I. INTRODUCTION

Since the initial discovery and characterization of the transient receptor potential (TRP) channel in Drosophila phototransduction mutants, a growing body of work has demonstrated the diversity, tissue distribution, and remarkable range of functions performed by these cation channels in mammals. Twenty-eight mammalian TRP homologs, distributed throughout the body, have been described. TRP channels are critically involved in sensory and signal transduction processes in excitable and nonexcitable cells present in virtually every organ system. A common theme regarding the specific physiological roles of these channels is their significant contribution to the regulation of intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and distribution, which directly influence a multitude of cellular functions. The biophysics, molecular and cellular biology, and pathophysiological involvement of these “truly remarkable proteins” have recently been reviewed in considerable depth (95, 249).

The importance of TRP channels in the cardiovascular system has been clearly demonstrated over the past 15 years. TRP channels in the heart are involved in normal pacemaker function and contractility and contribute to mechanisms underlying pathologies such as cardiac hypertrophy, fibrotic disease, and arrhythmias. In the circulatory system, TRP channels are present and functional in smooth muscle cells and endothelial cells constituting the vascular wall, and are also expressed in perivascular neurons and astrocytes, which are closely associated with cerebral parenchymal arterioles. TRP channels contribute to mechanosensation and G protein-coupled receptor (GPCR)-initiated signaling pathways that modulate vasoconstrictor and vasodilator activity and cellular proliferation. In vascular endothelial cells, Ca\(^{2+}\) entry through TRP channels is an important contributor to endothelium-dependent vasodilation, vascular wall permeability, and angiogenesis. TRP channels present in perivascular sensory nerves and astrocytes participate in vasodilator responses in some tissues. Altered TRP channel function has been linked to a number of common vascular disorders, including hypertension, vascular occlusive disease,
neurogenic inflammation, neointimal injury, and pulmonary edema.

This review is organized into three main sections. The first provides a brief overview of TRP channel structure and function. The second considers the normal physiological roles of TRP channels in vascular smooth muscle cells, endothelial cells, and perivascular cells (i.e., neurons and astrocytes). And the last reviews evidence supporting the involvement of TRP channels in vascular disease.

II. A BRIEF REVIEW OF TRP CHANNELS

A. Discovery

The discovery of the TRP superfamily can be traced to Cosens and Manning’s seminal study of *Drosophila* phototransduction mutants that were able to navigate normally under low light conditions but behaved as if blind under bright lights (63). These flies also displayed characteristic abnormalities in electrical responses in the retina during prolonged light exposure. Minke et al. (224) further characterized the electrophysiological properties of photoreceptors from these mutants and reported that, in contrast to the steady-state depolarization induced by bright light recorded from wild-type flies, light-induced depolarization of the photoreceptor membrane potential in mutant flies was transient in nature. Consequently, the strain was designated *transient receptor potential* (*trp*) (224). Isolation of DNA encoding a portion of the affected gene by Montell et al. (230) and subsequent cloning of the full-length *trp* cDNA by Montell and Rubin (231) and Wong et al. (394) provided additional insight into the function of the *trp* gene product. Hydropathy plots of the predicted amino acid sequence suggested that *trp* encoded a membrane protein with intracellular NH2 and COOH termini and six to eight putative transmembrane domains. Montell et al. (231) noted that the membrane topology of the protein encoded by *trp* was reminiscent of previously characterized voltage-dependent ion channel proteins, and predicted that *trp* also encoded an ion channel. Vaca et al. (366) validated this hypothesis, reporting an electrophysiological analysis of *trp* and the related gene *trp-like* (*trpl*) in a heterologous expression system. When expressed in *S.9* insect cells, *trp* formed a Ca2+-permeable, nonselective cation channel that was activated by the sarco(endo)plasmic ATPase (SERCA) inhibitor thapsigargin. Evidence that *trp* homologs are present in vertebrates was reported almost simultaneously by Petersen et al. (268), who found that sequences related to *trp* are present in *Xenopus* oocytes and mouse brain. More comprehensive studies were performed by Wes et al. (388) and Zhu et al. (425), who cloned and characterized the first mammalian *trp* homologs, and Zhu et al. (426), who reported the first evidence of a receptor-operated mammalian TRP channel. The first mammalian TRP to be cloned is now known as TRPC1. Wes et al. (388) were prescient in predicting a larger role for multiple *trp* homologs in humans, concluding that “it will be interesting to determine the full size of the human TRP family and whether the human TRPs have different functional characteristics . . .” Indeed, the discovery of the first human TRPs spurred a decade-long race to clone all of the TRP genes and characterize the functional properties of the encoded proteins in heterologous expression systems. All members of the TRP superfamily now appear to be accounted for, and current efforts are focused on understanding how TRP channels affect the physiology and pathophysiology of different organs and tissues. Detailed insight into the early days of TRP channel research is provided by reviews and commentaries by the pioneers in this field (130, 223, 229).

B. TRP Superfamily Organization and Channel Structure

Mammalian genomes encode 28 distinct TRP protein subunits, which together comprise the TRP superfamily (FIGURE 1, TABLE 1). A standard nomenclature was established (61), grouping these genes and their products into six subfamilies based on amino acid sequence homology. These subfamilies are designated canonical (TRPC), vanilloid (TRPV), melastatin (TRPM), ankyrin (TRPA), mucolipin (TRPML), and polycystin (TRPP) (FIGURE 1, TABLE 1). One member of the TRPC subfamily, TRPC2, is present in rodents and is important for pheromone sensing (206), but is a nonexpressed pseudogene in humans (409).

The terminology used to describe the TRPP subfamily requires some clarification. The polycystin-1 protein encoded by *PKD1* is sometimes referred to as TRPP1 (or TRPP4). This is confusing, as *PKD1* encodes a protein with 11 transmembrane domains and a long extracellular NH2 terminus that does not form an ion-conducting pathway and is structurally unrelated to authentic TRP channels (390). The name “TRPP4” was also briefly used to describe the product of the *PKDREJ* (polycystic kidney disease and receptor for egg jelly related protein) gene in an early report. But *PKDREJ* is also unrelated to the mammalian TRP superfamily, and the name “TRPP4” is no longer in use. TRPP2, TRPP3, and TRPP5 have also been used to describe the products of the *PKD2* (polycystic kidney disease 2) gene (gene ID: 5,311; mRNA: NM_000297), *PKD2L1* gene (gene ID: 9,033; mRNA: NM_016112), and *PKD2L2* gene (gene ID: 27,039; mRNA NM_014386), respectively. In this review, we use TRPP1 for *PKD2*, TRPP2 for *PKD2L1*, and TRPP3 for *PKD2L2*. The current International Union of Basic and Clinical Pharmacology (IUPHAR) database (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=78) currently uses both nomenclatures, adding to confusion.

All TRP subunits are expressed as polypeptides of 553–2,022 amino acids (~64–230 kDa) containing six trans-
membrane domains (S1–S6) and intracellular NH₂ and COOH termini of variable length (FIGURE 2). An ~25-amino acid conserved element called the TRP domain is present immediately distal to the S6 domain in members of the TRPC, TRPM, and TRPV subfamilies. The TRP domain contains two highly conserved, six-residue TRP boxes bordering a central region that has greater sequence variability. The function of the TRP domain is not completely understood, but it may be involved in phosphatidylinositol 4,5-bisphosphate (PIP₂) binding (287) or subunit assembly (105). Multiple regulatory elements, interaction sites, and enzymatic domains are present in the intracellular termini. Numerous ankyrin repeat domains are present on the NH₂ terminus of TRPC, TRPV, and TRPA subunits. These are particularly prominent in TRPA1 subunits (~15–19 repeats), where they contribute to channel regulation. The COOH terminus of TRPC channels encodes calmodulin/IP₃R-binding (CIRB) domains, Ca²⁺-binding EF hands and, for TRPC4, a PDZ domain. A domain known as the TRPM homology region is present in the NH₂ terminus of TRPM subfamily members. Functional serine-threonine kinase domains are present in the COOH terminus of TRPM6 and TRPM7, and an ADP-ribose-binding NUDIX phosphohydrolase domain is present in the COOH-terminal region of TRPM2. TRPP and TRPML channels share homology through the transmembrane region, including a large extracellular loop between the S1 and S2 domains that is not present in other subfamilies. Transcriptional splice variants, some with functionally distinct properties, have been described for almost all TRP subunits (373).

Functional TRP channels arise from the assembly of four subunits, with the S5 and S6 transmembrane domains forming the ion-conducting pore. All TRP subunits, with the possible exception of TRPC1 (328), are capable of forming functional homomeric cation channels. Many cell types express multiple TRP subunits, and heteromultimeric channels composed of two or three related subunits can form and are present in vivo. Heteromultimeric channels display conduction properties and regulatory mechanisms that are distinct from those of homomeric channels. Formation of heteromultimeric channels is best characterized for TRPC channel subunits. TRPC1 subunits can form heteromultimeric channels with TRPC3, TRPC4, TRPC5, TRPC6, and TRPC7 subunits. TRPC4 and TRPC5 can heteromultimerize, as can combinations of TRPC3, TRPC6, and TRPC7 subunits (141). Other arrangements include channels composed of the closely related subunits TRPV5 and TRPV6 (138), TRPM6 and TRPM7 (186), and TRPML1 and TRPML3 (367). There is less evidence supporting interactions between channels from different subfamilies, although formation of TRPC1/TRPV4 heteromultimeric channels in the endothelium (207), TRPV1/TRPA1 channels in sensory nerves (93, 290), and other combinations (263) has been reported.

The most detailed study of TRP channel structure to date was provided by Liao et al. (192) and Cao et al. (43), who used cryoelectron microscopy to determine the structure of TRPV1 channels at high resolution (3.4 Å). These studies revealed that four TRPV1 subunits are symmetrically ar-
ranged around a central ion-permeable pore in a configuration reminiscent of previously characterized voltage-gated ion channels. Six transmembrane α-helices (S1–S6) span the lipid bilayer, with a pore-forming loop between the S5 and S6 domains. The TRP domain forms an interfacial helix distal to the S6 domain that is positioned to interact with the region preceding the S1 domain and the linker region between the S4 and S5 domains. The outer pore of the assembled channel is very wide. The selectivity filter is short and narrow in the inactive configuration, but dilates substantially when the channel is activated by capsaicin. This work confirmed the general structural features of TRP

Table 1. The human transient receptor potential cation channel superfamily

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channels that had been derived using lower-resolution cryo-electron microscopy methods in studies of TRPV1 (227), TRPV4 (313), and TRPC3 (225) channels. Not surprisingly, these studies, as well as the few available crystallography analyses of the ankyrin repeat domain of TRPV2 (150, 215), revealed specific structural differences in both intracellular and extracellular domains that are clearly associated with the unique signaling partners and sensory modalities served by individual TRP channel family members.

C. Functions in Mammalian Cells

TRP channels influence numerous important biological processes by controlling the flux of cations across the plasma membrane. Several TRP family members, including TRPP2, TRPM8, TRPV1, and TRPA1, are also present and active on the membranes of intracellular organelles and are involved in protein trafficking and vesicular ionic homeostasis (see Ref. 71 for review). Cation permeability has substantial biological significance. The distribution of the monovalent cations Na$^+$ and K$^+$ is particularly important for the regulation of cellular membrane potential and excitability. The divalent cations Ca$^{2+}$ and Mg$^{2+}$ also function as intracellular second messengers and as required cofactors for enzymatic activity. Although TRP channels are often described as “nonselective cation channels,” this definition is too broad and rather imprecise. TRPV5 and TRPV6 channels are highly selective for Ca$^{2+}$, with relative permeability of Ca$^{2+}$ versus Na$^+$ ($P_{Ca}/P_{Na}$) of ~100:1 (253, 368,

![Figure 2](https://example.com/figure2.png)

**FIGURE 2.** Membrane topology and functional domains of TRP subunits.
are selective for monovalent cations (Na\(^+\) and K\(^+\)) and are essentially impermeant to Ca\(^{2+}\) and other divalent cations. Ion substitution experiments suggest that the remaining TRP channels are permeant to both mono- and divalent cations, but with ionic preference ranging from essentially nonselective (i.e., TRPC1; Ref. 334) to significantly selective for Ca\(^{2+}\) (e.g., TRPV3; \(P_\text{Ca} : P_\text{Na} \sim 12:1\); Ref. 401) or preferentially selective for Mg\(^{2+}\) under physiological conditions (TRPM6 and TRPM7) (186). Selectivity for particular monovalent species also varies, with certain channels favoring Na\(^+\) versus K\(^+\) (e.g., TRPC3, TRPC6) and others with the opposite bias (e.g., TRPM4, TRPV4). This property is significant, since the reversal potential of channels with high K\(^+\) permeability occurs at negative membrane potentials in physiological ionic gradients; thus activation of these channels will have only a small direct effect on membrane potential depolarization. Membrane depolarization is greater for channels with a higher Na\(^+\) conductance, which diminishes the electrochemical driving force for Ca\(^{2+}\) influx in nonexcitable cells. Ultimately, this leads to channels with greater K\(^+\) permeability (e.g., TRPV4) conducting mixed currents having a higher fraction of Ca\(^{2+}\) compared with channels with higher Na\(^+\) permeability (e.g., TRPC6). Among the channels that conduct currents with a significant (>20%) Ca\(^{2+}\) fraction under physiological conditions are TRPV1-4 and TRPA1; in contrast, the fractional contribution of Ca\(^{2+}\) to TRPC channel currents is expected to be very low. Native currents are often not apparent from patch-clamp records reported in the literature, since these experiments are usually performed using a symmetrical cationic distribution. Few studies have actually attempted to record and characterize mixed currents under physiological ionic conditions.

### 1. Membrane potential regulation

Asymmetric concentration gradients maintained by cellular ion transporters and pumps establish the resting membrane potential. TRP channels contribute to membrane potential regulation in both excitable cells (i.e., those expressing voltage-gated Na\(^+\) or Ca\(^{2+}\) channels), such as neurons, glia, myocytes, and endocrine cells, and nonexcitable cells (i.e., those lacking voltage-gated ion channels), such as epithelial and endothelial cells, by regulating the flux of cations across the plasma membrane.

TRPM4 channels have a well-documented influence on membrane potential regulation in excitable and nonexcitable cells. These channels have a unitary conductance of \(~25\ pS\) and are selective for monovalent cations and impermeant to Ca\(^{2+}\) ions, and are activated by high levels of intracellular Ca\(^{2+}\) (179). TRPM4 channels are equally permeant to Na\(^+\) and K\(^+\), resulting in a reversal potential of \(~0\ mV\) in physiological solutions. At the hyperpolarized resting membrane potentials that are typical for most cells, these channels conduct inward currents that are primarily composed of Na\(^+\) when activated by global or localized increases in [Ca\(^{2+}\)], resulting in membrane depolarization. TRPM4-mediated Na\(^+\) currents influence the membrane excitability of pancreatic β-cells (53), detrusor (258, 321, 322) and cerebral artery (64, 76, 83, 104, 114, 115, 117, 118) smooth muscle cells, atrial cardiomyocytes (317), and pre-Botzinger neurons (226). In addition, membrane potential regulation in nonexcitable mast (369) and dendritic cells (26) is disrupted in TRPM4−/− mice. Membrane hyperpolarization associated with TRPM4 knockout and loss of depolarizing Na\(^+\) currents increases the driving force for Ca\(^{2+}\) influx, resulting in Ca\(^{2+}\) overload and impaired chemokine-dependent migration of dendritic cells (26).

### 2. Ca\(^{2+}\) signaling

Changes in the intracellular concentration of free Ca\(^{2+}\) influence muscle contraction, hormone secretion, gene expression, cell proliferation, and many other critical processes (32). The cytosolic free [Ca\(^{2+}\)] is normally maintained at low levels (~100–300 nM) by stationary and mobile Ca\(^{2+}\)-buffering proteins and by the activity of Ca\(^{2+}\)-pumping ATPases located on the plasma membrane and other cellular compartments. Changes in intracellular Ca\(^{2+}\) occur as a result of the influx of Ca\(^{2+}\) from the extracellular space and/or through the release of Ca\(^{2+}\) from intracellular structures. The endoplasmic/sarcoplasmic reticulum (ER/SR) is the best characterized intracellular Ca\(^{2+}\) storage organelle, but the membranes of mitochondria, nuclei, and other structures also delimit regions with high [Ca\(^{2+}\)]. Ca\(^{2+}\) signals can be global, occurring throughout the cytosol, or can be localized, creating subcellular domains where [Ca\(^{2+}\)] is transiently elevated. Almost all TRP channels are permeable to Ca\(^{2+}\), and several have a strong influence on Ca\(^{2+}\)-dependent signaling pathways (62). TRP channels have been described as direct (i.e., “ionotropic”) Ca\(^{2+}\) influx pathways, metabotropic (receptor-operated) Ca\(^{2+}\) influx channels, or store-operated Ca\(^{2+}\) channels, although this latter property is disputed.

A) TRP CHANNELS AS IONOTROPIC CA\(^{2+}\) INFLUX PATHWAYS: TRP SPARKLETS. Elementary Ca\(^{2+}\) signals representing Ca\(^{2+}\) influx through single TRPV4 channels, called TRPV4 sparklets, have been recorded from airway (423) and vascular smooth muscle cells (221), endothelial cells (337), and the intact endothelium (324) (for review, see Ref. 336). Recordings of TRPA1 sparklets have also been reported for cerebral artery endothelial cells (338). Theoretically, all TRP channels with sufficient unitary conductance and Ca\(^{2+}\) permeability (i.e., TRPV1-6; TRPA1) should produce detectable sparklets. TRPV4 and TRPA1 sparklets are optically recorded from cells expressing Ca\(^{2+}\) biosensors or loaded with Ca\(^{2+}\)-indicator dyes using total internal reflection fluorescent (TIRF) or confocal microscopy (FIGURE 3). Individual TRPV4 sparklets are very large Ca\(^{2+}\) influx events, with the amount of Ca\(^{2+}\) entering estimated to be ~100 times that of a single L-type Ca\(^{2+}\) channel sparklet (221).
The unitary amplitude of TRPA1 sparklets (338) is approximately twice that of a TRPV4 sparklet recorded under identical conditions (337), suggesting that more Ca$^{2+}$/H$^{11001}$ enters during the opening of a single TRPA1 channel than is the case for a single TRPV4 channel. TRPV4 sparklets generate subcellular microdomains in which local Ca$^{2+}$/H$^{11001}$ levels are very high, leading to activation of a variety of Ca$^{2+}$/H$^{11001}$-dependent signaling cascades, including endothelium-dependent vasodilation (324), negative feedback inhibition of vasoconstrictor stimuli (221), and NFATc3-dependent smooth muscle proliferation (423). TRPA1 sparklets are linked to endothelium-dependent dilation of cerebral arteries (338).

**FIGURE 3.** TRPV4 sparklets in endothelial cells and vascular smooth muscle cells. **A:** time-lapse image of a typical TRPV4 sparklet recorded from a primary cerebral artery endothelial cell using TIRF microscopy. The cell was stimulated with the selective TRPV4 agonist GSK1016790A (GSK; 100 nM). **B:** TRPV4 sparklets recorded from a tsA-201 cell transfected with TRPV4 (top) and from a native cerebral artery myocyte (bottom). The middle and right panels demonstrate the single channel-like behavior of these events. TRPV4 sparklets were stimulated with GSK. **C:** TRPV4 sparklets recorded from the intact endothelium of cerebral arteries from mice expressing the Ca$^{2+}$/H$^{11001}$-indicator protein GCaMP2 exclusively in the endothelium using high-speed, high-resolution confocal microscopy before and after stimulation with GSK. Experiments were performed in the presence of cyclopiazonic acid (CPA) to eliminate interference from ER Ca$^{2+}$/H$^{11001}$-release events. Representative traces indicating single channel-like events are shown below. [A from Sullivan and Earley (336). B from Mercado et al. (221). C from Sonkusare et al. (323), with permission from American Association for the Advancement of Science.]
through receptor-operated channels (ROCs) is responsible for the sustained phase (FIGURE 4B). This latter mode of Ca\(^{2+}\) influx is called “receptor operated Ca\(^{2+}\) entry” (ROCE). TRPC3, TRPC6, and TRPC7 channels have been characterized as ROCs that are activated by GPCRs linked to phospholipase C (PLC) (144, 199, 266, 323). In response to receptor stimulation, PLC cleaves the membrane phospholipid PIP\(_2\) into the second messenger molecules inositol 1,4,5-trisphosphate (IP\(_3\)) and diacylglycerol (DAG), the latter of which activates protein kinase C (PKC). IP\(_3\) binds to IP\(_3\)Rs on the ER/SR to cause release of Ca\(^{2+}\) into the cytosol. DAG directly activates Ca\(^{2+}\) influx through TRPC3, TRPC6, or TRPC7 channels on the plasma membrane.

The PLC substrate PIP\(_2\) can directly influence, both positively and negatively, the activity of almost all TRP channels. For example, application of PIP\(_2\) to excised membrane patches has been shown to directly activate the ROCs discussed above (TRPC3, TRPC6, and TRPC7) in HEK cell expression systems (181). This extensive topic is the subject of several recent reviews (285, 286, 288).

C) TRP CHANNELS AS STORE-OPERATED CHANNELS. Store-operated Ca\(^{2+}\) entry (SOCE) is defined as Ca\(^{2+}\) influx that occurs in response to depletion of intracellular Ca\(^{2+}\) stores, typically through a mechanism involving the endoplasmic reticulum (ER) or sarcoplasmic reticulum (SR). depletion of Ca\(^{2+}\) in these subunits (140); ROCE through these channels accounts for sustained elevations in intracellular Ca\(^{2+}\).

The PLC substrate PIP\(_2\) can directly influence, both positively and negatively, the activity of almost all TRP channels. For example, application of PIP\(_2\) to excised membrane patches has been shown to directly activate the ROCs discussed above (TRPC3, TRPC6, and TRPC7) in HEK cell expression systems (181). This extensive topic is the subject of several recent reviews (285, 286, 288).

D) calcium-homeostasis mechanisms mediated by TRP channels. A: receptor-operated Ca\(^{2+}\) entry (ROCE). Agonist binding to GPCRs stimulates the activity of PLC, which cleaves the membrane phospholipid PIP\(_2\) into IP\(_3\) and DAG. IP\(_3\) binds to IP\(_3\)Rs on the ER/SR to cause release of Ca\(^{2+}\) into the cytosol. DAG directly activates Ca\(^{2+}\) influx through TRPC3, TRPC6, or TRPC7 channels on the plasma membrane. B: example trace of changes in intracellular Ca\(^{2+}\) following stimulation of a GPCR, showing the phasic increase resulting from ER/SR Ca\(^{2+}\) release and sustained elevation resulting from ROCE. C: store-operated Ca\(^{2+}\) entry (SOCE). Depletion of Ca\(^{2+}\) in the ER/SR causes clustering of STIM1 in the ER/SR membrane proximal to the plasma membrane. STIM1 interacts with Orai1 at the plasma membrane to promote Ca\(^{2+}\) influx. TRPC1, TRPC4, and/or TRPC5 channels, either independently or in association with STIM1/Orai1, may participate in SOCE. D: example of a typical SOCE experiment. Under Ca\(^{2+}\)-free conditions, cells are treated with the SERCA inhibitor thapsigargin, causing Ca\(^{2+}\) to leak from the ER/SR into the cytosol. Reintroduction of Ca\(^{2+}\) to the bathing solution after ER/SR stores are depleted causes SOCE.
accomplished experimentally by prolonged stimulation of cell surface receptors or blocking SERCA activity with pharmacological agents such as thapsigargin or cyclopiazonic acid (260, 278) (Figure 4). Ca\(^{2+}\) directly enters the ER/SR compartment during SOCE, providing a dedicated mechanism for refilling depleted intracellular Ca\(^{2+}\) stores. The ion channels that conduct SOCE are called store-operated channels. A number of early studies suggested that TRPC1, TRPC4, and/or TRPC5 channels are store-operated channels (6, 97, 122, 261, 396). However, the discovery that complexes formed by stromal interacting protein 1 (STIM1) and Orai1 are critically involved in SOCE (for review, see Ref. 21) casts doubt on an obligate role for TRP channels in this response. STIM1 is a single-transmembrane-domain protein present on both the plasma membrane and the ER/SR. The NH\(_2\) terminus of STIM1 contains an EF hand Ca\(^{2+}\)-sensing domain that resides in the ER/SR lumen and two coiled-coil domains in the cytosolic COOH terminus that mediate homomeric and heteromeric protein-protein interactions. Orai1 is a Ca\(^{2+}\)-selective ion channel present on the plasma membrane (275). Depletion of intracellular Ca\(^{2+}\) stores is sensed by the EF hand domain located in the ER/SR lumen and leads to rearrangement and clustering of STIM1 proteins at the surface of the ER/SR proximal to the plasma membrane. STIM1 clusters interact with Orai1 on the plasma membrane, thereby initiating Ca\(^{2+}\) entry. Studies have shown that SOCE can occur in the absence of TRPC1, TRPC4, and TRPC5 channel expression and that TRPC channels function independently of STIM1 and Orai1 (68), leading some to conclude that TRP channels are not involved in SOCE. However, other studies have reported that TRPC channels are required for SOCE in certain settings, and some investigators have noted that the structure of Orai1 is more reminiscent of a regulatory subunit than classically described ion-permeable channels (393). It has been proposed that Orai1 proteins are a regulatory component of a store-operated channel composed of TRPC1 subunits (or vice versa) (16), but this issue is currently unresolved. Experimental details are important in interpreting SOCE experiments. For example, in whole cell patch-clamp experiments, if cytosolic Ca\(^{2+}\) is not fully buffered, SOCE through the STIM1/Orai1 pathway can cause Ca\(^{2+}\)-dependent activation of certain TRP channels that is independent of store depletion. In this context, it has been proposed that SOCE through Orai1 causes TRPC1 channels to traffic to the plasma membrane where they mediate a secondary Ca\(^{2+}\) influx event (55). The precise functional relationships between STIM1, Orai1, and TRPC channels during SOCE remain to be determined.

3. Mg\(^{2+}\) homeostasis

Mg\(^{2+}\) are central to the maintenance of nucleic acid structure, regulation of certain Ca\(^{2+}\) and K\(^{+}\) channels, and the biological activity of ATP and hundreds of enzymes (for review, see Ref. 67). TRPM6 and TRPM7 channels are important pathways for Mg\(^{2+}\) influx (228, 303, 305). TRPM7 has a unitary conductance of \(~40–105\) pS, is abundantly expressed in many types of cells, and is constitutively active in heterologous expression systems (240). TRPM7 is permeable to both monovalent and divalent cations, although some reports suggest greater permeability of divalent cations under physiological conditions. The channel is inhibited by intracellular [Mg\(^{2+}\)] greater than 1 mM. Under physiological conditions, the channel may act as a Mg\(^{2+}\)/Ca\(^{2+}\) influx pathway, with intracellular [Mg\(^{2+}\)] providing negative feedback. The ion permeation and regulatory properties of TRPM6 are similar to those of TRPM7, but its unitary conductance is greater (\(~80\) pS). TRPM6 can form heteromultimeric channels with TRPM7 with properties distinct from those of the homomeric channels. Significantly, mutations within TRPM6 are associated with familial hypomagnesemia with secondary hypocalcemia (303), and TRPM7 deletion causes Mg\(^{2+}\) deficiency, indicating that TRPM6 and TRPM7 channels are vital for Mg\(^{2+}\) homeostasis. TRPM6 and TRPM7 channels are also permeable to other divalent cations, including the essential elements Zn\(^{2+}\), Co\(^{2+}\), and Mn\(^{2+}\), and toxic metals such as Sr\(^{2+}\), Cd\(^{2+}\), Ba\(^{2+}\), and Ni\(^{2+}\), and may be involved in transporting these ions across the plasma membrane (228).

III. PHYSIOLOGICAL ROLES OF TRP CHANNELS IN THE VASCULATURE

The primary function of the vasculature is to dynamically regulate blood flow and vascular permeability to ensure appropriate matching of oxygen and nutrient supply with the metabolic demands of the tissue. Vascular resistance and blood flow are primarily regulated by the contractile state of smooth muscle in the arterial circulation, but can also be chronically altered by cellular proliferation and vascular remodeling that occur during vascular development and in association with vascular diseases. Endothelial cells have a profound influence on vascular tone and permeability, an influence that can vary between physiological and pathophysiological conditions. Pervascular neurons and astrocytes closely apposed to blood vessels can also influence smooth muscle contractility and blood flow regulation. A growing body of evidence, reviewed below, clearly demonstrates the importance of TRP channels in the initiation, modulation, and integration of regulatory pathways that govern the contributions of these diverse cell types within the vascular wall to control of vasomotor tone and tissue blood flow.

A. TRP Channels in Vascular Smooth Muscle

Luminal diameter and hence vascular resistance are determined by the contractile state of vascular smooth muscle cells, which are arranged circumferentially around the lumen of all blood vessels. Vascular smooth muscle contraction is primarily regulated by the phosphorylation state of a myosin regulatory light chain (RLC) domain in the smooth
muscle myosin complex. The net balance of myosin light chain (MLC) kinase versus MLC phosphatase activity determines the phosphorylation state of the RLC. MLC kinase, a Ca$^{2+}$/calmodulin-dependent enzyme, is activated by increases in cytosolic Ca$^{2+}$ levels, but its phosphatase activity is regulated by intracellular signaling pathways that serve to modify the sensitivity of the contractile apparatus to intracellular Ca$^{2+}$. Smooth muscle [Ca$^{2+}$], is dynamically regulated by the relative activities of Ca$^{2+}$-entry, -release, and -extrusion mechanisms. The L-type voltage-dependent Ca$^{2+}$ channel (VDCC) is the primary Ca$^{2+}$-influx pathway in vascular smooth muscle cells. Thus membrane depolarization and subsequent influx of Ca$^{2+}$ through these channels is a primary mechanism of vascular smooth muscle cell contraction (FIGURE 5). Increased cation permeability associated with TRP channel activation results in membrane depolarization and Ca$^{2+}$ influx, which are the primary mechanisms by which these channels cause vasoconstriction. Ca$^{2+}$ influx through TRP channels with significant selectivity for Ca$^{2+}$ could theoretically directly activate the contractile process, but there is little experimental evidence in support of this possibility in the systemic circulation. However, some data suggest that SOCE through TRP channels can support a vasoconstrictor response in pulmonary arteries (see sect. IIIA2).

1. TRP channels and vascular smooth muscle contractility

Nearly a dozen TRP channels have been detected in vascular smooth muscle using RT-PCR, Western blotting, and immunolabeling approaches (TABLE 2). Lacking selective pharmacological blockers or activators, early researchers in the field relied on antisense nucleotide-mediated knockdown of TRP channel expression or antibodies that bind to epitopes on extracellular domains of the channels to inhibit cation currents. In some cases, the specificity of these treatments was not fully demonstrated; thus the conclusions drawn from the results of these studies should be carefully considered. In addition, many studies have attempted to study smooth muscle cell contractility using cultured cells, such as the aorta-derived A7r5 transformed cell line. This approach is questionable, as A7r5 cells (and other transformed cell lines) display little or no contractility and are phenotypically distinct from native vascular smooth muscle cells. Primary smooth muscle cells are also problematic since they rapidly (within a few hours) differentiate to a proliferative, noncontractile state under standard culture conditions. Accordingly, conclusions about regulation of smooth muscle cell contractility derived from data obtained using cultured cells should be interpreted with caution.

Even now, many studies continue to rely on nonselective pharmacological agents, such as trivalent cations (Gd$^{3+}$, La$^{3+}$), 2-aminoethoxydiphenyl borate (2-APB), ruthenium red, and SKF96365 without consideration of the broad range of targets affected by these compounds. Although these experimental tools have their place, knockout animals, small inhibitory RNA (siRNA) approaches, and selective pharmacological agents that have been developed in recent years are more definitive approaches for studying contractile responses.

A1 TRPC6. Compelling evidence indicates that TRPC6 channels are functionally important in arterial myocytes. TRPC6 channel mRNA and protein have been identified in vascular smooth muscle, both arterial and venous, from many species and in many vascular beds (145). In native cells, it is often difficult to determine whether the observed ion channel activity is attributable to a specific TRPC channel isoform, perhaps because many TRPC subunits form heteromultimeric associations, creating a challenge for characterizing endogenous TRPC channels. This is in contrast to expression systems, where the composition of channel isoforms is controlled. Furthermore, TRPC channels are often activated or modulated by diverse cellular signaling mechanisms, the activity and function of which are often altered by cell isolation procedures or the specific patch-clamp configuration (i.e., on-cell, whole-cell, perforated

![FIGURE 5. TRP channels contribute to vascular smooth muscle cell contractility. Cation influx through TRP channels depolarizes the plasma membrane ($\Delta E_m$), initiating Ca$^{2+}$ influx through VDCCs. Elevated Ca$^{2+}$ levels increase binding of calmodulin-Ca$^{2+}$ (Cd-Ca$^{2+}$) complexes to MLCK, stimulating phosphorylation of the regulatory myosin light chain (MLC$_{20}$). MLC$_{20}$ is dephosphorylated by myosin light-chain phosphatase (MLCP). Some studies suggest that Ca$^{2+}$ influx through TRP channels can directly initiate myocyte contraction.](http://physrev.physiology.org/ by 10.220.33.3 on June 25, 2017)
patch) employed. Nevertheless, single-channel activity that likely is determined primarily by TRPC6 channels has been observed in a variety of vascular smooth muscle types, including portal vein (9), mesenteric artery (293), and cultured aortic smooth muscle cells (144). Single-channel conductance values ranging from 33 to 45 pS have been reported in these studies. The presence of functional ion channels in mesenteric (293) myocytes that are composed, at least in part, of TRPC6 subunit proteins has been confirmed through the use of current-blocking antibodies that target TRPC6.

In work growing out of initial observations that TRPC channel activity in expression systems is linked to activation of GPCRs (62), Inoue et al. (146) provided the first evidence that TRPC6 channels are present and functionally active in native vascular smooth muscle. These seminal studies clearly demonstrated that a nonselective cation channel with biophysical and pharmacological properties resembling those of cloned TRPC6 channels expressed in heterologous systems is present in portal vein myocytes. The activity of this portal vein smooth muscle channel was substantially elevated following α1-adrenoceptor activation, and was greatly reduced or was absent in cells treated with TRPC6 subunit proteins has been confirmed through the use of current-blocking antibodies that target TRPC6.

In freshly isolated rabbit mesenteric artery myocytes, low levels (1 nM) of angiotensin II (ANG II) were shown to activate a nonselective cation channel that was inhibited by TRPC6 antibodies (293). Activation of TRPC6 in these studies followed stimulation of a GPCR and subsequent signaling via PLC and generation of DAG (327). The stimulatory effect of DAG on TRPC6 channel activity appeared to be direct and was independent of PKC activation (137, 140). Furthermore, there are apparent interactions between receptor-mediated and other signaling modalities that result in amplification of vascular TRPC6 channel activity under some conditions. Specifically, transmembrane Ca2+ mobilization through TRPC6 activation is amplified through combined receptor and mechanical stimulation via signaling pathways that involve contributions by both PLC and phospholipase A2 (PLA2) (144). Other examples of vascular TRPC6 coregulation by various cellular signaling molecules have been noted. Synergistic stimulatory effects on vascular TRPC6 channels through coactivation of IP3Rs and DAG (9, 147, 152) or IP3 and arginine vasopressin receptors (213) have been described, and it has been shown that protein kinase A (PKA) activity amplifies the activating effects of ANG II on TRPC6 channels (255). In contrast to findings obtained using a HEK cell expression system (181), PIP2 was shown to inhibit TRPC6 currents in native mesenteric artery smooth muscle cells (13). TRPC6 channels may also be inhibited by interactions with other TRPC channels, including TRPC1/C5 heteromultimeric channels (312).

Functional studies have linked the activation of TRPC6 channels in vascular smooth muscle cells with pressure-induced (myogenic) vasoconstriction. Myogenic constriction, which contributes to the autoregulation of blood flow in some vascular beds (28), is mediated by depolarization of

<table>
<thead>
<tr>
<th>TRP</th>
<th>Function</th>
<th>Tissue</th>
</tr>
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<tbody>
<tr>
<td>TRPC1</td>
<td>ROCE (ET-1 constriction), SOCE</td>
<td>Brain (124), aorta (344), lung (245-247)</td>
</tr>
<tr>
<td>TRPC3</td>
<td>GPCR-mediated vasoconstriction</td>
<td>Brain (4, 5, 283, 398), aorta (198), heart (266), kidney (296), lung (420)</td>
</tr>
<tr>
<td>TRPC4</td>
<td>SOCE</td>
<td>Aorta and mesentery (196, 197)</td>
</tr>
<tr>
<td>TRPC5</td>
<td>SOCE</td>
<td>Brain (403)</td>
</tr>
<tr>
<td>TRPC6</td>
<td>GPCR-mediated vasoconstriction, myogenic tone</td>
<td>Portal vein (146), aorta (323), brain (217, 387)</td>
</tr>
<tr>
<td>TRPV1</td>
<td>Vasoconstriction</td>
<td>Aorta, skeletal muscle (158, 201), mesentery (273), cephalic circulation (46)</td>
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<tr>
<td>TRPV2</td>
<td>Mechanosensitive Ca2+ influx</td>
<td>Aorta (238)</td>
</tr>
<tr>
<td>TRPV3</td>
<td>mRNA present in VSM but no known function</td>
<td>Aorta and lung (408)</td>
</tr>
<tr>
<td>TRPV4</td>
<td>Ca2+ influx, vasodilation</td>
<td>Brain (79, 221), mesentery (81)</td>
</tr>
<tr>
<td>TRPA1</td>
<td>Vasodilation</td>
<td>Aorta (405)</td>
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<tr>
<td>TRPM4</td>
<td>Depolarization, myogenic tone</td>
<td>Brain (64, 82, 83, 104, 114, 115, 117)</td>
</tr>
<tr>
<td>TRPM8</td>
<td>Vasoconstriction</td>
<td>Aorta (151)</td>
</tr>
<tr>
<td>TRPP2</td>
<td>Ca2+ entry, vasoconstriction, myogenic tone</td>
<td>Mesentery (279, 308), brain (243)</td>
</tr>
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ROCE, receptor-operated Ca2+ entry; SOCE, store-operated Ca2+ entry; ET-1, endothelin-1; GPCR, G protein-coupled receptor. Reference numbers are given in parentheses.
the arterial myocyte plasma membrane and Ca\(^{2+}\) entry through VDCCs (163). TRPC6 channels in vascular smooth muscle cells and expression systems are activated by mechanical forces such as cell swelling (387) (327) or application of suction to the plasma membrane (327). However, the exact mechanism responsible for mechanical activation of TRPC6 is a matter of controversy, with some studies indicating that TRPC6 channels are directly activated by application of force (327) and others reporting indirect activation downstream of mechanosensitive signaling pathways (217). Data from some laboratories point to GPCRs as the primary mechanosensors that activate TRPC6 channels through downstream signaling involving the formation of DAG (217, 302). A study by Welsh et al. (387) demonstrated that swelling-activated cation currents and intravascular pressure-induced smooth muscle cell depolarization and vasoconstriction were substantially reduced in cerebral arteries treated with antisense oligonucleotides targeting TRPC6, suggesting the critical involvement of this channel in the myogenic response in this vascular bed. It should be mentioned that neither agonist-evoked contraction nor myogenic tone were reduced in arteries from TRPC6\(^{-/-}\) mice (70, 302). However, upregulation of constitutively active (12) TRPC3 channels in TRPC6\(^{-/-}\) mice may provide a compensating depolarization and vasoconstrictor pathway in these animals.

TRPC6 appears to be an integral part of a force-sensing complex in cerebral artery smooth muscle cells that regulates myogenic vasoconstriction (118). Gonzales et al. (118) demonstrated that Ca\(^{2+}\) entry through TRPC6 channels, activated either by PLC-\(\gamma\)-dependent generation of DAG or by direct mechanical stimulation, reinforces IP\(_3\)-R-dependent Ca\(^{2+}\) release from the SR, which then activates nearby TRPM4 channels. TRPM4 channels, in turn, mediate smooth muscle cell depolarization, promoting Ca\(^{2+}\) influx through VDCCs and subsequent vasoconstriction (see below for more details regarding the role of TRPM4 channels in vasoconstriction) (FIGURE 6). This model clearly delineates the architecture, interactions, and mechanisms of action of a variety of signaling molecules recognized as critical players in the development and modulation of arterial tone in the brain circulation.

TRPC6 channels are also involved in vasoconstrictor responses in the pulmonary arterial circulation (385). Small pulmonary arteries constrict in response to hypoxic challenge, a response called hypoxic pulmonary vasoconstriction (HPV). HPV improves oxygenation of the blood by matching perfusion to well-ventilated regions of the lung. Weissmann et al. (385) found that robust HPV, present in the lungs of wild-type mice, was absent in TRPC6\(^{-/-}\) mice. Hypoxia-induced activation of Ca\(^{2+}\) influx as well as TRPC6-like cation currents were also absent in pulmonary artery myocytes from TRPC6\(^{-/-}\) mice. Interestingly, in contrast to the apparent compensatory upregulation of TRPC3 expression in nonpulmonary arteries previously documented in TRPC6\(^{-/-}\) mice (70), no change in the expression of TRPC3 or other TRPC channels (other than TRPC6) was reported in this study. A follow-up study by this group reported the clear involvement of DAG as a mediator of TRPC6 activation in pulmonary artery myocytes during hypoxia (99). Others have reported a key role for epoxyeicosatrienoic acid (EET) derivatives as mediators of enhanced TRPC6 contributions to HPV (272). Much of the ability of EETs to increase channel activity in pulmonary artery vascular smooth muscle cells is likely attributable to recruitment of TRPC6 channels to caveolae (159).

It is now abundantly clear that TRPC6 channels are fundamentally important for the onset, maintenance, and modulation of vascular tone in many different vascular beds. Additional studies are needed to further define the unique contributions of these channels in various regions within the cardiovascular system and to characterize other important modulatory inputs that may influence the activity of

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**FIGURE 6.** A force-sensitive signaling network involving TRPC6 and TRPM4 channels in cerebral artery smooth muscle cells. A stretch-sensing pathway in cerebral artery myocytes that includes 1) activation of AT\(_1\)R, Src tyrosine kinase, and PLC-\(\gamma\)1, leading to the generation of IP\(_3\); and 2) Ca\(^{2+}\) influx through TRPC6 channels onto IP\(_3\)Rs, promoting CICR that results in activation of TRPM4 channels, which are responsible for pressure-induced depolarization of the plasma membrane. PM, plasma membrane; \(\Delta V_m\), change in membrane potential. [From Gonzales et al. (118).]
these channels in various physiological and pathological contexts. For instance, more detail is needed about the regulation of TRPC6 by PLC-derived molecules, the influences of related TRPC channels that form heteromultimers with TRPC6, and the contributions of TRPC6-mediated Ca\(^{2+}\) entry to local and global cellular signaling processes.

B) TRPC3. Expression of TRPC3 channel mRNA and/or protein has been demonstrated by RT-PCR, Western blotting, and/or immunohistochemical approaches in vascular smooth muscle cells in cerebral (256, 283), cephalic (12), coronary (168, 199), renal (373), uterine (306), and pulmonary arteries (420) and in the aorta (202). However, the properties of TRPC3 currents in native vascular smooth muscle cells have been described only rarely. Albert et al. (12) recorded a constitutively active nonselective cation channel in vascular myocytes from the rabbit ear artery with pharmacological properties very similar to cloned TRPC3 channels. This channel appeared to have three distinct conductance levels (10), although none matched the single value reported for the cloned TRPC3 channel expressed in HEK cells (427). Heteromultimeric TRPC channel assembly, in particular between TRPC3 and TRPC7 channels, has been documented in coronary artery myocytes, and related diversity in channel properties has been described (266).

TRPC3 channels in vascular smooth muscle cells are involved in contractile responses, primarily evoked by receptor-ligand interactions. This function has been best described in cerebral and carotid arteries and the aorta. TRPC3 channels in vascular smooth muscle are activated by stimulation of a variety of GPCRs, including pyrimidine (283), endothelin-1 (ET-1) (266), and ANG II (199) receptors. In brain pial arteries, suppression of TRPC3 channel expression using antisense oligonucleotides was shown to greatly reduce inward currents, depolarization, and vasoconstriction induced by the purinergic receptor agonist uridine triphosphate (UTP) (283). Interestingly, in this case IP\(_3\) itself inhibited the TRPC3/C7 channel, demonstrating the potential differential control of homomeric versus heteromultimeric TRPC channels in cerebral artery smooth muscle cells. This activation pathway does not involve IP\(_3\)-mediated Ca\(^{2+}\) release from the SR, but rather reflects direct coupling between IP\(_3\)Rs and TRPC3 channels mediated by IP\(_3\). Coupling of TRPC3 channels and IP\(_3\)Rs in vascular smooth muscle cells leads to activation of TRPC3-mediated cation currents, followed by membrane depolarization, Ca\(^{2+}\) entry through VDCCs, and myocyte contraction. Close physical association of IP\(_3\)Rs with TRPC3 channels is orchestrated by the integral membrane protein caveolin-1. Activation of heteromultimeric TRPC3/TRPC7 channels by ET-1 in coronary artery myocyte (266) also involves products of phosphoinositide metabolism. Phosphatidylinositol-3-phosphate, formed by the actions of specific phosphatidylinositol-3-kinase isoforms, appears to be a direct activator of this channel (311). Interestingly, in this case IP\(_3\) itself inhibited the TRPC3/C7 heteromultimeric channel, demonstrating the potential differential control of homomeric versus heteromultimeric TRPC channels. In expression systems, TRPC3 trafficking, membrane localization, and activity are regulated byPIP\(_2\), which is the primary substrate for phosphoinositide metabolism (142, 181). Whether this holds true for native vascular smooth muscle cells has not been determined.

The primary mechanism for activation of TRPC3 downstream of receptor stimulation in expression systems (140) and in native vascular myocytes (11) was initially thought to be through the direct actions of DAG on the channel. However, work from Kiselyov et al. (162) showed that TRPC3 channels functionally interact with IP\(_3\)Rs in HEK cell expression systems, and Boulai et al. (35) showed that TRPC3 and IP\(_3\)Rs could be coimmunoprecipitated. Jaggar and colleagues (3–5, 398) showed that IP\(_3\)Rs are critical elements in the activation mechanism for TRPC3 channels in cerebral artery smooth muscle cells. This activation pathway does not involve IP\(_3\)-mediated Ca\(^{2+}\) release from the SR, but rather reflects direct coupling between IP\(_3\)Rs and TRPC3 channels mediated by IP\(_3\). Coupling of TRPC3 channels and IP\(_3\)Rs in vascular smooth muscle cells leads to activation of TRPC3-mediated cation currents, followed by membrane depolarization, Ca\(^{2+}\) entry through VDCCs, and myocyte contraction. Close physical association of IP\(_3\)Rs with TRPC3 channels is orchestrated by the integral membrane protein caveolin-1. Activation of heteromultimeric TRPC3/TRPC7 channels by ET-1 in coronary artery myocyte (266) also involves products of phosphoinositide metabolism. Phosphatidylinositol-3-phosphate, formed by the actions of specific phosphatidylinositol-3-kinase isoforms, appears to be a direct activator of this channel (311). Interestingly, in this case IP\(_3\) itself inhibited the TRPC3/C7 heteromultimeric channel, demonstrating the potential differential control of homomeric versus heteromultimeric TRPC channels. In expression systems, TRPC3 trafficking, membrane localization, and activity are regulated byPIP\(_2\), which is the primary substrate for phosphoinositide metabolism (142, 181). Whether this holds true for native vascular smooth muscle cells has not been determined.
ing TRPC3 knockout mice found no apparent link between TRPC3 function and cGMP- or PKG-induced relaxation of the aorta or the hindlimb circulation (202), perhaps suggesting species- or tissue-specific differences in this regard. In expression systems (360) and in arterial myocytes (8), TRPC3 channels are inhibited by PKC.

The weight of evidence supports the contention that TRPC3 channels in vascular smooth muscle play important roles in regulating vascular contractility. Constitutive activity of these channels contributes to basal vascular tone, and activation of TRPC3 channels by vasoconstrictor agonists causes further membrane depolarization and Ca\(^{2+}\) entry through VDCCs. Future studies will provide needed details of the mechanisms by which the basal and ligand-activated states of TRPC3 channels are achieved.

TRPC4. TRPM4 and the closely related TRPM5 are the only TRP channels that are essentially impermeant to Ca\(^{2+}\). However, because of their relative high Na\(^{+}\)-versus-K\(^{+}\) permeability and significant inward current amplitudes in standard ionic gradients and at physiological membrane potentials, TRPM4 channels have considerable capacity to affect membrane depolarization and thereby alter cellular function. TRPM4 channels have been reported to influence Ca\(^{2+}\) entry in excitable (125) and nonexcitable cells (178), primarily through their depolarizing influence. In excitable cells, depolarization leads to activation of VDCCs and a global increase in Ca\(^{2+}\), whereas in nonexcitable cell depolarization diminishes passive Ca\(^{2+}\) influx because it reduces the electrical driving force.

Molecular and functional studies have shown that TRPM4 channels are present in native smooth muscle cells. TRPM4 mRNA has been reported in smooth muscle cells in the rat aorta (145, 408), as well as mesenteric (145), pulmonary (408), and cerebral arteries (83, 145). Immunohistochemical and Western blot analyses have also demonstrated the presence of TRPM4 protein in cerebral artery myocytes (64, 114). Single-channel and whole cell currents with properties very similar to those recorded from HEK cells overexpressing TRPM4 channels have been detected in cerebral artery myocytes (82, 83, 234). The activity of TRPM4 channels is primarily regulated by the intracellular [Ca\(^{2+}\)] through interactions of the Ca\(^{2+}\)/calmodulin complex with defined sites near the COOH terminus (252). Activation of native and exogenously expressed TRPM4 channels requires a very high (micromolar) [Ca\(^{2+}\)] at the intracellular face of the channel (82) (251). However, global intracellular Ca\(^{2+}\) levels in vascular smooth muscle cells are typically in the range of 100–500 nM, suggesting that TRPM4 activation must occur within subcellular regions with locally elevated [Ca\(^{2+}\)]. Such domains are created when Ca\(^{2+}\) is released through ryanodine receptors (234) or IP\(_3\)Rs (114). Work by Gonzales et al. demonstrated that TRPM4 channels in native cerebral artery smooth muscle cells are activated by release of Ca\(^{2+}\) from IP\(_3\)Rs (114) and that channel activation is dependent on intracellular Ca\(^{2+}\) buffering (115).

Ca\(^{2+}\)-dependent activation of TRPM4 can be fine tuned by other signaling pathways. For example, Nilius et al. (252) demonstrated that the innate Ca\(^{2+}\) sensitivity of cloned TRPM4 channels expressed in HEK cells is elevated by PKC-dependent phosphorylation. Similarly, stimulation of PKC activity was shown to increase TRPM4 whole cell currents in cerebral artery myocytes and cause constriction of isolated cerebral arteries (82). This increase in Ca\(^{2+}\) sensitivity was found to result from PKC-δ-dependent translocation of TRPM4 channels to the plasma membrane of atrial myocytes (64, 104). PIP\(_2\) has been clearly implicated as an important regulator of TRPM4 channel activity in expression systems (250, 422). These studies demonstrated that the exogenous administration of PIP\(_2\) prevents desensitization of TRPM4 currents in response to high levels of Ca\(^{2+}\) (~10–100 μM) at the cytosolic face; however, the significance of this pathway in native vascular smooth muscle cells remains to be determined.

TRPM4 channels are critically important for cerebral artery function (76, 116). Suppression of TRPM4 expression in cerebral arteries was shown to result in a substantial reduction in smooth muscle membrane depolarization and vasoconstriction accompanied by increased intravascular pressure (83). This pioneering study was reinforced by a subsequent report showing that 9-phenanthrol, a highly specific pharmacological inhibitor of TRPM4 channels (119), inhibited pressure-induced depolarization and myogenic tone in isolated pial arteries (117). However, interpretation of these latter data may be called into question by a recent study suggesting that 9-phenanthrol can activate intermediate-conductance Ca\(^{2+}\)-activated K\(^{+}\) channels present in the endothelium to cause vasodilation of mesenteric arteries (107). Two additional studies have confirmed the involvement of TRPM4 channels in cerebral myogenic tone, in this case in the cerebral intraparenchymal arteriolar network (37, 188). The involvement of TRPM4 in pressure-induced vasoconstriction suggests that this channel is mechanically activated. Although one study reported that application of negative pressure to the recording electrode activated single cation channels in vascular smooth muscle cells with properties much like those in HEK cells overexpressing TRPM4 channels (234), a more recent study failed to detect inherent mechanosensitivity of TRPM4 (118). Recent work points to involvement of purinergic or ANG II type 1 receptor (AT\(_1\)R) GPCRs in arterial pressure-sensing. Along these lines, a compelling case has been made that TRPM4 channels are activated downstream of a mechanosensitive losartan-sensitive receptor (likely AT\(_1\)R) in cerebral artery smooth muscle cells (118) (FIGURE 6). Mechanooactivation of P2Y4 and P2Y6 purinergic receptors and downstream
activation of TRPM4 channels in cerebral intraparenchymal arteriolar myocytes have also been proposed (188).

Two studies have investigated the effect of TRPM4 activity on cardiovascular control in vivo. Using a knockdown approach, Reading et al. (282) demonstrated that TRPM4 channels in cerebral artery myocytes directly contribute to autoregulation of cerebral blood flow in intact animals, presumably through control of myogenic tone. However, another study (214) reported no difference in agonist- or pressure-induced contractile responses of the aorta or in hindlimb vascular preparations isolated from wild-type and TRPM4−/− mice. It is unclear if these discrepancies are due to differences in vascular beds or experimental preparations, or possible compensatory expression of other cation channels in the TRPM4−/− animals. Furthermore, the TRPM4−/− mice used for this study were mildly hypertensive, likely due to increased release of catecholamines from chromaffin cells. Thus it is possible that elevated catecholamine levels in TRPM4−/− mice compensate for the loss of TRPM4-induced smooth muscle contractility. Additional studies are needed to determine if vascular function in specific areas of the circulation, such as in the brain, is altered in TRPM4−/− animals, preferably using conditional and cell-specific deletion approaches.

TRPM4 activity also promotes excitability of detrusor smooth muscle in the urinary bladder (258, 321, 322), suggesting that this channel could have a broad role in the regulation of smooth muscle contractility.

D) TRPP CHANNELS. The TRPP subfamily consists of TRPP1 (PKD2), TRPP2 (PKD2L1), and TRPP3 (PKD2L2). TRPP1 is a Ca2+-permeable, nonselective cation channel with a large unitary conductance (135–175 pS) that may be localized to the plasma membrane or intracellular organelles. PKD1 is sometimes referred to as TRPP1, but it is structurally distinct from the other TRPP proteins and does not form a cation-permeable ion channel. PKD1 can interact with TRPP1 and may be a mechanically sensitive regulatory subunit. PKD1 and TRPP1 are both expressed in vascular smooth muscle cells (121, 243, 354). Early reports suggested that PKD1 and TRPP1 physically interacted and might be involved in regulating the development and organization of the cytoskeletal elements of vascular myocytes (308). A report by Shaif-Naeini et al. (308) demonstrated that overexpression of TRPP1 in COS-7 cells diminished the stretch-induced currents present in native cells. Mechanosensitive currents were restored by coexpression of PKD1 and TRPP1. Selective deletion of PKD1 in smooth muscle cells (Pkd1SMdel/del) decreased stretch-activated currents in mesenteric artery smooth muscle cells and diminished myogenic constriction of isolated mesenteric arteries. Surprisingly, knockdown of TRPP1 expression in Pkd1SMdel/del mice restored stretch-activated currents in isolated smooth muscle cells and myogenic constriction of mesenteric arteries. The authors of this study proposed that, in the absence of PKD1, TRPP1 inhibits mechanosensitive ion channels involved in the vascular smooth muscle depolarization associated with myogenic tone and that these responses are restored by physical interaction of PKD1 with TRPP1.

Phenylephrine-induced aortic and mesenteric resistance artery contractions are enhanced in TRPP1+/− mice due to increased actin and myosin expression (72, 279), suggesting that TRPP1 may exert inhibitory effects on vascular tone through inhibition of contractile protein structure and function. An age-dependent vascular phenotype was observed in Pkd1−/− mice, with old mice (40 wk), but not young mice, exhibiting an increase in phenylephrine-mediated aortic contractility (133). This finding also suggests vascular bed-specific roles for TRPP isoforms, although clarification of this possibility will require additional detailed studies. It has also been proposed that the physical interaction of PKD1 with TRPP1, and related membrane association of these proteins, is an important element and causal factor in some of the vascular pathologies observed in patients with polycystic kidney disease (280).

Jaggar et al. (243) reported a different take on TRPP1 channels, concluding that TRPP1 cation currents directly activate myogenic tone in the cerebral circulation. This study showed that TRPP1 and PKD1 are present in cerebral artery myocytes and localize to the plasma membrane, and further indicated that TRPP1 mRNA is much more abundant than PKD1 mRNA. Knockdown of TRPP1 reduced swelling-activated cation currents in isolated vascular smooth muscle cells and suppressed the development of myogenic tone in isolated cerebral arteries. The reasons for the apparently divergent functions of TRPP1 channels in cerebral and mesenteric arteries in particular, and the physiological roles of TRPP proteins in vascular smooth muscle cells in general, are not clear and will require further investigation.

E) TRPV1. Expression of TRPV1 channels in aortic and skeletal muscle arteriolar myocytes from rats has been reported (201, 158, 355). These studies showed that the TRPV1 activator capsaicin increases hindlimb vascular resistance in vivo and causes substantial endothelium-independent vasoconstriction of skeletal muscle arterioles in vitro. Vasконstriction of isolated canine mesenteric arteries following direct activation of TRPV1 channels has also been reported (273). More recent work has revealed the presence of TRPV1 channels in murine arteriolar myocytes located in thermoregulatory tissues and has demonstrated a role for these channels as mediators of myocyte Ca2+ entry and contraction in intact auricular arterioles (46).

F) TRPV2. TRPV2 channels are present in murine aortic, mesenteric, and basilar artery smooth muscle cells (238). Hypotonic cell swelling of aortic myocytes was shown to activate nonselective cation channels and Ca2+ entry, effects
that were suppressed by treatment with TRPV2 antisense oligonucleotides. These findings led the authors of this study to propose a role for TRPV2 channels in myogenic vasoconstriction; however, functional studies investigating the role of TRPV2 channels in intact arteries have not been reported.

G) TRPM8. TRPM8 channels are thought to be involved primarily in sensory nervous system signaling activated by cold and menthol. A single study showed that TRPM8 mRNA and protein are present in rat aortic, mesenteric, tail, and femoral artery myocytes (151). In most reported experiments, menthol caused a dilatory response in the aorta, as well as in mesenteric and tail arteries, that was at least partly endothelium independent. However, in some cases menthol caused small contractions of endothelium-dependent aortic rings. The exact role of TRPM8 channels in the vasculature remains undefined.

H) TRPC1. TRPC1 channels are frequently linked to SOCE (30, 145), although this function has been disputed (68, 69, 274), and a few studies have investigated ROCE through TRPC1 channels in vascular smooth muscle cells. For example, ET-1 was shown to induce VDCC- and SOCE-dependent Ca2+ entry in cerebral arteries (124) and in cultured aortic smooth muscle cells (344) that may be mediated by TRPC1 channels (344). A series of studies by Large, Albert, and colleagues investigated receptor-operated regulation of TRPC1 and heteromultimeric channels containing TRPC1 subunits in vascular smooth muscle cells (291, 309, 310, 312). These studies suggested that TRPC1 channels are activated downstream of PLC- and PKC-dependent signaling pathways. A model proposed in another report suggests an association between TRPC1, IP3Rs, and STIM1 that could account for Ca2+-influx and contraction of vascular smooth muscle independent of SOCE (34), although supporting experimental evidence is meager. The biophysical properties of TRPC1 channels suggest that the fractional Ca2+-component of TRPC1 currents under physiological conditions is very small. This argues against involvement of direct Ca2+-influx, but is it possible that TRPC1-mediated cation influx could cause membrane depolarization and Ca2+ influx through VDCCs in excitable cells. However, vasoconstriction in response to elevated intravascular pressure (69), adrenoceptor activation, or K+-induced depolarization (304) is unaltered in TRPC1−/− mice.

2. SOCE in vascular smooth muscle cells

Many studies have investigated the contribution of TRPC-mediated SOCE in vascular smooth muscle cells to cellular differentiation, proliferation, and excitation-contraction coupling (183). In considering these reports, it is important to bear in mind that SOCE is not active in all types of native, contractile smooth muscle cells, and its presence may be an indicator of phenotypic differentiation to a proliferative state or adaptation to cell culture conditions (274). In addition, as noted above, several lines of evidence argue against the involvement of TRPC channels in SOCE in vascular smooth muscle and other cell types (68, 274). While the issue remains controversial, there is some evidence supporting the involvement of TRPC channel activity in SOCE in smooth muscle cells, as discussed below.

A) TRPC1. The contribution of TRPC1 to SOCE activity has been investigated using siRNA-mediated knockdown in A7r5 cells (40) and neutralizing antibody approaches (30, 402) in smooth muscle from several tissues, including pulmonary arteries (245–247) and the portal vein (14). SOCE via TRPC1 channels is also thought to be involved in proliferation of pulmonary artery myocytes in culture systems (216, 348). However, conflicting evidence is provided by studies using TRPC1−/− mice, which found no evidence for the involvement of TRPC1 in SOCE in myocytes isolated from cerebral arteries or the thoracic aorta (69). Work by Saleh et al. (292) suggests that TRPC1 currents activated by the SERCA pump inhibitor cyclopiazonic acid (interpreted as SOCE) require PIP2 for activation.

A few studies have attempted to link SOCE through TRPC1 with smooth muscle cell contraction. In the pulmonary vasculature, SOCE has been shown to cause vasoconstriction (174), and some evidence suggests that hypoxia-induced pulmonary vasoconstriction could be mediated by SOCE through TRPC1 channels (187, 205). One report suggested that SOCE via TRPC1 leads to the activation of large-conductance, Ca2+-sensitive K+ (BK) channels and relaxation of arterial smooth muscle (176). In this study, smooth muscle cell hyperpolarization and decreased global Ca2+ in response to activation of SOCE were greatly inhibited by a TRPC1-specific antibody or by a selective inhibitor of BK channels. Coinmunoprecipitation and immunohistochemical analyses indicated that TRPC1 and BK channels are colocalized in arterial myocytes. The authors of this study proposed that TRPC1 and BK channels form a vasodilator-signaling complex in vascular smooth muscle cells, a conclusion supported by the subsequently reported interaction between TRPC1 and BK channels in aortic smooth muscle cells (166). Although it remains difficult to reconcile how the small fractional Ca2+-currents generated by TRPC1 channels (~2 pS; Ref. 177) could produce localized domains with Ca2+ levels high enough to activate BK channels, such physical interactions may provide an enabling mechanism.

B) TRPC4. Two studies in cultured cells, one using aortic and one using mesenteric artery cells, suggested the involvement of TRPC4 channels in SOCE in smooth muscle cells. Cyclic stretch of these cells in culture reportedly caused a decrease in TRPC4 expression that was correlated with a decrease in SOCE (197). A second report from this group demonstrated that downregulation of TRPC4 was associated with reduced SOCE in mesenteric artery smooth muscle cells from
normotensive WKY rats, but not spontaneously hypertensive rats (SHR) (196).

C. TRPC5. Some evidence supporting a role for TRPC5 in SOCE in vascular smooth muscle cells, likely in heteromultimeric combinations with TRPC1, TRPC6, and/or TRPC7, has been presented. For example, an anti-TRPC5 antibody has been demonstrated to have a significant inhibitory effect on store-depletion-induced cation currents in freshly isolated cerebral pial arterioles (403). Similarly, Saleh et al. (294) showed that currents evoked by administration of the SERCA pump inhibitor cyclopiazonic acid were attenuated by anti-TRPC5, anti-TRPC1, and anti-TRPC7 antibodies in smooth muscle cells from coronary and mesenteric arteries as well as the portal vein (294).

3. TRPV4 channels in vascular smooth muscle cells and vasodilation

The presence of TRPV4 in cerebral artery smooth muscle cells has been demonstrated by immunohistochemistry (211) and RT-PCR (79). Earley et al. (79) reported the surprising finding that Ca\(^{2+}\) influx through TRPV4 channels in cerebral arterial myocytes caused vasodilation rather than vasoconstriction. This study showed that Ca\(^{2+}\) influx through TRPV4 channels stimulated by 11,12-EET activation and decreased Ca\(^{2+}\) activated BK channels, leading to myocyte hyperpolarization and decreased Ca\(^{2+}\) entry through VDCCs, causing vasodilation. Thus, although previous reports clearly demonstrated a key role for BK channels in the mechanism of vasodilation induced by EETs (42, 143), an indirect activation pathway involving TRPV4 channels appears to be a major mechanism of EET-induced activation of BK channels. A similar role for smooth muscle TRPV4 channels in regulating mesenteric artery contractile activity in vitro and in vivo has been described (81). In this study, ~50% of 11,12 EET-induced vasodilation was due to activation of TRPV4 channels in the endothelium and 50% was due to activation of the channel in vascular smooth muscle cells. A subsequent study by Macado et al. (221) demonstrated that ANG II acts through AT\(_1\)R to increase TRPV4 activity, recorded in the form of TRPV4 sparklets, in isolated cerebral artery smooth muscle cells. In intact cerebral arteries, Ca\(^{2+}\) influx through TRPV4 channels opposes the vasoconstrictor effects of ANG II through the Ca\(^{2+}\) spark-dependent mechanism described above. Using selective pharmacology and an elegant optogenetic approach, these authors demonstrated that the effects of AT\(_1\)R activation on TRPV4 sparklet activity are dependent on PKC. This study further demonstrated that the dynamic interactions between TRPV4 channels and PKC necessary for AT\(_1\)R-dependent regulation of TRPV4 sparklets are coordinated by AKAP150 (a kinase anchoring protein 150). Collectively, these findings reveal the formation of an AKAP150-organized subcellular signaling complex involving AT\(_1\)Rs, PKC, and TRPV4 channels that is capable of finely tuning local [Ca\(^{2+}\)] and CICR to influence vascular contractility (FIGURE 7).

B. TRP Channels in the Vascular Endothelium

The vascular endothelium is composed of a single layer of metabolically dynamic endothelial cells that line all of the blood vessels in the body. This tissue is located between the vascular lumen and underlying smooth muscle cells, allowing the endothelium to sense blood-borne substances and shear stress resulting from fluid flow along the vessel wall. The principal functions of the endothelium are to promote smooth muscle cell relaxation and arterial dilation, control vascular permeability, exert an antithrombotic effect, and regulate angiogenesis. Endothelial dysfunction is a hallmark of common cardiovascular diseases such as hyperten-

![Figure 7](https://physrev.physiology.org/doi/10.2200.33.3.on June 25, 2017 http://physrev.physiology.org/ Downloaded from http://physrev.physiology.org/)
TRP channels and endothelium-dependent vasodilation

The endothelium exerts a profound relaxing effect on underlying smooth muscle cells that, balanced by tonic constrictor influences, maintains optimal vascular tone (FIGURE 8). Nitric oxide (NO) (100) and prostacyclin (PGI2) are well-characterized vasoactive substances produced by the endothelium that diffuse to and relax smooth muscle to cause arterial dilation. Endothelium-dependent vasodilation persists when NO and PGI2 production are blocked, a response initially attributed to an unidentified “endothelium-derived hyperpolarizing factor” (EDHF) (48). The molecular basis of EDHF is now thought to encompass multiple diffusible substances, including K\(^+\) (86), EETs (42, 94), C-type natriuretic peptide, carbon monoxide, hydrogen peroxide (H\(_2\)O\(_2\)), hydrogen sulfide (H\(_2\)S), and others (87). In addition, vasodilatory responses originally attributed to EDHF are now known to result from direct electrotonic spread of membrane hyperpolarization from endothelial cells to smooth muscle cells through myoendothelial gap junctions (FIGURE 8). This factorless mechanism is referred to as endothelium-dependent hyperpolarization (EDH) to avoid confusion with EDHF-type pathways involving diffusible substances (106).

Table 3. TRP channels in the vascular endothelium

<table>
<thead>
<tr>
<th>TRP</th>
<th>Function</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPC1</td>
<td>Angiogenesis, permeability</td>
<td>Embryonic tissues (411), lung (39, 218)</td>
</tr>
<tr>
<td>TRPC3</td>
<td>Angiogenesis, endothelium-dependent dilation</td>
<td>Mesentery (198, 306), mammary artery (102)</td>
</tr>
<tr>
<td>TRPC4</td>
<td>Angiogenesis, endothelium-dependent dilation, permeability</td>
<td>Umbilical vein (19), aorta (97), lung (59, 352)</td>
</tr>
<tr>
<td>TRPC5</td>
<td>Angiogenesis</td>
<td>Umbilical vein (110)</td>
</tr>
<tr>
<td>TRPC6</td>
<td>Angiogenesis, permeability</td>
<td>Umbilical vein (110), epidermis (128)</td>
</tr>
<tr>
<td>TRPV1</td>
<td>Endothelium-dependent dilation</td>
<td>Mesentery (57, 406), heart (36, 123)</td>
</tr>
<tr>
<td>TRPV3</td>
<td>Endothelium-dependent dilation</td>
<td>Brain (78)</td>
</tr>
<tr>
<td>TRPV4</td>
<td>Angiogenesis, endothelium-dependent dilation, permeability</td>
<td>Aorta (381), mesentery (81, 295, 324, 417), skeletal muscle (169), heart (220, 371), lung (15), brain (132, 211)</td>
</tr>
<tr>
<td>TRPA1</td>
<td>Endothelium-dependent dilation</td>
<td>Brain (77, 281), Sullivan et al., unpublished data</td>
</tr>
</tbody>
</table>

Reference numbers are given in parentheses.
contain gap junctions have been referred to as myoendothelial junctions (MEJs), whereas those that lack gap junctions have been termed myoendothelial close contacts (MECCs). Connexin proteins (MEJs only), intermediate-conductance Ca\(^{2+}\)-activated K\(^+\) (IK) channels (77, 298), IP\(_3\)Rs (180), TRPA1 (77), TRPV4 (325), and other signaling proteins are present within MEPs. In mesenteric arteries, transient, localized Ca\(^{2+}\) signals are generated within MEPs by release of Ca\(^{2+}\) from the ER through IP\(_3\)Rs. These signals, which occur both spontaneously and in response to activation of GPCRs, are called Ca\(^{2+}\) pulsars and stimulate EDH by activating SK (small-conductance) and IK Ca\(^{2+}\)-activated K\(^+\) channels (180) (FIGURE 9).

The Ca\(^{2+}\) influx pathways that stimulate endothelium-dependent vasodilation remained largely unknown until the discovery of TRP channels (248). To date, TRPV1, TRPV3, TRPV4, TRPA1, TRPC3, and TRPC4 channels have been reported to be involved in endothelium-dependent vasodilation (TABLE 3) (77, 78, 307, 416, 417).

TRPV1. TRPV1 channels are activated by capsaicin, a substance found in hot peppers, and by noxious heat (45). The single-channel conductance of TRPV1 is 35 pS at −60 mV and 77 pS at +60 mV. TRPV1 channels are selective for Ca\(^{2+}\) versus Na\(^+\) (P\(_{Ca}/P_{Na}\) ≈ 9.6). Several studies have provided evidence of TRPV1 involvement in endothelium-dependent vasodilation. Poblete et al. (271) demonstrated that activation of TRPV1 channels with anandamide increased the release of endothelium-derived NO but did not cause dilation of rat mesenteric arteries. Work from Yang et al. (406) demonstrated that capsaicin stimulated Ca\(^{2+}\) influx and endothelial nitric oxide synthase (eNOS) phosphorylation, and elevated NO production. Capsaicin also caused endothelium-dependent relaxation of isolated mesenteric arteries, an effect that was absent in arteries from TRPV1\(^{-/-}\) mice. In addition, this study showed that endothelial function was improved and systolic blood pressure was slightly lower in SHRs receiving dietary administration of capsaicin for 7 mo compared with animals fed a normal diet. A subsequent study by Ching et al. (57) reported that capsaicin-induced Ca\(^{2+}\) influx through TRPV1 channels

![FIGURE 9. Ca\(^{2+}\) pulsars in the endothelium. A: time course of a three-dimensional Ca\(^{2+}\) pulsar originating from within a hole in the IEL (white circle) shown in the leftmost image. Scale bar, 5 µm. B: Ca\(^{2+}\) pulsars recorded from a pressurized artery (80 mmHg) expressing the Ca\(^{2+}\)-indicator protein GCaMP2. An endothelial cell and its nucleus are outlined (dotted lines), with the initiation sites indicated by red arrows. Scale bar, 10 µm. C: model of the functional effects of endothelial Ca\(^{2+}\) pulsars. IP\(_3\)-R dense ER stores follow portions of the endothelial cell membrane that evaginate through holes in the IEL and interface with underlying SM cell membranes. Repetitive localized Ca\(^{2+}\) events (pulsars) originate from these deep Ca\(^{2+}\) stores, which are regionally delimited to the myoendothelial junction and the base of the endothelial cell. These ongoing dynamic Ca\(^{2+}\) signals are driven by constitutive IP\(_3\) production and are inherently dependent on the level of endothelial stimulation. The left detail depicts a single endothelial projection through the IEL. IK (K\(_{Ca\,3.1}\)) channels in the plasma membranes of these endothelial projections are in very close proximity to Ca\(^{2+}\) pulsars, eliciting persistent Ca\(^{2+}\)-dependent hyperpolarization of the membrane potential at the myoendothelial junctions. The right detail illustrates the endothelial influence on the smooth muscle membrane potential at the myoendothelial interaction site where Ca\(^{2+}\) pulsars activate K\(_{Ca\,3.1}\) channels and hyperpolarize the endothelial membrane. This hyperpolarization can be transmitted to the SM through gap junction channels or by activation of SM K\(_{Ca}\) channels by K\(^+\) ions released by endothelial K\(_{Ca\,3.1}\) channels. Membrane hyperpolarization promotes relaxation of SM through a decrease in VDCC open probability. [From Ledoux et al. (180).]
caused TRPV1 to physically interact with eNOS through a pathway requiring phosphatidylinositol 4,5-bisphosphate 3-kinase, Akt, and calmodulin-dependent protein kinase II (CamKII), resulting in increased NO production (57). Bratz et al. (36) demonstrated that capsaicin dilated coronary arteries from pigs and showed that this response was diminished by the TRPV1 antagonist capsazepine and inhibition of NO production and was blunted in coronary arteries from obese swine. Further work from this group showed that capsaicin increased myocardial blood flow in mice in vivo, a response that was absent in TRPV1−/− animals and blunted in the db/db model of type II diabetes (123). Together, these studies support a model in which Ca2+ influx through TRPV1 channels increases the production of endothelium-derived NO to cause vasodilation. This mechanism may be disrupted during metabolic syndrome and diabetes, contributing to the endothelial dysfunction associated with these conditions.

A study by Chen et al. (50) showed that capsaicin caused an endothelium-dependent dilation of mesenteric arteries that was absent in TRPV1−/− mice. In contrast, capsaicin had no TRPV1-dependent effects on the main renal arteries, suggesting a heterogeneous distribution of TRPV1 channels in the endothelium throughout the vasculature.

B) TRPV3. TRPV3 channels have a large unitary conductance (∼150–200 pS) (58) and are highly permeable to Ca2+ (P_Ca:P_Na ∼12:1) (401). TRPV3 is activated by innocuous heat (401) and dietary monoterpenes such as carvacrol (derived from oregano), eugenol (clove oil), and thymol (found in thyme) (400). The channel is present in the skin and oral and nasal epithelium and is involved in chemosensation (400). Earley et al. (78) found that TRPV3 channels are also present in the endothelium of rat cerebral arteries and showed that carvacrol activated ruthenium red-sensitive cation currents and increased intracellular Ca2+ levels in native cerebral artery endothelial cells. Carvacrol administration evoked an endothelium-dependent dilation of isolated cerebral arteries that was not altered by NOS or COX inhibition; however, it was sensitive to block of SK, IK, and inwardly rectifying K+ (KIR) channels, and was associated with smooth muscle cell hyperpolarization. These data suggest that Ca2+ influx through TRPV3 activates EDH (78). The cardiovascular effects of TRPV3 knockout have not been reported, and the functional significance and endogenous regulation of TRPV3 in the endothelium remain unknown.

C) TRPV4. TRPV4 channels are more permeable to Ca2+ than Na+ (P_Ca:P_Na ∼6:1) (332) and are activated by cell swelling (193), warm temperatures, and chemical agonists (351, 381). The unitary conductance of the TRPV4 channel is ∼30–60 pS at −60 mV and ∼88–100 pS at +60 mV. The involvement of TRPV4 channels in endothelium-dependent vasodilation has been extensively studied, in part because of the availability of selective pharmacological activators, including 4α-phorbol 12,13-didecanoate (4α-PDD) (381) and GSK1016790A (351), and blockers such as RN-1732 (371) and HC-067047 (89).

I) TRPV4 and EET-induced dilation. Wantanabe and co-workers discovered that TRPV4 channels are present in freshly isolated mouse aortic endothelial cells and could be activated by the phorbol compound 4α-PDD (381), heat (25–43°C) (383), and EETs (382). The EETs are a family of four regioisomers, 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET, generated from arachidonic acid by cytochrome P-450 (CYP) epoxygenase enzymes. These compounds have significant biological activity in many tissues, and when produced by endothelial cells may act as EDHFs in some vascular beds (42, 80, 88, 94). Earley et al. (81) demonstrated that exogenous administration of 11,12-EET dilated isolated mesenteric arteries from wild-type, but not TRPV4−/−, mice. Approximately 50% of this response was lost when endothelial function was disrupted, suggesting that EETs can act on TRPV4 channels in both endothelial cells and smooth muscle cells (81). This study also showed that 11,12-EET-induced vasodilation was associated with smooth muscle cell hyperpolarization and was not affected by NOS or COX inhibition, but was blocked by inhibition of BK, IK, or SK channels, supporting an EDHF or EDH mechanism. A subsequent study by Vriens et al. (372) established that EETs generated by the CYP2C9 epoxygenase isoform could activate TRPV4 in an autocrine manner in cultured endothelial cells, providing support for the hypothesis that endogenously produced EETs activate Ca2+ influx through TRPV4 channels in an autocrine/paracrine manner to elicit dilation.

II) TRPV4 and flow-induced dilation. Increases in blood flow velocity or blood viscosity augment the level of laminar shear stress at the vascular wall. The endothelium senses this stimulus, initiating a vasodilator response known as “flow-induced dilation” (66). The molecular nature of the flow-sensing mechanism remains incompletely understood. An early study showing that TRPV4 was activated by hypotonic conditions, possibly by stretch of the plasma membrane associated with cell swelling (193), stimulated interest in this channel as a flow sensor in the endothelium. However, a subsequent study applying a cell-attached patch-clamp approach reported that TRPV4 channels were not directly activated by suction applied to the plasma membrane (332), suggesting that cell swelling activates the channel indirectly through a force-sensitive signaling cascade.

A number of studies support the possibility that TRPV4 channels are involved in flow-induced dilation. Kohler et al. (169) reported that administration of the TRPV4 agonist 4α-PDD or application of shear stress dilated rat gracilis arteries, and showed that these responses were blunted by...
the nonselective TRPV blocker ruthenium red. NOS inhibition attenuated flow-induced dilation but did not affect 4α-PDD-induced dilation, which was diminished by block of SK and IK channels. These results suggest that shear stress-induced Ca\(^{2+}\) influx through TRPV4 channels causes dilation through increased NO production, but pharmacological activation of the channel initiates EDHF or EDH pathways (169). A further study by this group demonstrated that carotid arteries from TRPV4\(^{-/-}\) mice lack shear stress-induced dilation (132). Zhang and colleagues (220) reported that TRPV4 is critically important for flow-mediated dilation of mouse mesenteric arteries and showed that shear stress induces Ca\(^{2+}\) influx through TRPV4 channels in human coronary artery endothelial cells. Bubloz and co-workers showed that flow-induced dilation of human coronary arteries was blunted by ruthenium red, the selective TRPV4 blocker RN-1732 (371), and siRNA-mediated downregulation of TRPV4 expression (41), providing strong evidence for a TRPV4 influence in shear stress-induced responses in this vascular bed.

It remains unclear if TRPV4 channels are directly activated by shear stress or respond to signaling pathways activated by a flow-sensing apparatus. In support of the latter possibility, a few studies have provided evidence that TRPV4 channels are activated by metabolites produced in response to laminar shear stress. Loot et al. (204) showed that inhibition of CYP epoxygenase attenuated flow-induced dilation of mouse carotid arteries, suggesting that EETs produced in response to shear stress are responsible for TRPV4-mediated dilation. These findings are supported by reports showing that cell-swelling-induced activation of TRPV4 is blocked by selective inhibition of CYP2C9 (372) and flow-induced dilation of mouse carotid arteries is impaired by inhibition of PLA\(_2\) (132), an enzyme that liberates arachidonic acid from the plasma membrane. Together, these studies suggest that the molecular nature of the endothelial cell flow sensor responsible for activation of TRPV4 in response to increased shear remains unresolved. The AT\(_1\)R, which is present in the endothelium and can be activated by a mechanical stimulus independently of ANG II, is a potential candidate for this function. AT\(_1\)Rs are functionally coupled to TRPV4 channels in vascular smooth muscle cells (221), but this linkage has not yet been demonstrated in endothelial cells.

III) TRPV4 and agonist-induced dilation. Several studies have demonstrated involvement of TRPV4 channel activity in endothelium-dependent relaxation of systemic arteries in response to muscarinic and purinergic receptor agonists. Saliez et al. (295) reported that EDHF/EDH and NO-dependent relaxation in response to carbachol are diminished in mesenteric arteries from TRPV4\(^{-/-}\) mice compared with arteries from wild-type animals, indicating that TRPV4 channels are involved in the response. Using pressure myography and in vivo approaches, Gutterman and colleagues (417) confirmed that acetylcholine (ACh)-induced dilation was blunted in mesenteric arteries from TRPV4\(^{-/-}\) animals. This study also demonstrated that ACh-induced Ca\(^{2+}\) influx and NO production in endothelial cells isolated from TRPV4\(^{-/-}\) mice were decreased compared with that in wild-type controls (417). Senadheera et al. (306) showed that block of TRPV4 with RN-1734 blunted ACh-induced dilation of radial uterine arteries, producing a greater effect in arteries from pregnant rats. Marrelli et al. (211) found that UTP-induced endothelial cell Ca\(^{2+}\) influx and dilation of cerebral arteries was blunted by ruthenium red and block of PLA\(_2\), suggesting that PLA\(_2\) may serve to generate arachidonic acid, which activates TRPV4-mediated Ca\(^{2+}\) influx and leads to arterial dilation through an EDHF or EDH mechanism. Chen et al. (50) showed that GSK1016790A induced a dilation of isolated renal arteries and perfused kidneys that was largely endothelium dependent and sensitive to NOS inhibitors.

TRPV4 channels are also present in the pulmonary artery endothelium. The selective TRPV4 agonist GSK1016790A relaxes pulmonary artery rings in an endothelium-dependent manner, an effect that is blocked by the selective TRPV4 antagonist HC-067047 and is largely dependent on SK and IK channel activity. These data indicate that pharmacological activation of TRPV4 causes EDH in pulmonary arteries. However, ACh-induced relaxation of pulmonary artery rings was not affected by block of TRPV4 channels with HC-067047 or RN1734 in this study, indicating that TRPV4 channels are not involved in muscarinic agonist-induced responses in isolated pulmonary arteries. Similar results have been obtained in vivo (257), indicating that TRPV4 channels are probably not involved in dilation of pulmonary arteries in response to muscarinic agonists.

IV) TRPV4 channels and subcellular Ca\(^{2+}\) signaling in the endothelium. An elegant study by Nelson and colleagues (324) reported that TRPV4 sparklets could be recorded from the intact endothelium of mesenteric arteries isolated from mice expressing a genetically encoded Ca\(^{2+}\) indicator protein exclusively in the endothelium. The amplitudes of TRPV4 sparklets stimulated by 4α-PDD, 11,12-EET, or GSK1016790A displayed a quantal distribution and exhibited cooperative gating, with simultaneous activation of two, three, or four channels occurring more frequently than predicted by binomial distribution models (324). Events with amplitudes greater than four quantal levels were not observed, suggesting that TRPV4 is present in a four-channel metastructure in endothelial cells. 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.
Although the amplitude of individual TRPV4 sparklets is quite large, the total number of active sites per cell is surprisingly low. Both Sonkusare et al. (324) and Sullivan et al. (337) showed that only a few (~3–8 sites) TRPV4 sparklets are active in individual endothelial cells during maximal, agonist-induced dilation. TRPV4 sparklets stimulated IK and SK currents in native endothelial cells, indicating that TRPV4 sparklets activate an EDH-dependent vasodilator pathway (324). Muscarinic receptor activation was shown to increase TRPV4 sparklet activity, supporting the hypothesis that large-amplitude Ca\(^{2+}\) signals generated by coupled TRPV4 channels activate proximal SK and IK channels to cause vasodilation (FIGURE 10) (324).

A report from the Dora lab (22) suggested that Ca\(^{2+}\) influx through TRPV4 channels could trigger CICR in the endothelium. This study showed that TRPV4 channels cluster in MEPs in mesenteric arteries and are spatially coupled with Ca\(^{2+}\) pulsars sites. Ca\(^{2+}\) pulsar activity was greater at very low intraluminal pressure (5 mmHg) compared with physiological levels (80 mmHg). The effects of low pressure on Ca\(^{2+}\) pulsar activity were attenuated by TRPV4 inhibition. Block of TRPV4 channels also increased myogenic tone at low levels of intraluminal pressure (20 and 40 mmHg), suggesting that the channel mediates a Ca\(^{2+}\)-pulsar-dependent vasodilator influence at these pressures. The authors proposed that Ca\(^{2+}\) influx through TRPV4 channels stimulated by low intraluminal pressure is amplified by CICR from IP3Rs to generate large-amplitude Ca\(^{2+}\) pulsars that promote EDH and thereby suppress myogenic constriction. The significance of these data is unclear, but it is conceivable that activation of endothelial cell TRPV4 channels at low intraluminal pressure and subsequent EDH and/or increases in vascular permeability contribute to the precipitous drop in blood pressure observed during hypovolemic shock. In contrast to the findings of Bagher et al. (22), Senadheera et al. (306) did not detect enriched TRPV4 expression within IEL fenestrations of uterine arteries from control or pregnant rats, suggesting that the site of TRPV4 expression in endothelial cells may differ between vascular beds.

V) TRPV4 function in vivo. Many of the studies cited above used ex vivo preparations to demonstrate that TRPV4 channels present in the endothelium contribute to vascular regulation in response to shear stress, receptor-dependent agonists, and autocrine/paracrine factors. These data are at odds with a number of in vivo studies showing little involvement of TRPV4 channels in cardiovascular control. Two reports showed that systemic activation of TRPV4 with GSK1016790A caused a significant drop in mean arterial pressure (MAP) that was independent of changes in cardiac output (257, 389), indicating that activation of the channel lowered total peripheral resistance in whole animals. These findings suggest that loss of TRPV4 activity in vivo should increase total peripheral resistance and MAP. However, basal MAP did not differ between wild-type and TRPV4\(^{-/-}\) mice (342) or was slightly lower in TRPV4\(^{-/-}\) mice (254). Furthermore, acute or chronic (8 days) administration of the selective TRPV4 blocking compounds GSK2193874 and GSK2263095 had no effect on MAP or heart rate, indicating that inhibition of the channel has no significant effect on basal cardiovascular regulation (350). GSK2193974 also had no effect on decreases in MAP following intravenous injection of ACh, suggesting that TRPV4 channels are not involved in systemic muscarinic receptor-induced vasodilation in vivo (257). The only reported cardiovascular phenotype of TRPV4\(^{-/-}\) mice is a mildly enhanced sensitivity to hypertensive stimuli (81, 254). Together, these in vivo studies suggest that TRPV4 channels in the endothelium are essentially inactive in healthy animals under basal conditions and exert only a modest effect on vascular resistance and MAP. The reason for the inconsistency between in vivo and ex vivo data is not clear, but it is possible that the influence of TRPV4 channels is confined to specific vascular beds, with other segments rapidly compensating for the loss of channel activity during pharmacological inhibition in whole animal studies.
D) TRPA1. TRPA1 channels have a large unitary conductance (~98 pS) (241), are highly permeable to Ca\(^{2+}\) \((P_{\text{Ca}}:P_{\text{Na}} \sim 7.9)\) (157), and are activated by electrophilic compounds, including the dietary molecules allicin (208), found in garlic, and allyl isothiocyanate (AITC) (24), derived from mustard oil. The channel acts as a receptor for these pungent substances in sensory neurons and may also be involved in responses to noxious cold (329). Recent studies have demonstrated that TRPA1 contributes to vascular regulation (for review, see Ref. 75). Bautista et al. (27) reported that allicin dilated mesenteric arteries by activating TRPA1 channels in perivascular nerves to cause the release of calcitonin gene-related peptide (CGRP). Earley and co-workers subsequently found that TRPA1 channels are present in the cerebral artery endothelium of rodents and humans (77), but were not detectable in mesenteric, coronary, dermal, or renal arteries (338). This study further reported that AITC caused a robust endothelium-dependent dilation and smooth muscle cell membrane hyperpolarization in pressurized cerebral arteries that was blocked by the selective TRPA1 inhibitor HC-030031. These AITC-induced responses were insensitive to NOS and COX blockade but were diminished by inhibition of SK, IK, or K\(_\text{IR}\) channels (77). This study also showed that TRPA1 channels are abundant in MEPs in the cerebral endothelium and are colocalized with IK channels in this tissue (77). Further insight into TRPA1-mediated cerebral artery dilation was provided by Qian et al. (281), who demonstrated that activation of TRPA1 channels provoked dilation by recruiting large, dynamic Ca\(^{2+}\) signals in the cerebral artery endothelium. These signals, generated by the release of Ca\(^{2+}\) from IP\(_3\)Rs present on the endothelial cell ER, are distinguished from sparklets and Ca\(^{2+}\) pulsars by amplitude, spatial spread, and duration and appear to mediate AITC-induced vasodilation.

It is unlikely that TRPA1 channel activity in the cerebral endothelium is regulated by AITC or allicin under physiological conditions. TRPA1 channels are activated by reactive oxygen species (ROS), such as H\(_2\)O\(_2\), and ROS-derived metabolites, including 4-hydroxy-2-nonenal (4-HNE), 4-oxo-nonenal (4-ONE), and 4-hydroxylhexenal (4-HHE) that are generated by peroxidation of \(
\text{\oe}\) polyunsaturated fatty acids (17, 363). Sullivan et al. (338) showed that TRPA1 channels and the ROS-generating enzyme NOX2 (NADPH oxidase isoform 2) colocalize in the endothelium of cerebral arteries. This study also showed that the amplitude of TRPA1 sparklets recorded from cerebral artery endothelial cells was approximately twice that of TRPV4 sparklets, consistent with the channel’s larger unitary conductance (~98 vs. 60 pS) and higher Ca\(^{2+}\) selectivity (~7.9:1 vs. 6:1). Stimulation of NOX2 activity increased the frequency of TRPA1 sparklets and dilated isolated cerebral arteries. These responses were dependent on the generation of extracellular H\(_2\)O\(_2\) and hydroxyl radicals replicated by administration of exogenous 4-HNE. Together, these data suggest that superoxide anions (O\(_2^\cdot\)) generated by NOX2 activity proximal to TRPA1 channels increase TRPA1 sparklet activity to cause vasodilation. This mechanism appears to be present only in the cerebral circulation and could provide compensatory increases in blood flow during cerebrovascular diseases associated with oxidative stress.

E) TRPC3. TRPC3 channels have a unitary conductance of ~60 pS, are essentially equally permeable to Ca\(^{2+}\) and Na\(^+\), and are more permeable to Na\(^+\) than K\(^+\) (156). The channel is activated by GPCR pathways, is directly sensitive to DAG (and stable analogs), and may be stimulated by elevated intracellular [Ca\(^{2+}\)]\(_i\). Reports from several laboratories suggest involvement of TRPC3 in endothelium-dependent vasodilation. An early study of isolated rat mesenteric arteries showed that injection of animals with TRPC3 antisense oligonucleotides diminished flow- and bradykinin-induced vasodilation (198). A later report by Gao et al. (102) provided evidence for expression of TRPC3 in the endothelial and smooth muscle layers of the human internal mammary artery (IMA), and showed that the TRPC3 blocker Pyr3 modestly diminished muscarinic agonist-induced relaxation of preconstricted IMA rings. Kochukov et al. (166) used knockout animals to demonstrate that TRPC1 and TRPC3 subunits form heteromultimeric channels involved in muscarinic agonist-induced Ca\(^{2+}\) influx and relaxation of aortic ring segments. A subsequent study by Sandow et al. (307) reported participation of TRPC3 channels in endothelium-dependent dilation of rat and mouse mesenteric arteries. This latter study demonstrated that TRPC3 channels are present in the mesenteric arterial endothelium, with ~70% of the channels localizing to MEPs. The selective TRPC3 blocker Pyr3 blunted acetylcholine-induced vasorelaxation and endothelial cell hyperpolarization in the presence of the NOS inhibitor L-NAME, the guanylyl cyclase inhibitor ODQ, and the COX inhibitor indomethacin. The IK channel blocker TRAM34 and the SK channel blocker apamin also attenuated vasodilatory responses. Together, these data suggest that TRPC3 channel activity stimulates EDH in mesenteric arteries following muscarinic receptor activation. A study by Kirby et al. (161) reported the presence of TRPC3 as well as SK and IK channels within IEL fenestrations of rat popliteal and first-order skeletal muscle arteries from the gastrocnemius muscle, but the function of TRPC3 in this arterial bed was not assessed. Marrelli and co-workers (165) demonstrated that TRPC3 channels contribute to EDH-mediated, SK and IK channel-dependent dilation of cerebral arteries in response to ATP. Interestingly, this study provided evidence that receptor activation leads to rapid recruitment of TRPC3 channels to the plasma membrane (165).

Taken together, the findings cited above support a mechanism whereby activation of muscarinic GPCRs stimulates TRPC3 activity, mostly likely through PLC-dependent gen-
eration of DAG. TRPC3 activity leads to increases in intracellular Ca\(^{2+}\) that activate SK and IK channels to cause EDH. However, the Ca\(^{2+}\) fraction of TRPC3-mediated mixed cation currents is predicted to be small at the endothelial cell resting membrane potential, arguing against a direct Ca\(^{2+}\) influx mechanism for SK and IK channel activation. The explanation for this apparent discrepancy is not clear, but it is possible that TRPC3 subunits form part of a heteromultimeric, Pyr3-sensitive channel with greater Ca\(^{2+}\) permeability. Another possibility, reviewed by Eder et al. (84), is that Na\(^{+}\) influx through TRPC3 indirectly activates Ca\(^{2+}\) influx through the Na\(^{+}/Ca^{2+}\) exchanger (NCX) acting in reverse mode. This mechanism requires colocalization of NCX and TRPC3 to produce subcellular domains with locally elevated [Na\(^{+}\)] capable of activating reverse-mode Ca\(^{2+}\) entry without causing membrane depolarization, which would diminish the electrical driving force for Ca\(^{2+}\) entry. Support for TRPC3-mediated NCX reverse-mode Ca\(^{2+}\) entry is provided by a study from Rosker et al. (289), who showed that elevated extracellular [Na\(^{+}\)] caused Ca\(^{2+}\) influx in HEK cells overexpressing TRPC3 channels. Na\(^{+}\)-induced Ca\(^{2+}\) influx was blocked by KB-R7943, a compound that preferentially inhibits reverse-mode NCX activity. Using a coimmunoprecipitation approach, these authors (289) also demonstrated that endogenously expressed NCX1 binds to the COOH terminus (amino acids 742–848) of heterologously expressed TRPC3. It has also been reported that TRPC3 associates with NCX1 and KB-R7943 blocks ANG II-induced Ca\(^{2+}\) influx in adult cardiomyocytes (85). However, KB-R7943 has off-target effect: it is a potent blocker of TRPC3, TRPC5, and TRPC6 channels (171) and a strong Ca\(^{2+}\)-independent activator of BK channels in smooth muscle cells (191). Thus a convincing demonstration of the proposed TRPC3-NCX Ca\(^{2+}\)-entry pathway and possible functional consequences awaits the development of more selective pharmacological agents.

F) TRPC4. TRPC4 channels are equally permeable to Na\(^{+}\) and Ca\(^{2+}\) and are activated by GPCR pathways, although the specific mechanisms are not fully understood. Freichel et al. (97) reported involvement of TRPC4 channels in endothelium-dependent vasodilation. This study demonstrated that SOCE could not be evoked in endothelial cells isolated from TRPC4\(^{-/-}\) mice, and ACh-induced Ca\(^{2+}\) entry in these cells was diminished compared with that in wild-type controls. Aortic ring sections from TRPC4\(^{-/-}\) mice displayed blunted endothelium-dependent relaxation in response to ACh, suggesting that TRPC4-mediated Ca\(^{2+}\) influx contributes to endothelium-dependent dilation in conduit arteries (97). The authors proposed that SOCE through TRPC4 stimulates eNOS activity and NO production to induce vasodilation (98). This conclusion is tempered by subsequent reports demonstrating that TRPC4 channels are not necessary for SOCE in endothelial cells (2, 316).

2. Permeability of the vascular endothelium

The vascular endothelium forms a semi-permeable barrier that regulates passage of water, ions, and macromolecules between the blood and the interstitium (219, 222). The degree of permeability varies considerably among vascular beds and segments and is influenced by physiological and pathophysiological conditions. Substances are transferred through the vascular wall by passive diffusion and active transport mechanisms, and through clefts between neighboring endothelial cells by bulk flow. Transport across the endothelium by bulk flow is governed by mechanical coupling between adjacent endothelial cells imparted by two types of protein complexes, tight junctions and adherens junctions, linked to the actin cytoskeleton. Ca\(^{2+}\)-dependent cytoskeletal reorganization of these interendothelial cell junctions dynamically regulates permeability of the endothelium in response to GPCR agonists such as thrombin, bradykinin, and histamine. TRPC1, TRPC4, TRPC6, and TRPV4 channels have been reported to influence endothelial permeability, as discussed below.

A) TRPC1. TRPC1 channels have a unitary conductance of ~5 pS and are not selective for Ca\(^{2+}\) relative to Na\(^{+}\) in heterogeneous expression systems. It is unclear if homomeric TRPC1 channels form in native cells, but TRPC1 subunits have been shown to form heteromultimeric channels with TRPC5 (334), TRPC4 (334), TRPC3 (333), TRPP2 (164, 419), and TRPV6 (301). TRPC4, TRPC1, and/or heteromultimeric channels containing TRPC1 subunits have been implicated in the control of endothelial permeability in the pulmonary vasculature through regulation of SOCE. Inhibition of SERCA pump activity causes a large increase in the permeability of the pulmonary endothelium (56) that is associated with a change in the shape of pulmonary artery endothelial cells (232). Brough et al. (39) showed that SOCE in pulmonary artery endothelial cells was diminished following antisense-mediated knockdown of TRPC1 expression, suggesting involvement of the channel in this response. Mehta et al. (218) provided evidence that SOCE in cultured endothelial cells was diminished by inhibition of RhoA activity and also showed that RhoA physically interacts with IP\(_3\)Rs and TRPC1 channels (218). In cells overexpressing TRPC1, stimulation with thrombin was found to result in PKC-\(\alpha\)-mediated phosphorylation of TRPC1 channels in association with SOCE and increased transendothelial permeability (6). In human umbilical cord endothelial cells in culture, the cytokine tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) was shown to increase TRPC1 expression accompanied by increased SOCE and stress fiber formation (261).

B) TRPC4. Evidence supporting a role for TRPC4 channels in the regulation of pulmonary endothelial permeability was first provided by Tiruppathi et al. (352), who demonstrated that sustained increases in intracellular Ca\(^{2+}\) in response to
thrombin receptor activation were diminished in endothelial cells from the lungs of TRPC4−/− mice compared with controls. Diminished thrombin-induced Ca2+ influx was associated with decreased actin-stress fiber formation and endothelial cell retraction. In isolated lung preparations, basal microvascular permeability did not differ between control and TRPC4−/− mice, but thrombin receptor activation-induced increases in lung permeability were significantly diminished in lungs from TRPC4−/− mice. The authors concluded that Ca2+ store depletion following thrombin receptor stimulation causes a TRPC4-mediated influx of Ca2+ that contributes to cytoskeletal rearrangement and formation of intercellular gaps in the endothelium. Cioffi et al. (59) demonstrated that the interaction between spectrin-protein 4.1 complexes and TRPC4 channels was required for SOCE in pulmonary artery endothelial cell cultures. The formation of intercellular endothelial cell adhesions promotes recruitment of TRPC4 channels to the plasma membrane. The authors of this study speculated that Ca2+ influx through TRPC4 may contribute to the maturation of junctional complexes (120).

C) TRPC6. Several studies have implicated TRPC6 channels in the regulation of endothelial permeability. Singh et al. (320) reported that TRPC6 knockdown diminished thrombin-induced stress fiber formation and endothelial barrier function in pulmonary artery endothelial cells. Samapati et al. (297) provided evidence that TRPC6 channels contribute to the regulation of pulmonary vascular permeability. This study demonstrated that TRPC6 channels are enriched in pulmonary endothelial cell caveoli. The TRPC6 activator hyperforin (182, 237) elevated intracellular Ca2+ levels in primary lung endothelial cells and increased the wet weight of isolated perfused rat lungs. Furthermore, platelet-activating factor (PAF)- and sphingomyelinase (ASM)-induced increases in endothelial cell Ca2+, lung vascular filtration coefficient, and wet weight gain were absent in TRPC6−/− mice. The effects of PAF on endothelial cell Ca2+ levels and hyperforin-induced inward cation currents were diminished by administration of an NO donor or a membrane-permeant cGMP analog, and hyperforin-induced increases in the wet weight of isolated perfused lungs were augmented by NOS blockade. The authors concluded that NO (via cGMP) inhibits TRPC6 channels in endothelial cells. Stimulation of the PAF/ASM signaling pathway recruits TRPC6 channels to caveoli and silences eNOS activity, allowing TRPC6-mediated Ca2+ influx to increase vascular permeability.

D) ARE TRPC1, TRPC4, AND TRPC6 CHANNELS NECESSARY FOR ENDOTHELIAL CELL SOCE? Although many studies cited above have put forward the concept that SOCE mediated by TRPC1 and TRPC4 channels can increase the permeability of the vascular endothelium, this interpretation is called into question by work from the Trebak lab showing that endothelial cell SOCE does not require TRPC1, TRPC4 or TRPC6 expression (2, 316), despite the fact that knockdown of these TRPC channels blocks thrombin-induced increases in endothelial permeability (316). This latter study also demonstrated that STIM1 independently controls endothelial barrier function by a mechanism that does not require TRPC1 or TRPC4 channels. Orai1, or Ca2+ entry (316). These data indicate that TRPC channels contribute to the regulation of endothelial permeability by a mechanism that is independent of SOCE. A conflicting study by Cioffi et al. (60) reported that heteromultimeric channels containing TRPC4 and TRPC1 subunits are present in pulmonary artery endothelial cells and physically interact with Orai1 via TRPC4; they further showed that Orai1 activity is necessary for the formation of interendothelial cell gaps. In a similar vein, Sundivakkam et al. (341) showed that STIM1 interacts with TRPC1 and TRPC4 to form a store-operated channel in cultured endothelial cells. Although the contradictory results regarding TRPC1 and TRPC4 channels and SOCE currently remain unresolved, strong evidence supports the concept that endothelial cell SOCE can occur without TRPC1, TRPC4, and TRPC6 expression (359). The findings of the Trebak laboratory are supported by the results of unbiased high-throughput siRNA screens, which revealed the necessity for STIM1 and Orai1 in SOCE but did not demonstrate the involvement of any TRP channels (370). It has also been reported that Ca2+ release from internal stores persists in Purkinje neurons from TRPC1/C4/C6 triple-knockout mice, suggesting that these channels are not required for the maintenance of intracellular stores via SOCE (131). Furthermore, the biophysical properties of TRPC channels suggest that fractional Ca2+ currents are probably very small under physiological conditions. It should be noted that many of the studies linking SOCE with endothelial permeability have presented only data showing changes in global intracellular Ca2+ and provide little or no evidence for direct TRPC channel activation using biophysical or electrophysiological methods. For SOCE studies that have used the patch-clamp technique, the apparent involvement of TRPC channels under the conditions used could reflect PLC-mediated activation of TRPC channels downstream of store depletion (2). In addition, many of the studies supporting a role for TRPC channels in SOCE-mediated Ca2+ influx were performed using endothelial cells in culture, and in some cases, overexpression systems. A study by Bergdahl et al. (31) showed that culture conditions significantly increased TRPC1 and TRPC6 expression and SOCE in smooth muscle cells. The effects of culture conditions on endothelial cell TRPC4 expression have not been reported, but phenotypic transformations that influence SOCE and permeability regulation are possible. Additional work using intact artery preparations is needed to fully resolve these issues.

E) TRPV4. TRPV4 is present in pulmonary endothelial cells in both humans and rodents (15), and studies from several laboratories have convincingly demonstrated an important
role for the channel in the regulation of pulmonary vascular permeability. A study from by Townsley and colleagues (15) showed that treatment of isolated lung preparations from wild-type mice with 4α-PDD, 5,6-EET, or 14,15-EET increased microvascular permeability. That this increased microvascular permeability was mediated by TRPV4 channels, specifically TRPV4, was confirmed by the fact that it was attenuated by the nonselective TRPV blocker ruthenium red and was unaltered in response to 4α-PDD in lungs from TRPV4−/− mice. However, SOCE-induced permeability responses did not differ between TRPV4−/− and wild-type mice. The overall 4α-PDD and EET-induced increases in permeability were comparable to those induced by SOCE, but these treatments influenced different vascular segments. TRPV4 agonists primarily affected alveolar septal microvessels, whereas the effect of SOCE was greatest in extra-alveolar vessels. Moreover, TRPV4 agonists caused breaks in the barrier and blebbing of endothelial cells, whereas SOCE caused the formation of gaps at cellular junctions. This study clearly showed that TRPV4-mediated Ca2+ influx and SOCE have differential effects on the pulmonary endothelium.

The concept of functional specificity of Ca2+-influx pathways in the pulmonary endothelium was further explored in a study that compared the effects of endothelial cell Ca2+ entry through T-type Ca2+ channels with that of TRPV4. This study showed that Ca2+ entry via T-type Ca2+ channels, but not TRPV4-mediated Ca2+ entry, induced surface expression of the adhesion molecule P-selectin in pulmonary capillary endothelium. In contrast, TRPV4-dependent, but not T-type Ca2+ channel-dependent, Ca2+ influx increased capillary permeability (397). Inhibition of myosin light-chain kinase (MLCK) was shown to diminish cell surface levels of TRPV4 protein and decrease 4α-PDD-induced Ca2+ influx and cation currents in pulmonary vascular endothelial cells (264), suggesting that MLCK activity is involved in trafficking of TRPV4 channels to the plasma membrane in the pulmonary endothelium. Using real-time Ca2+ imaging in isolated perfused lungs, Yin et al. (410) provided evidence that TRPV4 channels mediate increases in endothelial cell [Ca2+]i in response to elevations in left atrial pressure. Increases in NO production induced by increases in left atrial pressure were absent in TRPV4−/− mice, and wet-to-dry weight ratios of lungs subjected to pressure elevation were smaller in TRPV4−/− mice compared with those in wild-type animals. This study also showed that inward cation currents stimulated by 4α-PDD in pulmonary microvascular endothelial cells were attenuated by pretreatment with a cGMP analog. The authors proposed that pressure-induced activation of TRPV4 channels causes Ca2+ influx, which elevates endothelial permeability and increases NO production. NO, acting through cGMP, acts as a negative-feedback mechanism to govern TRPV4-mediated Ca2+ influx.

3. Angiogenesis

Angiogenesis, the process of new blood vessel development, occurs in a tightly regulated manner during embryonic growth and wound healing, and during pathological conditions such as diabetes, arthritis, and cancer (96). Angiogenesis is initiated by remodeling of the basement membrane followed by proliferation and migration of endothelial cells, resulting in the generation of new capillary tubes. The angiogenic process is stimulated by several growth factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF). Blocking Ca2+ entry diminishes endothelial cell adhesion, motility, and tube formation in culture and angiogenesis in vivo (170), but the influx pathways responsible are not well characterized. However, a few studies have demonstrated the involvement of TRPC1, TRPC3, TRPC4, TRPC5, TRPC6, and TRPV4 channels.

A) TRPC1. Using an in vivo zebrafish model, Yu et al. (411) demonstrated that knockdown of TRPC1 disrupted angiogenesis in developing larvae. This study showed that TRPC1 is a downstream target of VEGF-a, and its expression is necessary for extracellular signal-regulated kinase (ERK) activation. In contrast to these findings, knockdown of TRPC1 expression had no significant effect on the in vitro formation of human umbilical vein-derived endothelial tubes (19), and the vasculature was reported to develop normally in TRPC1−/− mice (304).

B) TRPC3, TRPC4, AND TRPC5. Antigny et al. (19) reported that siRNA-mediated silencing of TRPC3, TRPC4, or TRPC5 expression diminished spontaneous Ca2+ oscillations in human umbilical vein-derived endothelial cells and inhibited endothelial tube formation in vitro, suggesting a possible role for these TRPC channels in angiogenesis.

C) TRPC6. Endothelial cell TRPC6 channels are activated by binding of the pro-angiogenic factor VEGF to VEGF receptors (54), suggesting that TRPC6-mediated cation currents could be involved in the angiogenic response. In agreement with this possibility, Hamdollah Zadeh et al. (128) reported that VEGF-induced migration and tube sprouting of microvascular endothelial cells was diminished in cultures expressing a dominant-negative form of TRPC6 (110). Further support is provided by a study by Kini et al. (160), who showed that TRPC6 associates with PTEN (phosphatase and tensin homologue) protein in human pulmonary artery endothelial cells and demonstrated that this interaction is required for surface expression of TRPC6 and thrombin-induced proliferation and tube formation. In contrast, neither TRPC6 gain-of-function mutations in humans (391) nor TRPC6 gene deletion in mice (70) was reported to have any overt effect on blood vessel structure.

D) TRPV4. Several studies have examined the concept that activation of TRPV4 channels contributes to angiogenesis.
Knockdown of TRPV4 expression was shown to prevent cyclic strain-induced Ca\(^{2+}\) influx and capillary endothelial cell reorientation, suggesting involvement of the channel in migration and sprouting associated with angiogenesis (349). Interestingly, administration of the TRPV4 agonist 4a-PDD enhanced collateral growth of cerebral blood vessels, suggesting involvement of the channel in cerebral angiogenesis (300). Total and surface TRPV4 expression and channel activity were found to be elevated in breast tumor-derived endothelial cells compared with normal dermal microvascular endothelial cells (92), and activation of TRPV4 channels with arachidonic acid promoted migration of tumor-derived, but not normal endothelial cells. Collectively, these observations suggest that TRPV4 channels may be involved in tumor angiogenesis.

C. TRP Channels in Perivascular Cells

TRP channels are present in perivascular neurons and astrocytes. Several studies have indicated that TRPV1, TRPA1, and perhaps TRPV4 channels in perivascular neurons can cause vasodilation, and one study demonstrated that TRPV4 channels present in astrocytic endfeet enveloping parenchymal cerebral arteries influence neurovascular coupling.

1. TRPV1 in perivascular sensory nerves

Neuronally evoked vasodilation is a common feature of the vasculature in many organ systems, including the brain, cutaneous tissues, and the gastrointestinal tract (33). A model describing a vasodilator role for TRPV1 channels located on perivascular sensory nerves has emerged during the past decade. Data from several research groups have demonstrated that activation of TRPV1 channels causes release of the potent vasodilator CGRP from sensory nerves, leading to vasodilation. This interaction between TRPV1 channels and CGRP activity is most frequently reported for the mesenteric resistance vasculature (335, 430, 376, 378), but also clearly applies to other vascular beds, such as the dural (7) and cerebral (430) circulations. The endogenous endocannabinoid anandamide has been strongly implicated as a potential physiological activator of the above TRPV1/CGRP vasodilator pathway, acting through direct activation of TRPV1 channels on sensory nerves rather than through activation of cannabinoid-specific receptors (7, 38). A similar vasodilatory mechanism in the coronary circulation, initiated by ethanol, has been described (109). Activation of TRPV1 channels has also been reported to inhibit sympathetic vasoconstrictor tone through the release of CGRP (412). Similar roles for TRPA1 (27, 429) and TRPV4 (101) channels in mesenteric perivascular sensory nerves have been reported (FIGURE 11).

2. TRPV4 channels and neurovascular coupling

TRPV4 activity in astrocytes has been linked to the local control of cerebral blood flow. Brain parenchymal astrocytes form close associations with neurons and with penetrating arterioles that link pial arteries on the surface of the brain with the subsurface microcirculation. Astrocytes have been proposed to serve as “middlemen” in the functional hyperemic process of neurovascular coupling, which closely matches blood perfusion with neuronal activity (318). Astrocytic endfeet envelop the parenchymal arterioles and can mediate profound vasodilation in response to elevated synaptic activity. A number of vasoactive factors, including EETs, NO, and K\(^{+}\), have been implicated in this response (74). Work from the Nelson laboratory has shown that K\(^{+}\) efflux through BK channels present on astrocytic endfeet acts through elevation of [K\(^{+}\)] in the restricted space between the endfoot and the arteriolar wall to activate K\(_{IR}\) channels on smooth muscle cells, causing membrane hyperpolarization and vasodilation (91) (FIGURE 11). This response is dependent on increased astrocytic intracellular [Ca\(^{2+}\)] (330). Ca\(^{2+}\) entry through activated TRPV4 channels located in astrocytic endfeet (29) stimulates CICR from intracellular stores and enhances arterial dilation (73). TRPA1 (314, 315) and TRPC3 (331) channels are also present in astrocytes and regulate intracellular Ca\(^{2+}\), but their involvement in neurovascular regulation has not been reported.

IV. INVOLVEMENT OF TRP CHANNELS IN VASCULAR PATHOLOGIES

Given the clear physiological roles for TRP channels in vascular smooth muscle, the endothelium, and perivascular cells, it is not surprising that there are multiple examples where the function or dysfunction of various TRP channels contributes to cardiovascular disease (see TABLE 4). The literature describing the involvement of TRP channels in vascular-related diseases is discussed below.

A. Hypertension

Although the causes of essential hypertension are still not established, alterations in central cardiovascular control mechanisms as well as cardiovascular, hormonal, and fluid and electrolyte adjustments typically associated with renal disease are clearly involved in the development of this disease (44). Specific features of vascular dysfunction are prevalent in virtually all situations where sustained blood pressure elevations are present. The primary manifestation of hypertension in the vasculature is an increase in peripheral resistance resulting from enhanced vascular contractility, differing degrees of vascular remodeling in the resistance vasculature, or both. Although TRP channel dysfunction may be involved in hypertension driven by neural (339), humoral (214), or renal (395) mechanisms, the following discussion will focus entirely on evidence that TRP channels in the vasculature contribute to the pathophysiological processes associated with elevated blood pressure.
There are multiple examples of altered TRPC channel expression and function, primarily TRPC3 and TRPC6, associated with hypertension.

A) TRPC3. Several studies have reported that TRPC3 expression is upregulated in arteries from hypertensive animals, a phenomenon most clearly documented in the SHR model of hypertension. Increased TRPC3 expression in vascular smooth muscle cells isolated from SHRs is correlated with enhanced ANG II-induced Ca\(^{2+}\)/H\(^{1001}\) influx that is independent of VDCCs (199). A similar increase in carotid artery smooth muscle TRPC3 channel expression in SHRs accompanied by enhanced vascular contractility has also been described (256). Interestingly, this latter study found that TRPC1 expression was decreased. Others have reported increased expression of TRPC3 as well as TRPC1 and TRPC5 in mesenteric arterioles from SHRs compared with normotensive controls, with an associated increase in vaso-motion (51). Enhanced ET-1-induced contractions of SHR mesenteric resistance arteries were apparently due in part to increased TRPC3 expression, which was associated with greater coupling of ET-1 receptor activity to Ca\(^{2+}\)/H\(^{1001}\) entry through direct physical interactions between IP\(_3\)Rs and TRPC3 channels (4). Although there are no specific reports of TRPC3 upregulation in the vascular smooth muscle cells of humans with hypertension, TRPC3 levels were found to be elevated in monocytes from patients with essential hypertension in association with enhanced migration activity described (256).

B) TRPV4 channels contribute to neurovascular coupling. Stimulation of metabotropic glutamate receptors (mGluR) on parenchymal astrocytes leads to the generation of IP\(_3\), which causes the release of Ca\(^{2+}\) from the endoplasmic reticulum (ER), stimulating K\(^{+}\) efflux through BK channels. The resulting increase in [K\(^{+}\)]\(_{\text{extracellular}}\) in the space between the astrocytic end-foot and cerebral arteriole activates K\(^{+}\) efflux through K\(_{\text{ir}}\) channels present on vascular smooth muscle cells to cause dilation. Production of EETs or prostaglandins (e.g., PGE\(_2\)) may also contribute. Ca\(^{2+}\)/H\(^{1001}\) influx through TRPV4 channels contributes to the propagation of Ca\(^{2+}\) waves throughout the endfoot.
of these cells (424). Also, although TRPC3 expression levels and hypertension were positively correlated in each of these studies, cause and effect analyses, for instance, examination of TRPC3 expression in prehypertensive or anti-hypertensive-treated SHRs, have not been reported. See Reference 377 for a comprehensive review of TRPC3 and hypertension.

TRPC6. Upregulation of TRPC6 in vascular smooth muscle in association with hypertension has also been reported. In a rat model of ouabain-induced hypertension, cellular levels of TRPC6, TRPC1, the α2 subunit of the Na+/K+-ATPase pump, and the Na+/Ca2+ exchanger are increased in mesenteric artery vascular smooth muscle cells (277). Increased and coordinated activity among these various Ca2+ transport systems is correlated with increases in [Ca2+]i, which could increase vascular contractility and contribute to elevated blood pressure. Similar changes in the expression of TRPC6 and the Na+/Ca2+ exchanger in mesenteric myocytes, and altered Ca2+ homeostasis occur in Milan hypertensive rats (195, 428). MAP is slightly elevated in TRPC6−/− mice compared with wild-type animals (70). This is unexpected, but may be due to the (apparently compensatory) upregulation of TRPC3 channels observed in TRPC6−/− mice.

Table 4. TRP channels involved in vascular pathologies

<table>
<thead>
<tr>
<th>TRP</th>
<th>Pathology</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRPC1</td>
<td>Hypertension, vascular remodeling/VSM proliferation</td>
<td>Lung (113, 194, 200, 374), large veins (184, 347), brain (31)</td>
</tr>
<tr>
<td>TRPC3</td>
<td>Hypertension, vascular remodeling/VSM proliferation</td>
<td>Mesentry (4, 51), brain (256), lung (413)</td>
</tr>
<tr>
<td>TRPC4</td>
<td>Diminished risk of MI, vascular remodeling/VSM proliferation</td>
<td>Endothelium (153), lung (421)</td>
</tr>
<tr>
<td>TRPC6</td>
<td>Hypertension, I/R-induced pulmonary edema, vascular remodeling/VSM proliferation</td>
<td>Mesentry (277, 428), lung (194, 413)</td>
</tr>
<tr>
<td>TRPV1</td>
<td>Hypertension, chronic hypoxia-induced proliferation, cardiac and cerebral I/R injury, neurogenic inflammation</td>
<td>Systemic (185, 379), lung (380), heart (375), brain (239, 265)</td>
</tr>
<tr>
<td>TRPV3</td>
<td>Cerebral I/R injury</td>
<td>Brain (235)</td>
</tr>
<tr>
<td>TRPV4</td>
<td>Hypertension, APP-associated endothelial dysfunction, pulmonary edema</td>
<td>Brain (418), lung (127), mesentry (325)</td>
</tr>
<tr>
<td>TRPA1</td>
<td>Oxidative stress response, neurogenic inflammation</td>
<td>Brain (Sullivan et al., unpublished data), sensory nerves (18, 244, 346, 361)</td>
</tr>
<tr>
<td>TRPM2</td>
<td>Oxidative stress response</td>
<td>Endothelium (135, 345)</td>
</tr>
<tr>
<td>TRPM4</td>
<td>Cerebral ischemia</td>
<td>CNS microvessels (112), brain (203)</td>
</tr>
<tr>
<td>TRPM7</td>
<td>Hypertension, neuronal death following cerebral ischemia, vascular remodeling/VSM proliferation</td>
<td>Mesentry (357, 358), brain (339), epidermis (134)</td>
</tr>
</tbody>
</table>

VSM, vascular smooth muscle; MI, myocardial infarction; I/R, ischemia reperfusion; CNS, central nervous system. Reference numbers are given in parentheses.

Pulmonary hypertension is characterized by pulmonary arterial pressures greater than 25 mmHg and is due in part to heightened contractility of pulmonary artery smooth muscle cells as well as substantial cellular proliferation and remodeling of the pulmonary arterial wall. Both processes contribute to sustained increases in pulmonary artery resistance. Several studies have indicated that both TRPC6 and TRPC1 channels contribute to the mechanisms underlying chronic hypoxia-induced pulmonary hypertension (194, 374). For example, chronic (3–4 wk) hypoxia in rats was shown to increase TRPC6 and TRPC1 channel expression in pulmonary artery smooth muscle cells by two- to threefold (194). Chronic hypoxia also increased ROCE and SOCE; these responses were correlated with enhanced pulmonary artery contractile activity (399) and were inhibited by molecular or genetic suppression of TRPC6 and TRPC1 expression (194, 374). Others have demonstrated a central role for EETs, in particular, 11,12-EET, in the increased expression and activity of TRPC6 channels in this system, predominantly through enhanced trafficking and localization of TRPC6 channels to the plasma membrane in response to hypoxia (159, 272). A similar upregulation of TRPC6 and TRPC3 channels has been demonstrated in pulmonary artery myocytes from humans with idiopathic pulmonary hypertension (413, 414). Suppression of TRPC6 expression in pulmonary vascular smooth muscle cells cultured from these patients was shown to substantially reduce cellular proliferation. A role for TRPC6 in hypoxia-induced pulmonary hypertension is less apparent in mice, where TRPC1 may make a significant contribution (209, 385). TRPC1 channel expression and Ca2+ entry are both elevated in pulmonary arteries of rats with monocrotaline-induced pulmonary hypertension (200). Taken together, the preceding observations suggest that the common theme of upregulated TRPC6 and/or TRPC1 channel function and accompanying heightened vascular reactivity is associated with nearly all forms of hypertension.
2. TRPV channels

A) TRPV1. TRPV1 channel function appears to be altered in response to hypertension and has a significant impact on the regulation of MAP. The TRPV1 channel agonist capsaicin was shown to decrease MAP in normal Wistar rats (185) and salt-sensitive Dahl rats (379) fed a high-salt diet; TRPV1 channel expression was also increased in these latter animals. Interestingly, MAP was further elevated in hypertensive rats on a high-salt diet following treatment with the TRPV1 channel antagonist capsazepine. These observations have led to the conclusion that TRPV1 receptors, likely those present predominantly on perivascular sensory nerves, are activated in animals with salt-sensitive hypertension. The associated release of CGRP from sensory nerve terminals provides a compensatory vasodilator response that opposes the elevation of blood pressure in this model of hypertension. There is no significant difference in systolic or diastolic blood pressures in TRPV1+/− compared with wild-type mice (212). This supports the hypothesis that altered TRPV1 activity occurs primarily in response to elevated blood pressure, but probably is not involved in the etiology of hypertension.

B) TRPV4. A few studies have indicated the involvement of TRPV4 channels in pulmonary and systemic hypertension. In rat pulmonary arteries, chronic hypoxia-induced pulmonary hypertension was shown to upregulate TRPV4 channels (407). This increase in TRPV4 expression produced a feed-forward effect on pulmonary hypertension whereby Ca²⁺ entry through the expanded population of TRPV4 channels in pulmonary artery myocytes increased global [Ca²⁺]i, which enhanced pulmonary vascular contractility and pulmonary hypertension.

Elegant work from Nelson and colleagues (325) has shown that the coupling efficiency between endothelial cell TRPV4 channels and activation of EDH and dilation of mesenteric resistance arteries is disrupted in a mouse model of ANG II-induced chronic hypertension. Much of this effect was attributed to a profound decrease in the PKC-anchoring protein AKAP150 in the vicinity of myoendothelial gap junctions in arteries from normotensive mice, and the resulting disruption in the AKAP150-dependent clustering of TRPV4 channels that is responsible for the high degree of cooperative gating of localized TRPV4 channels. The authors of this groundbreaking study proposed that these hypertension-induced changes in TRPV4 function could contribute to the mechanisms by which high blood pressure alters local blood flow regulation.

3. TRPM7

Abundant evidence supports the hypothesis that altered regulation of Mg²⁺ transport contributes to the development and maintenance of hypertension (reviewed in Ref. 326). TRPM6 and TRPM7 play key roles in Mg²⁺ transport in numerous tissue types, including vascular smooth muscle. Recent observations point to a substantial role for TRPM channels, particularly TRPM7, and altered TRPM channel function in various forms of hypertension. TRPM7 expression and activity, as well as [Mg²⁺], are reduced in mesenteric artery smooth muscle from SHR compared with WKY rats (358). Decreased intracellular Mg²⁺ is associated with heightened contractility, which contributes to hypertension. Disrupted Mg²⁺ regulation involving altered TRPM7 activity is also correlated with changes in cellular inflammation, proliferation, and remodeling, each of which is associated with vascular disease in hypertension (357).

B. Vascular Remodeling, Intimal Hyperplasia, and Vascular Occlusive Disease

Vascular remodeling occurs in the context of a number of cardiovascular diseases, including hypertension, coronary artery disease, atherosclerosis, vascular injury, and inflammatory diseases. Increased intracellular [Ca²⁺]i and activation of the δ isoform of Ca²⁺/CamKII seems to be a common mechanism associated with this type of cellular proliferative response. Signaling mechanisms downstream of CamKII activation are highly diverse, but likely involve altered expression of inhibitors and activators of cell-cycle progression, and altered gene transcription regulated by nuclear factor of activated T cells (NFAT) and cAMP response element binding protein (CREB) (319). TRP channels can facilitate increases in intracellular Ca²⁺ associated with activation of CamKII that are linked to vascular disease and remodeling.

1. TRPC1

Several studies provide evidence for the involvement of TRPC1 channels in disease-associated smooth muscle cell proliferation. In cultured human pulmonary artery myocytes, stimulation with serum-derived growth factors was shown to increase TRPC1 expression and Ca²⁺ influx in association with enhanced cellular proliferation (113). In cultured human saphenous vein myocytes, TRPC1 channel activity has been linked to smooth muscle cellular proliferation, an effect that is partly dependent on interactions with STIM1 (184). Other studies have shown that TRPC1 channel expression is upregulated in rodent models of vascular injury and myocyte proliferation in association with enhanced Ca²⁺ entry (31, 173). In the latter of these two studies, administration of an antibody targeting an extracellular loop of TRPC1 substantially reduced smooth muscle proliferation and Ca²⁺ entry in cultured human saphenous veins. Similarly, chronic stimulation of cultured human coronary artery smooth muscle cells with ANG II was shown to increase TRPC1 expression and SOCE in association with cellular hypertrophy, effects that were suppressed by siRNA-mediated knockdown of TRPC1 (347).
2. Other TRPCs

A few studies have implicated other members of the TRPC subfamily in smooth muscle cell proliferation. One report demonstrated that the proliferative response of human pulmonary artery smooth muscle to extracellular ATP, a mitogenic stimulant, occurred via CREB-mediated upregulation of TRPC4 and enhanced SOCE (421). Pulmonary artery smooth muscle from a subset of pulmonary hypertensive patients with idiopathic pulmonary arterial hypertension displayed enhanced expression of both TRPC3 and TRPC6. Proliferation of pulmonary artery myocytes cultured from these patients was greatly attenuated by suppression of TRPC6 expression (413). In addition, one group demonstrated that TRPC3 contributes to the cellular proliferation that occurs in the setting of stent-induced remodeling of large arteries (168).

3. TRPV1

One group reported that the proliferative response of cultured human pulmonary myocytes to chronic hypoxia was correlated with increased expression of TRPV1 channels and elevated SOCE (380). A role for TRPV1 in the remodeling of venous tissue exposed to altered pressure and flow has also been reported (52).

4. TRPM6 and TRPM7

Numerous studies have demonstrated that Mg\(^{2+}\) have a substantial impact on vascular smooth muscle and endothelial cell function (for review, see Ref. 326). In particular, changes in cytosolic Mg\(^{2+}\) levels may be involved in regulating vascular proliferation. TRPM6 and TRPM7 are critically important for Mg\(^{2+}\) transport in vascular smooth muscle and are involved in vascular diseases characterized by cellular proliferation and inflammation. Exposure to ANG II or aldosterone for 24 h was shown to substantially increase TRPM7 mRNA and protein levels in primary cultured aortic and mesenteric arterial smooth cells, an increase that was correlated with an increase in intracellular [Mg\(^{2+}\)] (134). The effects of ANG II on TRPM7 expression, Mg\(^{2+}\) influx, and accompanying cellular proliferation were inhibited by siRNA-mediated TRPM7 downregulation (134). The authors of this work concluded that a TRPM7-dependent influx of Mg\(^{2+}\) is directly linked to the vascular proliferative response to activation of ANG II receptors. Interestingly, basal levels of TRPM7 and intracellular [Mg\(^{2+}\)] were found to be reduced in cultured mesenteric vascular smooth muscle cells from SHRs compared with those from WKY rats (358). Furthermore, ANG II was shown to increase TRPM7 expression and intracellular [Mg\(^{2+}\)] in WKY, but not SHR, cells. These observations suggest that changes in TRPM7 expression and Mg\(^{2+}\) homeostasis are better correlated with enhanced contractility than with vascular smooth muscle cell proliferation in the SHR model of hypertension. However, direct causal links between TRPM7 expression, intracellular [Mg\(^{2+}\)], and differential effects on vascular contractility or smooth muscle proliferation are presently difficult to define given the cell-type-dependent, model-related, and tissue-specific differences in these parameters that have been reported (189, 259).

An exciting recent report demonstrated a novel mechanism for the regulation of gene expression, differentiation, and development involving TRPM7 channels (172). In this study, Kravivinsky et al. (172) showed that the COOH-terminal serine/threonine kinase domain of TRPM7 is proteolytically cleaved from the cation-conducting domain and translates to the nucleus in many different types of cells. The kinase domain participates in chromatin remodeling by phosphorylating histone proteins at specific sites important for cell differentiation and development. This study also showed that TRPM7 channels control cytosolic [Zn\(^{2+}\)], which in turn regulates binding of the cleaved kinase fragments to transcription factors containing zinc-finger domains. These data suggest that the kinase and ion-conducting domains of TRPM7 can together control epigenetic modifications and gene expression. However, it is not currently known if this system is functional in smooth muscle cells or participates in cellular proliferation.

C. Oxidative Stress and Ischemia/Reperfusion Injury

ROS generated by biological systems include H\(_2\)O\(_2\), O\(_2\), and hydroxyl radicals (OH\(^-\)). ROS act as signaling molecules, but when produced in excess can cause oxidative damage to membrane phospholipids, proteins, and DNA and diminish the bioavailability of endothelium-derived NO in the vasculature. Oxidative stress, defined as an imbalance between the generation of ROS by a biological system and the ability of that system to remove toxic metabolites and repair damage, is associated with numerous pathological conditions, including common cardiovascular diseases such as hypertension (65), atherosclerosis, and stroke. Reperfusion of tissue following ischemia (IR injury) is well recognized as a source of oxidative stress in the brain following stroke and in the heart following myocardial infarction. Multiple TRP channels have been implicated in responses to ROS and oxidative stress in various organ systems.

1. TRPC4

A study by Jung et al. (153) identified a single-nucleotide polymorphism (SNP) in the coding region of TRPC4 (I957V) that is associated with a diminished risk of myocardial infarction (MI) (153). Ca\(^{2+}\) imaging and patch-clamp analysis indicated that the mutant channel is more sensitive to muscarinic receptor stimulation compared with
the wild type. The authors speculate that elevated Ca\(^{2+}\) influx through the I957V variant lowered the incidence of MI by enhancing endothelium-dependent relaxation in coronary arteries.

2. TRPC6

Weissmann et al. (386) demonstrated that pulmonary edema caused by I/R injury is diminished in lungs from TRPC6\(^{-/-}\) mice compared with wild-type controls and TRPC1\(^{-/-}\) and TRPC4\(^{-/-}\) mice. This study further demonstrated that TRPC6 activation and I/R injury occur downstream of NOX2-generated ROS and PLC-\(\gamma\)-generated DAG.

3. TRPV1

A study by Wang et al. (375) demonstrated that recovery of cardiac function following I/R injury was diminished in TRPV1\(^{-/-}\) mice or mice treated with the TRPV1 inhibitor capsazepine compared with controls. Impairment of functional recovery was worse in animals treated with capsazepine compared with controls. These data suggest that TRPV1 channel activity attenuates I/R-induced damage in the heart. An apparent protective effect of TRPV1 channel activation on I/R injury has also been demonstrated in the kidney (269, 362, 364, 365).

Several studies have examined the effects of TRPV1 activity on I/R injury in the brain. Capsaicin administered 5 min after restoration of flow was shown to diminish I/R injury-induced neuronal damage in a global cerebral ischemia model, suggesting that activation of TRPV1 in this context is neuroprotective (265). Xu et al. (404) extended these findings, showing that chronic dietary capsaicin diminished hypertrophy of the cerebral vasculature and delayed onset of stroke in stroke-prone SHRs, likely due to enhanced production of NO. In addition, a study by Muzzi et al. (239) showed that systemic administration of the TRPV1 agonist rinvanil following I/R injury caused hypothermia in mice in association with diminished neuronal damage. In contrast, Gauden et al. (108) reported that blocking TRPV1 with capsazepine reduced I/R injury-induced increases in endothelial permeability in the brain, suggesting that TRPV1 activity can have negative consequences following cerebral I/R injury (108).

4. TRPV3

A study by Moussaieff and colleagues demonstrated that incensole, a TRPV3-activating compound derived from trees of Boswellia species (235), diminished brain infarct volume and neurological deficits induced by I/R injury following carotid artery occlusion (236). The neuroprotective effects of incensole were diminished in TRPV3\(^{-/-}\) mice and in mice pretreated with the nonselective TRPV inhibitor ruthenium red, suggesting that activation of TRPV3 channels mediates a portion of this response. The mechanism of action is unknown, but TRPV3 channels are present in the cerebral artery endothelium, and activation of the channel with carvacrol causes endothelium-dependent vasodilation (78), consistent with the possibility that incensole protects against ischemic damage by promoting cerebral blood flow.

5. TRPV4

Zhang et al. (418) showed that the selective TRPV4 activator GSK1016790A and the muscarinic receptor agonist ACh caused endothelium-dependent dilation of mouse cerebral arteries, an effect that was blocked by the selective TRPV4 antagonist HC-067047 and by inhibition of SK and IK channels. Interestingly, block of TRPV4 had little effect on ACh-induced dilation of arteries isolated from mice expressing mutant forms of the human amyloid precursor protein (APP), constitutively active tumor growth factor (TGF)-\(\beta\), or both transgenes. This effect was reversed by superoxide dismutase or catalase in arteries from APP mice, but not those from other groups. The authors concluded that endothelial TRPV4 function is impaired by the oxidative stress that is specifically associated with APP overexpression.

6. TRPA1

TRPA1 is present in the endothelium of cerebral arteries (77) and in the perivascular nerves of mesenteric arteries (27). A number of studies have demonstrated that TRPA1 channels are able to detect cellular oxygen status and oxidative stress. Work by Takahashi et al. (346) demonstrated that TRPA1 channels are activated by both hypoxic and hypoxic conditions, suggesting that the channel is intimately involved in oxygen sensing. TRPA1 channels are also activated by substances such as 4-HNE, 4-ONE, and 4-HHE that are generated by ROS-induced peroxidation of \(\omega\)6 polyunsaturated fatty acids (17, 363). A study by Sullivan et al. (338) demonstrated that endogenously generated oxidized lipids cause endothelium-dependent dilation of cerebral arteries, suggesting that TRPA1 channels may have a protective role in the cerebral vasculature during oxidative stress.

7. TRPM2

The unitary conductance of TRPM2 is \(\sim 52–60\) pS at negative holding potentials and \(\sim 76\) pS at positive potentials. Ionic selectivity is temperature dependent, with \(P_{Na^+}/P_{K^+}\) ratios of \(\sim 0.7:1\) at 20°C and \(\sim 6:1\) at 35°C (267, 299, 353). TRPM2, which is broadly expressed and is present in vascular smooth muscle and endothelial cells (135), is activated by ADP-ribose (ADPR) (267, 299) and \(H_2O_2\) (129, 384). Several studies have indicated that TRPM2 is critically involved in ROS-mediated signaling and responses to oxida-
OGD-induced Ca\textsuperscript{2+} influx and apoptosis of cultured endothelial cells is caused by PKC-\alpha-dependent activation of TRPM2 (135). H\textsubscript{2}O\textsubscript{2}-induced Ca\textsuperscript{2+} influx and apoptosis of cultured endothelial cells was diminished by siRNA-mediated knockdown of TRPM2 channels (135). H\textsubscript{2}O\textsubscript{2}-induced Ca\textsuperscript{2+} influx and apoptosis of cultured endothelial cells is caused by PKC-\alpha-dependent activation of TRPM2 (135). H\textsubscript{2}O\textsubscript{2}-induced Ca\textsuperscript{2+} influx and apoptosis of cultured endothelial cells was diminished by siRNA-mediated knockdown of TRPM2 channels (135).

8. TRPM4

Trauma-induced expression of TRPM4 channels in the endothelium has been linked to neuronal damage. Gerzanich et al. (112) demonstrated that TRPM4 expression is increased in tissue surrounding the site of spinal cord injury, particularly in microvessels and capillaries. Downregulation of TRPM4 expression reduced secondary hemorrhage at the injury site and improved neurobehavioral performance. A study by Loh et al. (203) demonstrated that I/R injury induced by middle cerebral artery occlusion (MCAO) promoted the expression of TRPM4 in the cerebral artery endothelium. Knockdown of TRPM4 expression enhanced angiogenesis and reduced infarct volume after MCAO, effects that were accompanied by improved motor function. These studies identify TRPM4 blockade as a possible prophylactic strategy against injury- and stroke-induced neuronal damage.

9. TRPM7

A study by Aarts et al. (1) demonstrated that exposure of cultured neurons to oxygen-glucose deprivation (OGD), an in vitro model of I/R injury, stimulated a cation current that was diminished by siRNA-mediated knockdown of TRPM7 expression. TRPM7 knockdown also decreased OGD-induced Ca\textsuperscript{2+} influx and reduced cell death. A follow-up study by Sun et al. (339) used targeted injection of adeno-associated virus to selectively diminish TRPM7 expression in CA1 hippocampal neurons in vivo. The resulting decrease in TRPM7 expression was associated with diminished delayed neuronal death following global cerebral ischemia and improved neurological outcomes. These data indicate an important role for TRPM7 in neuronal damage following I/R injury and suggest that block of the channel after stroke may be neuroprotective.

D. Pulmonary Edema

Pulmonary edema can result from congestive heart failure, ventilator-induced mechanical injury, prolonged exposure to high altitude, and other causes. Several studies have demonstrated that TRPV4 channels are involved in the development of pulmonary edema. A study by Hamanaka et al. (127) showed that ventilation-induced lung injury was diminished in lungs from TRPV4\textsuperscript{--/--} mice compared with controls, suggesting that TRPV4 channel activity contributes to mechanically induced vascular injury in the lung. The authors proposed that stretch-induced activation of TRPV4 contributes to this process. This report also demonstrated that infusion of macrophages isolated from wild-type mice into lungs isolated from TRPV4\textsuperscript{--/--} mice restored the permeability response to high ventilation pressure, suggesting that mechanical activation of TRPV4 channels present in macrophages is responsible for high pressure injury (126). Jian et al. (149) showed that elevated pulmonary endothelial permeability resulting from high vascular pressures was diminished in lungs from TRPV4\textsuperscript{--/--} mice compared with controls. This response was blocked by inhibition of CYP activity, suggesting that biosynthesis of EETs during exposure to high pressures induces activation of TRPV4 channels in the pulmonary microvascular endothelium. Ventilator-induced lung edema was blocked by inhalation of nanoparticles that release the nonselective TRPV blocker ruthenium red (155), further implicating TRPV4 channels in this response. An intriguing translational study by Thorneloe et al. (350) demonstrated that novel orally active TRPV4 blockers can prevent and resolve pulmonary edema associated with heart failure in rats, suggesting that block of TRPV4 channels may have important therapeutic effects. TRPV4 blockade has also been shown to prevent lung injury in mice exposed to acid and chlorine gas (23).

E. Neurogenic Inflammation

Neurogenic inflammation is characterized by vasodilation and edema that results in redness, warmth, hypersensitivity, and swelling. This condition is initiated by substances released from small-diameter sensory neurons, such as substance P, CGRP, glutamate, and prostaglandins, that act on endothelial cells, mast cells, immune cells, and vascular smooth muscle cells (284). Neurogenic inflammation is associated with many diseases, including migraine, arthritis, asthma, inflammatory bowel disease, and chronic obstructive pulmonary disease. Several studies have implicated TRPV1 and TRPA1 channels in neurogenic inflammation (for review, see Refs. 20, 90).

1. TRPV1

Many studies have demonstrated that TRPV1 channels are involved in neuropathic pain and neurogenic inflammation (for review, see Ref. 111). Among these studies is a report by Ji et al. (148) demonstrating that TRPV1 protein levels were elevated by inflammatory stimuli in dorsal root ganglion (DRG) neurons, resulting in hypersensitivity to heat. Genetic models have been used to show that mustard oil (25) and capsaicin (356) induce significantly less inflammation in TRPV1\textsuperscript{--/--} mice than in wild-type mice. The signaling pathways responsible for TRPV1 channel activation by inflammatory mediators have been extensively characterized and include sensitization mediated by PLC-\gamma, PKA-\gamma, and PKC-dependent signaling cascades (270). There is consid-
erable interest in TRPV1 blockade and desensitization as a potential treatment for chronic pain and neurogenic inflammation (233, 343).

2. TRPA1

TRPA1 channels are often coexpressed with TRPV1 in primary sensory neurons and may play an important role in the vascular component of neurogenic inflammation. This possibility was first suggested by Trevisani et al. (361), who showed that the TRPA1 agonist 4-HNE caused edema when injected into the hind paws of rats. Additional evidence for an inflammatory role for TRPA1 was provided by a study demonstrating that, in guinea pigs, tracheal plasma extravasation resulting from cigarette smoke inhalation was attenuated by inhibition of TRPA1 with HC-030031 (18). Furthermore, increases in capillary permeability were absent in TRPA1−/− mice administered an aqueous extract of cigarette smoke (18). N-acetyl-p-benzo-quinoneimine (NAPQI), a metabolite produced following administration of large doses of acetaminophen, was shown to activate TRPA1 channels and cause an extravasation of tracheal plasma in rats and mice that was sensitive to HC-030031 and TRPA1 knockout (244). NAPQI application also induced an increase in plasma protein extravasation in the skin conjunctiva of rats and mice that was sensitive to HC-030031 and TRPA1 knockout (244). These findings suggest that inhibitors of TRPA1 may be useful for treating increased capillary permeability and edema associated with certain inflammatory conditions.

V. SUMMARY AND CONCLUSIONS

TRP channels are critically involved in normal and pathological physiological responses in the vasculature. Strong evidence demonstrates that TRP channels contribute to mechanical- and agonist-induced vascular smooth muscle contractility and proliferation. TRP channels present in endothelial cells contribute to endothelium-dependent vasodilation, regulation of vascular permeability, and angiogenesis. TRP channels in other cell types, such as perivascular neurons and parenchymal astrocytes, are also important in the regulation of vascular function, primarily through the control of vascular tone. The primary regulatory paradigm for most of these functions is the control of global [Ca\(^{2+}\)], or the propagation of subcellular Ca\(^{2+}\) signaling events that modulate cellular activity. These effects can result directly from TRP channel-mediated Ca\(^{2+}\) influx; can be secondary to cation influx-induced membrane depolarization or activation of GPCRs; or can be caused by CICR from internal sources. Compelling evidence supports substantial roles for TRP channels in vascular disease states such as hypertension, neointimal injury, oxidative stress response, neurogenic inflammation, and pulmonary edema. These disease mechanisms involve increased expression or activation of TRP channels leading to elevated contractility, vascular hypertrophy, hyperplasia, or other forms of remodeling within the vascular wall. In light of these findings, selective pharmacological blockers of TRP channels may present exciting opportunities for the development of new therapies to prevent and treat vascular-related diseases.

Considerable work is needed to more fully understand the structural, functional, and mechanistic aspects of vascular TRP channel biology. In particular, important features of the organization and interactions between closely related, colocated TRP channels and other signaling modalities in vascular cells remain largely unknown. Several recent studies have highlighted the importance of such signaling networks in native smooth muscle and endothelial cells, and further advances in this area will greatly enrich our understanding of vascular function. Proteomic analyses and super-resolution imaging approaches may be useful in providing a more complete understanding of the interactions between TRP channels and cellular elements, such as the cytoskeleton and membrane phospholipids. Addressing controversies surrounding the relative fractional Ca\(^{2+}\) composition of TRP currents in vivo will require additional efforts to record TRP currents from native vascular cells under physiological ionic gradients. Such experiments may help to resolve controversies surrounding the role of TRP channels in SOCE. Successful completion of these studies will likely require the development of a more extensive array of selective pharmacological tools for modifying TRP channel activity, such as those that are currently available for probing TRPV4 channel function. In addition, little is currently known about how TRP channel gene expression is regulated under normal conditions, during development, or during disease. Finally, advanced genetic approaches, including the development of cell-specific, conditional knockouts targeted to the vasculature, genetically encoded indicators, and the application of optogenetic techniques to modulate channel activity in specifically targeted locations, are needed to address deeper questions regarding the physiological significance of TRP channels. These new approaches will allow a more comprehensive appreciation of the molecular and cellular complexity of TRP channels in the vasculature, providing substantial insight into fundamental mechanisms of vascular physiology and pathophysiology.

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Address for reprint requests and other correspondence: J. E. Brayden, Dept. of Pharmacology, Univ. of Vermont College of Medicine, 89 Beaumont Ave., Burlington, VT 05405 (e-mail: Joe.Brayden@uvm.edu).
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