonstrated that thrombin treatment of cultured HUVEC evoked SOCE and increased permeability through induction of protein kinase Cα (PKCα)-dependent phosphorylation of TRPC1 (4). Tauseef and colleagues noted that thrombin increased the permeability of endothelial cells from wild-type mice, but not $TRPC1^{-/-}$ mice (273). Furthermore, monolayers of cultured endothelial cell from $TRPC1^{-/-}$ mice were shown to express significantly higher levels of the adheren junction molecule vascular endothelial cadherin (273). Several reports implicate sphingosine-1-phosphate, produced through sphingosine kinase 1-mediated phosphorylation, anneals adheren junctions to stabilize the endothelium barrier (229, 274). Tauseef et al. proposed that Ca^{2+} entry through TRPC1 suppresses sphingosine kinase 1, resulting in disruption of endothelial cell barrier stability (273).

TRPC4—Tiruppathi and colleagues were the first to describe a role for TRPC4 channels in the permeability of the pulmonary vasculature. Treatment of cultured mouse pulmonary endothelial cells with either thrombin or a protease-activated receptor (PAR-1) agonist resulted in a sustained increase in intracellular Ca^{2+}, a response that was attenuated in lung endothelial cells from $TRPC4^{-/-}$ mice (280). This reduction in Ca^{2+} influx was associated with reduced actin-stress fiber formation and endothelial cell retraction. Although basal permeability did not differ between perfused lungs isolated from wild-type or $TRPC4^{-/-}$ mice, PAR-1 stimulation increased the vascular filtration coefficient of wild-type lungs, but not that of $TRPC4^{-/-}$ lungs (280). The authors proposed that TRPC4 is activated following intracellular store depletion and facilitates Ca^{2+} entry and subsequent rearrangement of the endothelium layer, a supposition confirmed in a subsequent follow up study. Disruption of the endothelial barrier by intracellular store depletion was prevented by siRNA-mediated knockdown of TRPC4 and was absent in endothelial cells from $TRPC4^{-/-}$ mice (268). In cultured pulmonary artery endothelial cell, direct association between spectrin protein 4.1 and TRPC4 channels was shown to be required for SOCE (52).

Channels formed through heteromeric association of TRPC1 and TRPC4 subunits may underpin SOCE and increases in microvascular permeability in the pulmonary circulation. Channels comprised of both subunits have been identified in cultured bovine aortic endothelial cells (14). Cioffi et al. characterized the individual subunit composition of TRPC1/TRPC4 heteromeric channels using Förster Resonance Energy Transfer. They determined that these channels contained one TRPC1 subunit and at least two TRPC4 subunits (53). Furthermore, Orai1 was found to associate with TRPC4, and loss of Orai1 function was shown to improve endothelial barrier properties in cultured rat pulmonary endothelial cells (53). Additionally, TRPC1 and TRPC4 were found to colocalize with STIM1 in cultured mouse pulmonary endothelial cells, implicating this heteromeric channel in SOCE (268). However, considering the controversies surrounding TRPC channels and SOCE as previously discussed, conclusions must be approached cautiously.

TRPC6—TRPC6 channels have a unitary conductance of ~30 pS, a P_{Ca}:P_{Na} ratio of ~5, and are activated by the second messenger DAG following G_qPCR stimulation (119,126,154). In

an early study, Singh et al. reported that actin stress fiber formation and endothelial barrier dysfunction induced in human pulmonary artery endothelial cells by thrombin or a DAG analog was prevented by siRNA-mediated knockdown of TRPC6 (244). Samapati and colleagues showed that TRPC6 expression in caveoli of pulmonary endothelial cells was increased following treatment of isolated perfused rodent lungs with platelet-activating factor (PAF) (228). This study also showed that the selective TRPC6 agonist hyperforin increased endothelial Ca^{2+} concentration and pulmonary wet weight. Furthermore, PAF increased the intracellular Ca^{2+} concentration in endothelial cells as well as the vascular filtration coefficient and edema formation in isolated lungs of wild-type mice, effects that were significantly diminished in lungs from TRPC6$^{-/-}$ mice (228). The effects of PAF and hyperforin were attenuated by NO donors and a membrane-permeable cGMP analog, whereas the increase in wet lung mass in response to hyperforin was augmented by NOS blockade. The authors proposed that NO inhibits Ca^{2+} entry through TRPC6 channels, but does not affect their recruitment to caveolin-rich microdomains in endothelial cells (228).

TRPM2—Although TRPM2 is expressed in the endothelium (86, 113), its role in regulating endothelial permeability is uncertain. Hecquet et al. provided early evidence that H_2O_2 increases intracellular Ca^{2+} entry and reduces transendothelial electrical conductance in cultured human pulmonary endothelial cell monolayers, a response that was sensitive to inhibition with an anti-TRPM2 blocking antibody, siRNA-mediated knockdown of TRPM2, or overexpression of a dominant-negative short TRPM2 isoform (113), suggesting TRPM2 increases permeability. However, investigations performed *in vivo* have provided conflicting results. In TRPM2$^{-/-}$ mice challenged with LPS, Di and colleagues observed an increase in pulmonary edema formation and reduced survival (64), suggesting TRPM2 maintains barrier integrity contrasting observations made *in vitro*. Moreover, Hardaker et al. reported no change in TRPM2$^{-/-}$ mice following LPS treatment (108). Although the reason for the discrepancies between these studies *in vivo* is not clear, it may be related to the mouse strain employed in the generation of TRPM2-deficient mice. Specifically, Di et al. created TRPM2$^{-/-}$ mice on a C57BL/6 background, whereas Hardaker et al. used a BALB/c mouse strain in generating their TRPM2$^{-/-}$ mice. This difference is notable because sensitivity to lung injury following LPS challenge is reported to be lower in BALB/c mice (57, 66). However, the precise role of TRPM2 in endothelial barrier function remains unresolved.

TRP channels and angiogenesis and arteriogenesis

TRPV1, TRPA1, TRPV4, TRPC1, TRPC3, TRPC4, TRPC5, and TRPC6 channels have been implicated in the formation of new blood vessels.

TRPV1 and TRPA1—Su et al. demonstrated that 14,15-EET increased Ca^{2+} influx and NO production in cultured human microvascular endothelial cells, a response that was blocked by the TRPV1 antagonist capsazepine or overexpression of inactive TRPV1 (261). In this study, application of 14,15-EET was found to increase endothelial tube formation, an effect that was abolished by capsazepine. Utilizing Matrigel plug *in vivo* angiogenesis assays, these authors also showed that hemoglobin content was reduced in in TRPV1$^{-/-}$ mice and wild-type animals treated with capsazepine, indicative of reduced neovascularization (261). A subsequent study from this group further demonstrated the

involvement of TRPV1 channels in simvastatin-induced angiogenesis, showing that Ca^{2+} entry through TRPV1 channels increased eNOS activity through CaMKII/Akt-dependent phosphorylation (262). Interestingly, the authors showed that the selective TRPA1 inhibitor HC-030031 diminished simvastatin-induced eNOS phosphorylation and NO production and reduced vascularization of Matrigel plugs from $TRPA1^{-/-}$ mice, suggesting the involvement of pathways downstream of TRPA1 (262). A separate report from Hofmann et al. reported that TRPV1 is necessary for anandamide uptake into cultured human endothelial cells and subsequent induction of tube formation in culture, further implicating TRPV1 in angiogenesis. However, this latter study proposed a Ca^{2+}-independent mechanism (117), differing from that hypothesized by Su et al. (261,262).

TRPV4—siRNA-mediated TRPV4 channel knockdown was shown to attenuate cyclic strain-induced Ca^{2+} influx and bovine capillary endothelial cell cytoskeletal remodeling and reorientation, suggesting the possible involvement of TRPV4 in migration and sprouting responses associated with angiogenesis (276). The TRPV4 agonist 4α-PDD induced an increase in Ca^{2+} influx in cultured human brain capillary endothelial cells and promoted their proliferation, an effect that was partially attenuated by TRPV4 knockdown (112). Schierling et al. observed increased growth of the rat cerebral collateral circulation in response to 4α-PDD (233). Troidl et al. also observed increased collateral growth of the rabbit hindlimb circulation following 4α-PDD application, further implicating TRPV4 in arteriogenesis (285). Fiorio Pla et al. noted increased TRPV4 channel expression and greater subsequent Ca^{2+} influx in cultured endothelial cells derived from human breast carcinomas compared with human microvascular endothelial cells (87). Stimulation of TRPV4 with either arachidonic acid or 4α-PDD promoted migration of breast cancer endothelial cells, but not control endothelial cells (87). Adapala et al. provided further support for the importance of TRPV4 channels in tumor angiogenesis. Using a syngenic tumor model, Thodeti and colleagues found that $TRPV4^{-/-}$ mice exhibited an increase in tumor growth that was associated with increased vascularization and vessel diameter. In contrast, cotreatment of wild-type mice with the TRPV4 agonist GSK1016790A and the anticancer agent cisplatin reduced tumor formation by inducing vessel maturation, whereas treatment with GSK1016790A alone did not (2). Collectively, these data suggest that Ca^{2+} entry through TRPV4 channels has a greater impact on tumor-associated angiogenesis than on physiological angiogenesis.

TRPC1—Using an *in vivo* zebrafish model, Yu et al. demonstrated that knockdown of TRPC1 channels using targeted morpholino oligonucleotides disrupted angiogenic sprouting of interseg-mental vessels in larvae, an effect that was prevented by overexpression of a morpholino-resistant TRPC1 form (331). These authors further found that TRPC1 channels act downstream of VEGF-A signaling and are required for ERK signaling (331). In contrast, knockdown of TRPC1 in cultured HUVECs was shown to minimally reduce tube formation(13), whereas $TRPC1^{-/-}$ mice were reported to exhibit normal vascular growth (235), suggesting that TRPC1 channels have minimal effects on angiogenesis in mammals.

TRPC3—VEGF has been reported to activate TRPC3 channels in cultured human microvascular endothelial cells, suggesting a potential role for this channel in angiogenesis

(47). Antigny et al. demonstrated that siRNA-mediated knockdown of TRPC3 reduced endothelial tube formation in human endothelial cell cultures, an effect that was associated with reduced spontaneous Ca^{2+} oscillations (13). Moreover, Andrikopoulos et al. recently demonstrated that VEGF signaling activates Ca^{2+} influx through TRPC3 channels in cultured HUVEC (11). The authors reported functional coupling of TRPC3 channels with NCX1, and showed that inhibition or knockdown of TRPC3 reduced protein kinase C-α and ERK1/2 activation, partially suppressed Ca^{2+} influx, and significantly attenuated *in vitro* tube formation in response to VEGF (11). These authors further showed that an analog of DAG induced Ca^{2+}- and Na^{+}-mediated activation of ERK1/2 and promoted tube formation. Interestingly, they also reported that Ca^{2+} transients were sensitive to both inhibition of TRPC3 and reverse-mode NCX1, whereas Na^{+} influx was only sensitive to TRPC3 inhibition (11). These data suggest that VEGF initiates Na^{+} influx through TRPC3, resulting in Ca^{2+} influx through reverse-mode NCX1 and promotion of tube formation via ERK1/2 signaling.

TRPC4 and TRPC5—In addition to TRPC3 channels, TRPC4 and TRPC5 channels have also been implicated in tube formation and Ca^{2+} oscillations in human endothelial cell cultures. In this report from Antigny et al. (13), only TRPC1 and TRPC4 were reported to be present in this immortalized endothelial cell line, and siRNA-mediated knockdown of TRPC4 was shown to significantly reduce spontaneous Ca^{2+} oscillations and tube formation (13). Zhu et al. reported that TRPC5 is highly expressed in human breast cancer cells following long-term chemotherapy treatment, and further demonstrated that siRNA-mediated TRPC5 knockdown reduced VEGF expression, tumor growth, and vessel density in a mouse xenograft model (339). In this study, TRPC5 activity was shown to promote nuclear translocation of hypoxia-inducible factor 1-α and subsequent induction of VEGF production, leading to increased tumor growth (339). Taken together, these observations suggest that TRPC4 may have a role in physiological angiogenesis, whereas TRPC5 channels are more likely involved in tumor angiogenesis.

TRPC6—It was reported that rat pulmonary microvascular endothelial cell TRPC6 channels are activated by VEGF (47), suggesting that Ca^{2+} influx through these channels may regulate blood vessel formation. A subsequent study from this laboratory confirmed this, showing that overexpression of TRPC6 increased the proliferation and migration of cultured microvascular endothelial cells, whereas overexpression of a dominant-negative isoform of TRPC6 reduced proliferation, migration, and sprouting (106). Moreover, Kini et al. reported in cultured human pulmonary artery endothelial cells that TRPC6 associates with PTEN (phosphatase and tensin homolog), a lipid-protein phosphatase that is required for thrombin-induced migration and tube formation (137). Although these studies proposed a role for TRPC6 channels in angiogenesis based on results obtained using an *in vitro* approach, contrasting evidence has been provided *in vivo*, where gain-of-function mutations of TRPC6 in humans (310) or genetic deletion of TRPC6 in mice (65) produced no change in blood vessel structure.

TRP channels and control of the inflammatory response by the endothelium

TRPV1, TRPV4, TRPA1, and TRPM2 have been associated with anti-inflammatory properties, whereas TRPC1, TRPC3, and TRPC6 exert pro-inflammatory affects.

TRPV1—The role of TRPV1 in neurogenic inflammation is well established. In particular, activation of TRPV1 in sensory neurons has been shown to cause the release of vasoactive neuropep-tides, including CGRP and tachykinins (e.g., substance P) and evoke a pro-inflammatory response. However, there is evidence that TRPV1 channels in non-neuronal tissue may have anti-inflammatory properties. Activation of TRPV1 channels is associated with increased production of NO, which is a known anti-inflammatory mediator. This was demonstrated by Wang et al., who used cultured HUVECs to show that activation of TRPV1 channels with capsaicin not only increased eNOS phosphorylation and NO production through the phosphatidylinositol-4,5-bisphosphate 3-kinase/Akt pathway, but also produced a number of anti-inflammatory effects associated with LPS treatment, including reduced production of cytokines and chemokines, decreased expression of adhesion molecules, activation and translocation of nuclear factor-κB (NF-κB), and increased monocyte adhesion to endothelial cells (299). These authors further reported that the anti-inflammatory effects of capsaicin were abrogated by inhibition of TRPV1 or NOS, or sequestration of Ca^{2+}, suggesting the Ca^{2+} influx through TRPV1 channels promotes NO production and subsequent inhibition of the inflammatory process (299). The authors also observed that TRPV1 exerted anti-inflammatory properties in renal microvascular endothelial cells isolated from hypertensive mice (299). Lee et al. observed that a derivative of rutaecarpine, a COX-2 inhibitor, increased expression of TRPV1 and eNOS phosphorylation in cultured human aortic endothelial cells, and reduced pulmonary neutrophil infiltration in mice challenged with ovalbumin, further suggesting a role for TRPV1 channels in protection against inflammation (152). Interestingly, not only did Wilhelmsen and colleagues observe that inhibition of TRPV1 with AMG9810 augmented inflammation associated with LPS in cultured human endothelial cells, indicative of its anti-inflammatory properties, they found that AMG9810 also abrogated the reduction in inflammatory cytokines associated with endocannabinoid treatment (305). This suggests that TRPV1 is anti-inflammatory under physiological conditions, but may also attenuate anti-inflammatory mechanisms associated with other factors.

TRPV4—Although TRPV4 channels have been implicated in regulating the interendothelial integrity of the pulmonary vasculature, they do not appear to be involved in the recruitment of cytokines. Ca^{2+} influx mediated by EETs-activated TRPV4 channels did not affect the expression of the adhesion molecules, VCAM (vascular cell adhesion molecule), ICAM (intercellular adhesion molecule) (282), or P-selectin (315). However, in certain vascular beds, Ca^{2+} entry through TRPV4 channels may be associated with a reduction in inflammation. Xu et al. reported that treatment of mice with the TRPV4 channel activator GSK1016790A inhibited monocyte adhesion in cultured HUVEC and *in vivo* in mice treated with tumor necrosis factor-α (TNF-α) (322). Furthermore, oral administration of GSK1016790A reduced the formation of atherosclerotic plaques in $ApoE^{-/-}$ mice. The authors proposed that increased intracellular Ca^{2+} through TRPV4 channels increases eNOS activity and NO production, thereby evoking an anti-inflammatory phenotype (322). In

contrast, ischemia/reperfusion injury in the renal microcirculation resulted in aggravated tubular damage in TRPV4$^{-/-}$ mice, but recruitment of granulocytes and macrophages was comparable to that in wild-type mice (174), suggesting that the anti-inflammatory actions of TRPV4 channels are localized to specific regions.

TRPA1—The TRPA1 channel activator cinnamaldehyde has been reported to exert anti-inflammatory affects. Liao et al. reported that treatment of cultured human endothelial cells with TNF-α increased monocyte adhesion, a response that was attenuated by cinnamaldehyde (161). Moreover, cinnamaldehyde reduced the expression of cell adhesion molecules and attenuated NF-κB signaling in endothelial cells (161). These authors concluded that cinnamaldehyde exerted its anti-inflammatory effects by preventing the degradation of IκBα (nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor α), an endogenous inhibitor of NF-κB, and subsequently inducing the protective transcription factor Nrf2 (nuclear factor erythroid-derived 2-like 2) (161). However, the anti-inflammatory properties of cinnamaldehyde have yet to be associated with Ca^{2+} influx through TRPA1 channels. The study by Liao et al. did not confirm whether cinnamaldehyde increased endothelial cell cytosolic Ca^{2+}. They also did not use TRPA1 antagonists on their preparations, an important consideration given that cinnamaldehyde is known to have off-target effects.

TRPC1—TRPC1 expression has been linked to TNF-α. During the cloning of human TRPC1, Paria et al. noted four NF-κB binding sites in the 5'-regulatory region, and showed that treatment of cultured microvascular endothelial cell with TNF-α increased TRPC1 promotor-luciferase activity and intra-cellular Ca^{2+} concentration following store depletion with thapsigargin (205). These changes were sensitive to NF-κB inhibition, leading the authors to propose that TNF-α increases endothelial TRPC1 expression through NF-κB, thereby increasing Ca^{2+} following intracellular store depletion (205). A separate investigation by Bodiga et al. reported that treatment of cultured HUVEC with cisplatin, an anti-cancer chemotherapeutic agent, increased the permeability and adherence of monocytes in culture, an effect that was associated with increased PKCα-mediated phosphorylation of TRPC1 channels (29). siRNA-mediated knockdown of PKCα, TRPC1, or the p65 subunit of NF-κB attenuated the increase in endothelial Ca^{2+} influx and permeability. The mechanism proposed to explain this effect was that cisplatin acted through PKCα to induce an NF-κB-dependent increase in TRPC1 channel expression and phosphorylation that facilitated Ca^{2+} influx and endothelial dysfunction (29). A follow-up study from this laboratory provided further support for this pathway(28). However, further investigations are required to determine if this mechanism operates *in vivo* following endogenous activation of TRPC1.

TRPC3—TRPC3 channels have been reported to exert a proinflamma-tory phenotype in macrophages and monocytes, but few studies have investigated their potential role in the endothelium. In an early study, Smedlund et al. reported that ATP-induced Ca^{2+} influx in cultured human coronary artery endothelial cells increased TRPC3 expression and was essential for VCAM-1 expression and monocyte adhesion, effects that were reduced by siRNA-mediated knockdown of TRPC3 (247). A subsequent follow-up study from this laboratory reported similar observations in endothelial cells treated with TNF-α (246). The

authors proposed that Ca^{2+} influx through TRPC3 channels promoted NF-κB activity through a calmodulin/CaMKII-dependent pathway (246). Perhaps most surprisingly was that TRPC3 expression was increased downstream of pro-inflammatory signaling, suggesting a positive feedback mechanism that augments the inflammatory phenotype. These findings were recently confirmed *in vivo* in $ApoE^{-/-}$ mice overexpressing human TRPC3 specifically in endothelial cells. After feeding a high-fat diet for 10 weeks, atherosclerotic lesions were comparable between $Apo^{-/-}$ and TRPV3-expressing $ApoE^{-/-}$ mice, but at 16 weeks, the latter mice exhibited increased lesion size, macrophage infiltration, VCAM-1 expression, and NF-κB activity compared with nontransgenic $Apo^{-/-}$ control mice (245,248).

TRPC6—TRPC6 channels have been associated with increased microvascular permeability, suggesting their possible involvement in the trafficking of leukocytes. Tauseef et al. demonstrated that treatment of cultured pulmonary endothelial cells from wild-type mice with LPS increased intracellular Ca^{2+} entry, an effect that was not observed in endothelial cells isolated from $TRPC6^{-/-}$ mice (275). In whole-animal studies, LPS increased lung permeability in association with increased leukocyte infiltration, responses that were blunted in $TRPC6^{-/-}$ mice (275). The authors proposed that Ca^{2+} entry through TRPC6 channels promotes NF-κB activation via MLCK to evoke an inflammatory response. Webber and colleagues further proposed that TRPC6 channels mediate leukocyte transendothelial migration during an inflammatory cascade. These authors reported that TRPC6 channels colocalized with platelet/endothelial cell adhesion molecule-1 (PECAM-1) in cultured HUVECs and formed clusters following engagement of PECAM-1 (304). Buffering intracellular Ca^{2+} prevented leukocyte transendothelial migration, as did expression of a dominant-negative TRPC6 and silencing of TRPC6 (304). Furthermore, activation of TRPC6 in the presence of a PECAM inhibitor rescued leukocyte trans-migration, suggesting that regulation of transmigration by TRPC6 channels occurs downstream of PECAM activity (304). Using topical application of croton oil on the ear, an *in vivo* model of transmigration, these authors observed reduced neutrophil transendothelial migration in irradiated $TRPC6^{-/-}$ mice treated with bone marrow from wild-type or $TRPC6^{-/-}$ mice, but had no effect on trafficking (304). Collectively, these data suggest that activation of PECAM stimulates TRPC6 channel-mediated Ca^{2+} influx, which promotes leukocyte transmigration during an inflammatory response.

TRPM2—The increased permeability of the pulmonary vasculature in $TRPM2^{-/-}$ mice challenged with LPS reported by Di and colleagues, noted above, was associated with increased expression of inflammatory cytokines, including TNF-α and ilterleukin-6 (64), suggesting that TRPM2 may have anti-inflammatory properties. However, because TRPM2 channels are also expressed in leukocytes, where they contribute to inflammatory responses, it is unclear whether this effect is mediated by TRPM2 channels in the endothelium (324).

TRP channels and endothelium-dependent regulation of thrombosis

Endothelial-derived NO and prostacyclin have anticoagulant properties that serve to regulate platelet aggregation *in vivo* (187, 188). Although several reports have suggested that Ca^{2+} entry through different TRP channels is associated with increased NOS activity and NO

production, these studies have not investigated possible associations between endothelial cell TRP channel activity and antithrombotic mechanisms.

Conclusions

TRP channels are critically important for many physiological and pathological responses within the vasculature (74). Strong evidence demonstrates an influential role of endothelial TRP channels in the regulation of endothelium-dependent dilation, vascular permeability, angiogenesis, and inflammation. These regulatory mechanisms are primarily governed through control of global changes in intracellular Ca^{2+} resulting from direct Ca^{2+} influx through TRP channels or regulation of sub-cellular Ca^{2+} signaling events involving intracellular Ca^{2+} stores. However, considerable work is still required to fully understand the mechanistic features of TRP channel signaling, including their direct interactions with signaling molecules and other TRP subtypes, and to address current discrepancies in the literature, for example the conflicting views of TRPC channels in SOCE. Nonetheless, selective pharmacological targeting of TRP channels offers a promising opportunity for potential new therapies to treat and prevent vascular diseases.

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Didactic Synopsis

Major teaching points

1. The primary function of the vascular endothelium involves:
   - **a.** Regulating vascular tone
   - **b.** Maintaining the vascular barrier
   - **c.** Angiogenesis
   - **d.** Control of the inflammatory response
   - **e.** Antithrombosis
2. Understand that Ca^{2+} is key signaling messenger in mediating a physiological response
3. Differences in structural and biophysical properties of TRP channels
4. Ca^{2+} influx through TRP channels are important in maintaining normal endothelium function

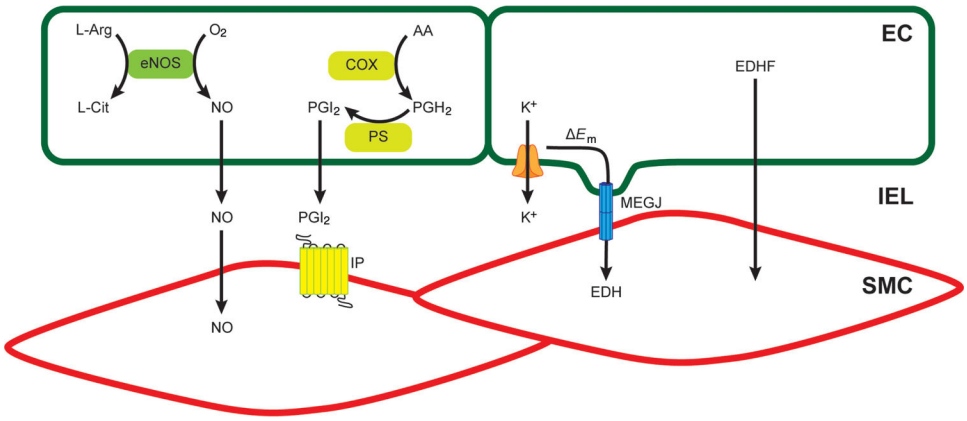

Figure 1.

Mechanism of endothelium-dependent dilation. Upon agonist stimulation, endothelial nitric oxide synthase (eNOS) activity increases to generate nitric oxide (NO). NO diffuses across the internal elastic lamina (IEL) to relax the underlying smooth muscle cells (SMC). Arachidonic acid (AA) metabolism by cyclooxygenase (COX) enzymes to produce prostaglandin H_2 (PGH_2). In turn, this is converted to prostacyclin (PGI_2) by prostacyclin synthase (PS), and mediates relaxation through the prostacyclin receptor (IP). Endothelial cell plasma membrane hyperpolarization generated by K^+ efflux (ΔE_m) spreads through myoendothelial gap junctions (MEGJ) to promote endothelium-dependent hyperpolarization (EDH) and relaxation. Several other factors, collectively termed as endothelium-derived hyperpolarizing factor (EDHF), hyperpolarizes smooth muscle cells to evoke relaxation.

Table 1

Function of Endothelial TRP Channels Summary

TRP	Vasodilation	Permeability	Vessel formation	Inflammation
TRPA1	↑		↑	↓
TRPV1	↑	↓	↑	↓
TRPV3	↑			
TRPV4	↑	↑	↑	↓
TRPC1	↑	↑	↑	↑
TRPC3	↑		↑	↑
TRPC4	↑	↑	↑	
TRPC5	↑		↑	
TRPC6		↑	↑	↑
TRPM2	↑	↑↓		↓
TRPP1	↑			

Table 2

Overview of the Biophysical and Pharmacological Properties of Endothelial TRP Channels

TRP	Unitary conductance	P_{Ca}:P_{Na}	Physiological activators	Pharmacological agonists (EC_{50})	Pharmacological antagonists (IC_{50})
TRPA1	~98ps(192)	7.9(135)	H_2O_2, 4-HNE, 4-ONE (10, 286), noxious cold <17°C (257))	Allyl isothiocyanate (rat 11 μmol/L, mouse 22 μmol/L) (19, 131), cinnamaldehyde (mouse 61 μmol/L) (19)	HC-030031 (human 0.7 μmol/L) (180)
TRPV1	35 pS at −60mV and 77 pS at +60mV (42)	9.6 (42)	Noxious heat >43°C (42), protons (12)	Capsaicin (mouse 9.1 nmol/L) (56)	Capsazepine (human 39 nmol/L, rat 0.22 μmol/L, mouse 1.4 μmol/L) (56, 179)
TRPV3	~150–200 pS (51)	~12 (320)	Heat 23–39°C (320), depolarization (67)	Carvacrol, eugenol, thymol (319)	Ruthenium red (211), isopentenyl pyrophosphate (239 nmol/L) (20)
TRPV4	~30–60 pS at −60mV and ~88–100 pS at +60mV (258,301,303)	~6 (258)	Heat 25–43°C (303), mechanical stimuli (164), EETs(302)	4α-PDD (11.7nM) (318), GSK1016790A (mouse 18 nmol/L, human 2.1 nmol/L) (279)	HC-067047 (human 48 nmol/L, rat 133nmol, mouse 17 nmol/L) (84), RN-1734 (human 2.3 μmol/L, rat 3.2 μmol/L, mouse 4 μmol/L) (293), GSK2193874 (278)
TRPC1	~5 pS (in combination with TRPC5) (260)	0.95 (in combination with TRPC5) (260)	Nitric oxide (330), stretch (176)		Gd^{3+} (340), La^{3+} (260), Pico 145 (human 200pmol/L, and in combination with TRPC5) (225)
TRPC3	~60 pS (134)	1.6(134)	Diacylglycerol (119)	OAG (119)	Pyr3 (mouse 0.7 μmol/L) (139), Gd^{3+} (human 20 nmol/L), La^{3+} (human 20 nmol/L) (105)
TRPC4	42 pS (232)	1.1–7.7 (25,232)	Nitric oxide (330)	(−)-Englerin A (human 11.2 nmol/L) (7), La^{3+} (μmol/L range) (132,232)	ML204 (mouse 0.96 μmol/L) (185), Picol45 (human 0.35 nmol/L) (225), La^{3+} (mmol/L range) (132,232)
TRPC5	40–66 pS (133,232)	1.8–9.5 (132,202, 232)	Nitric oxide, H_2O_2 (330), protons (238), stretch (99)	(−)-Englerin A (human 7.6 nmol/L) (7), La^{3+} (μmol/L range) (132,232)	ML204 (mouse <10 μmol/L) (185), Picol 45 (human 1.3 nmol/L) (225), La^{3+} (mmol/L range) (132)
TRPC6	~30 pS (119,126,154)	~5 (119, 126,154)	Diacylglycerol (119), stretch (255)	OAG (119), hyperforin(155)	Gd^{3+}(mouse 1.9 μmol/L), La^{3+} (mouse 4 μmol/L) (126), SH045 (104)
TRPM2	58 pS at negative membrane potentials and 76 pS at positive potentials (230)	~0.7–0.9 (230,317)	Heat 35°C (252), adenosine diphosphate ribose (213,230), arachidonic acid (107), H_2O_2 (145), nicotinic acid adenine dinucleotide phosphate (24)		Clotrimazole, econazole (115)
TRPP1	97 pS (140)	3.92 (140)	Intracellular Ca^{2+} (146), stretch (241)		Gd^{3+}, La^{3+}, Cd^{2+}, Ni^{2+} (212)

Note. Further details can be found at the International Union of Basic and Clinical Pharmacology (IUPHAR) database (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=78).