Published in final edited form as:

Microcirculation. 2010 May ; 17(4): 237–249. doi:10.1111/j.1549-8719.2010.00026.x.

Vanilloid and Melastatin Transient Receptor Potential Channels in Vascular Smooth Muscle

Scott Earley

Vascular Physiology Research Group, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO USA 80523

Abstract

The mammalian transient receptor potential (TRP) superfamily consists of six subfamilies that are defined by structural homology: TRPC (conventional or canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystin), and TRPML (mucolipin). This review focuses on channels belonging to the vanilloid (V) and melastatin (M) TRP subfamilies. The TRPV subfamily consists of six members (TRPV1–6) and the TRPM subfamily has eight (TRPM1–8). The basic biophysical properties of these channels are briefly described. All of these channels except TRPV5, TRPV6, and TRPM1 are reportedly present in arterial smooth muscle from various segments of the vasculature. Studies demonstrating involvement of TRPV1, TRPV2, TRPV4, TRPM4, TRPM7 and TRPM8 in regulation of arterial smooth muscle function are reviewed. The functions of TRPV3, TRPM2, TRPM3, and TRPM6 channels in arterial myocytes have not been reported.

Keywords

cation channels; smooth muscle; TRP channels

I. Introduction

The first evidence of the superfamily of cation channels now known as “transient receptor potential” or “TRP” channels was a report of *Drosophila* mutants that behaved as if they were blind in bright light(67). Subsequent studies demonstrated that this phenotype results from differences in photoreceptor membrane potential responses to sustained bright light stimulation(34). Changes in membrane potential are continuous in wild-type flies, but are transient and quickly return to baseline in these mutants. Because of this response, the gene responsible was named *trp* for *transient receptor potential* and it, and a homologous gene called *trp-like* (*trpl*) were cloned and found to encode functional cation channels (54). Genomic analysis demonstrated that *trp* homologs are present in many other organisms, including *C. elegans*(11), yeast(68), and mammals(98). These discoveries have spurred numerous studies demonstrating that TRP channels are critical for the function of many organ systems and are involved in pathophysiological processes(59).

The mammalian TRP superfamily consists of six subfamilies that are defined by structural homology: TRPC (conventional or canonical), TRPV (vanilloid), TRPM (melastatin), TRPA

Address Correspondence To: Scott Earley, PhD., Vascular Physiology Research Group, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO USA 80523, Phone: (970) 491-2062, Fax: (970) 491-7569, Scott.Earley@colostate.edu.

DISCLOSURES

None.

(ankyrin), TRPP (polycystin), and TRPML (mucoliptin). The names of the subfamilies are historical and are derived from properties of the founding members. Members of the TRPC subfamily were the first TRP channels to be identified in the mammalian genome and were initially referred to as “conventional” when other TRP subfamilies were discovered. TRPV1 was previously recognized as VR1, the extracellular receptor for vanilloid compounds. TRPM1 was initially identified as a protein called *melastatin* in mouse melanoma cell lines. This name was used because expression levels of *melastatin* are inversely proportional to the potential for tumor metastasis (13). The TRPA subfamily is named for the numerous ankyrin repeats that characterize the sole member’s protein structure. TRPP channels are linked to some forms of polycystic kidney disease and TRPML channels are associated with mucopolipidosis. There are a total of 28 TRP genes in the rat and mouse genomes, 27 in humans where TRPC2 is a pseudogene. All TRP channels are permeable to cations and impermeant to anions. Certain members are only permeable to monovalent cations, others have significant selectivity for Ca^{2+} , and many do not discriminate between mono- and divalent cation. TRP channels are activated by diverse stimuli, including temperature, light, pressure, changes in osmolarity, and chemical agents. TRP channels are prominent in sensory neurons and often serve as cellular sensors that mediate responses to changes in the extracellular environment.

This review focuses on channels belonging to the vanilloid (V) and melastatin (M) TRP subfamilies and their involvement in vascular smooth muscle cell function. The first section briefly describes the basic biophysical properties of these channels and the second section reviews what we currently know about how these channels influence vascular smooth muscle physiology. The reader is encouraged to seek out recent review articles for a more comprehensive assessment of TRP channel biology (1,12,69).

II. Biophysical Properties of TRPV and TRPM channels

A great deal of important information about the biophysical properties of TRP channels has been gained from patch clamp electrophysiology studies of cloned genes expressed in cultured cells. However, aspects of TRP channel biology make the *in vivo* situation more complex. TRP genes are expressed as six transmembrane spanning polypeptides and functional TRP channels are formed from the assembly of four of these TRP subunits. All cells express multiple TRP genes, and heteromultimeric channels with biophysical properties that are distinct from homomeric channels can occur (51). In addition, mRNA splice variants of several TRP subunits have been reported in smooth muscle cells (92). This molecular complexity almost certainly results in greater diversity of channel function in native cells compared with homomeric expression systems. Combinations of TRPV and TRPM subunits that are capable of forming heteromultimeric channels are noted in the following section.

TRPV Channels

There are six members of the TRPV subfamily. TRPV channels were initially characterized by chemosensitivity and are now known to be sensitive to temperature (TRPV1–4) and changes in osmolarity (TRPV2 and TRPV4). Properties of these channels are summarized in Table 1. The IUPHAR database (10) provides a more comprehensive summary of these properties.

TRPV1—TRPV1 (VR1) is the receptor for capsaicin, a substance found in hot chili peppers (7). The channel is modestly selective for Ca^{2+} vs. Na^{+} ions (~10:1) (7) and has a unitary conductance of ~35–80 pS in symmetrical cation solutions (7). TRPV1 channels are also activated by heat (>42°C) (6). The channel is selectively blocked by capsazepine (in the nM to low μM range) and, with less specificity, by ruthenium red (10 μM) (52).

TRPV2—TRPV2 (VRL-1) channels are insensitive to vanilloid compounds but are activated by high heat ($>50^{\circ}\text{C}$) (6) and hypotonicity-induced cell swelling (56). The channel has slight selectivity for Ca^{2+} vs. Na^{+} ions ($\sim 3:1$) (6). The unitary conductance has not been reported. TRPV2 channels are activated by 2-aminoethoxydiphenyl borate (2-APB) (35) and are blocked by ruthenium red (10 μM) (6).

TRPV3—TRPV3 channels are activated by heating, particularly at temperatures $>30^{\circ}\text{C}$, and are described as “warm-sensing” (71,80,102). TRPV3 channels are also activated by dietary molecules such as camphor, carvacrol, cinnamaldehyde, eugenol, menthol, and thymol (101). 2-APB sensitizes activation of TRPV3 by heat (9). The channel is somewhat selective for Ca^{2+} vs. Na^{+} ions ($\sim 12:1$) (102) and has a unitary conductance of ~ 170 pS, the largest of the TRPV channels (102). TRPV3 channels may form heteromultimeric channels with TRPV1 (80). The channel is blocked by ruthenium red (10 μM) (71).

TRPV4—TRPV4 (TRP12, OTRPC4) is activated by hypotonicity (82) and heat (>25 – 34°C) (23,96). TRPV4 is slightly selective for Ca^{2+} vs. Na^{+} ions ($\sim 6:1$) (82) and has a single channel conductance of 30 pS at -60 mV and 90 pS at $+60$ mV in symmetrical cation solutions (82). TRPV4 channels also exhibit chemosensitivity and are activated by the phorbol compound 4 α -phorbol 12,13-didecanoate (4 α -PDD) (94), epoxyeicosatrienoic acids (EETs) (95), and, at the single nM concentration range, the synthetic small molecule GSK1016790A (83,99). TRPV4 is blocked by ruthenium red (10 μM) (94).

TRPV5—TRPV5 (ECaC1) (30,72) channels are constitutively active and are highly selective for Ca^{2+} vs. Na^{+} ions ($\sim 100:1$) (87). In inside-out membrane patches, the channel has a single channel conductance of ~ 80 pS.

TRPV6—TRPV6 (ECaC2) channels are also highly selective for Ca^{2+} vs. Na^{+} ions ($\sim 100:1$). The single channel conductance is 42–58 pS (106). The channel is blocked by ruthenium red (31). TRPV5 and TRPV6 are closely related in structure and functions and can reportedly form heteromultimeric channels (32).

TRPM Channels

There are eight members of the TRPM subfamily. Certain properties of these channels are summarized in Table 2. The IUPHAR database (10) provides a more comprehensive synopsis.

TRPM1—TRPM1 is a non-selective cation channel that displays outward rectification. The channel is blocked by the trivalent ion La^{3+} (64).

TRPM2—TRPM2 (LTRPC2) has unitary conductance of ~ 60 – 80 pS in symmetrical cation solutions (73). At physiological temperatures, the channel is somewhat selective for Ca^{2+} vs. Na^{+} ions ($\sim 6:1$) but is nonselective at 20°C . The channel is activated by the second messenger ADP-ribose (73), cyclic ADP-ribose (43) and by H_2O_2 (24). Channel activity is blocked by 2-APB (84), miconazole (84), clotrimazole (28), econazole (28), and flufenamic acid (84). Interestingly, TRPM2 encodes a C-terminal ADP-ribose pyrophosphatase domain (73).

TRPM3—TRPM3 channels are equally permeant to monovalent and divalent cations and have a unitary conductance of ~ 73 – 133 pS under physiological conditions (22). TRPM3 channels are activated by sphingosine (22), high concentrations of the dihydropyridine compound nifedipine (90), and by the steroid compound pregnenolone sulphate (90).

TRPM3 channels are blocked by 2-APB (103), the trivalent ions Gd^{3+} and La^{3+} (21), and intracellular Mg^{2+} (65).

TRPM4—The commonly occurring splice variant of TRPM4 (sometimes referred to as TRPM4b) is selective for monovalent cations and has a single channel conductance of ~25 pS (45,60). TRPM4 requires high levels of intracellular Ca^{2+} for activation (45,60). Protein Kinase C (PKC) activity enhances the Ca^{2+} sensitivity of the channel (18,62). The membrane phospholipid phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) is also required for channel activation (58,108). Under conventional whole-cell and inside-out patch clamp conditions, high levels of intracellular Ca^{2+} are associated with rapid (~120 sec) inactivation of TRPM4 activity (60). Rundown of TRPM4 currents can be prevented by inhibition of phospholipase C (PLC), suggesting that inactivation results from increased activity of a Ca^{2+} -dependent PLC isoform closely associated with TRPM4 that locally depletes $PtdIns(4,5)P_2$ levels (58,108). Channel activity is potentiated by decavanadate (61). The channel is blocked by spermine (63,86), adenosine mono-, di-, and triphosphate (63), flufenamic acid (86) and the tricyclic compound 9-phenanthrol (20).

TRPM5—TRPM5 (Mtr1) is a Ca^{2+} -activated, monovalent selective cation channel with a single channel conductance of ~23–25 pS (33,74). The reported EC_{50} of Ca^{2+} for channel activation ranges from 840 nM (74) to 30 μ M (33).

TRPM6—TRPM6 (CHAK2) is a non-selective cation channel that is permeable to Mg^{2+} ions (78). The channel is unusual because it encodes both a functional ion channel and a protein kinase domain (78). The channel is inhibited by elevated levels of intracellular Mg^{2+} , providing a negative feedback mechanism for regulating cellular Mg^{2+} levels (88). Consistent with this function, mutations in TRPM6 are associated with hypomagnesemia, suggesting that the channel is critical for Mg^{2+} homeostasis (78,91). At negative holding potentials, the unitary conductance of TRPM6 is ~84 pS (46). TRPM6 is activated by 2-APB (46) and inhibited by ruthenium red (88). TRPM6 and TRPM7 can form heteromultimeric channels with biophysical properties that are distinct from homomeric TRPM6 and TRPM7 channels (8,46).

TRPM7—TRPM7 (TRP-PLIK) also encodes a C-terminal protein kinase domain (77). The channel is a Ca^{2+} -permeable, non-selective cation channel with a unitary conductance reported to be 40 pS (46) to 105 pS (77). TRPM7 is blocked by polyvalent cations (40) and the trivalent ion La^{3+} (77). TRPM7 is very similar in structure to TRPM6 and is also important for cellular Mg^{2+} homeostasis (79).

TRPM8—TRPM8 (CMR1) is a cold (maximum activation at 10°C, very low activity at 20°C) temperature activated channel that is also the receptor for menthol (53,70) and icillin (53). The channel is a Ca^{2+} permeable, non-selective cation channel (70) with a unitary conductance of 60 pS at 10°C and 75 pS at 30°C (4). The channel is blocked by 2-APB (35).

III. TRPV Channels in Vascular Smooth Muscle

Expression of TRPV1–4 mRNA has been reported in endothelium-denuded rat aorta and intralobar pulmonary arteries, suggesting that these channels may be present in smooth muscle cells in these vessels (105). TRPV5 and 6 have not been detected in vascular tissue. Quantitative RT-PCR was used to evaluate the relative levels of TRPV1–4 mRNA expression. In pulmonary arteries, TRPV4 is the most abundant of the TRPV channel, followed by TRPV2 (~50% of TRPV4 expression) (105). In rat aorta, TRPV2 and TRPV4 mRNA expression levels are about equal. In both pulmonary arteries and aorta, TRPV1 mRNA is present at low very levels compared with TRPV4 and TRPV2, and TRPV3 mRNA

expression is barely detectable in these vessels using quantitative RT-PCR (105). Evidence supporting functional roles for TRPV4, TRPV2, and TRPV1 channels in smooth muscle is as follows:

TRPV4

TRPV4 channels are activated by EETs (95), potent smooth muscle cell hyperpolarizing and vasodilatory factors that are produced by endothelial cells (5,16). As such, the involvement of TRPV4 channels in EET-induced vasodilation has generated significant interest. TRPV4 channel expression is detected in rat cerebral artery smooth muscle (15,50) and 11,12 EET- and 4αPDD-induced cation currents have been recorded from rat cerebral (15) and mouse mesenteric artery smooth muscle cells (17). 11,12 EET-induced currents are absent in mesenteric arterial myocytes isolated from TRPV4 knockout mice (17), demonstrating specificity of the response. TRPV4 expression was also detected in extra-alveolar vessels in human and rat, but not mouse, lung tissue by immunohistochemistry (2).

Activation of TRPV4 channels in smooth muscle cells causes membrane hyperpolarization and dilation of cerebral arteries through a novel signaling pathway involving altered intracellular Ca^{2+} dynamics and large-conductance Ca^{2+} -activated K^+ (BK_{Ca}) channels (15). Earley et al. showed that 11,12 EET increases the frequency of localized, intermittent Ca^{2+} release from ryanodine receptors on the sarcoplasmic reticulum (“ Ca^{2+} sparks”) and BK_{Ca} channel-dependent spontaneous transient outward currents (STOCs) in cerebral artery myocytes (15). Ca^{2+} spark-dependent activation of BK_{Ca} currents hyperpolarizes arterial smooth muscle cells promotes arterial relaxation (57). Stimulation of Ca^{2+} spark activity by 11,12 EET is attenuated by antisense-mediated down-regulation of TRPV4 expression and by removal of extracellular Ca^{2+} (15). These findings suggest that Ca^{2+} influx via TRPV4 stimulates increased Ca^{2+} spark frequency by a Ca^{2+} -induced Ca^{2+} release mechanism (44). Smooth muscle cell hyperpolarization and vasodilation in response to 11,12 EET were also diminished in endothelium-denuded cerebral arteries treated with TRPV4 antisense oligonucleotides (15), suggesting that the channel acts as a smooth muscle cell surface receptor for 11,12 EET (15). However, two studies suggest that EETs can act through Gαs-protein coupled cell surface receptors in other types of cells (100,104). The proposed mechanism for 11,12 EET-induced arterial relaxation through activation of smooth muscle cell TRPV4 channels is shown in Figure 1.

Further support for an important regulatory role for smooth muscle TRPV4 channels was provided by a report showing that EETs-induced vasodilation and smooth muscle cell hyperpolarization is present in mesenteric arteries isolated from wild-type mice but is absent in vessels from TRPV4 knockout mice (17). Approximately 50% the effects of 11,12 EET on wild-type arteries was lost when the endothelium was removed, suggesting that endothelium- and smooth muscle cell-dependent mechanism contribute equally to EETs-induced vasodilation (17).

TRPV2

TRPV2 expression was detected in mouse aortic, mesenteric artery, and basilar artery smooth muscle cells (56). Muraki et al. recorded ruthenium red-sensitive inward cation currents and elevations in intracellular $[\text{Ca}^{2+}]$ in response to hypotonicity-induced cell swelling in mouse aortic smooth muscle cells (56). Antisense-mediated down-regulation of TRPV2 resulted in diminished hypotonicity-induced cation currents and Ca^{2+} influx in mouse aortic smooth muscle cells, suggesting that TRPV2 is a swelling-activated channel in these cells (56). The authors of this study proposed, but did not test, the hypothesis that TRPV2 is responsible for arterial myocyte membrane depolarization in response to elevation in intraluminal pressure (56).

TRPV1

The TRPV1 agonist capsaicin causes transient, concentration dependent constriction of isolated gracilis arterioles (39). This effect is blocked by the TRPV1 antagonist capsazepine (47), suggesting specificity of the response. Capsaicin-induced constriction was slightly enhanced following removal of the endothelium, and neurofilament-positive nerve terminals were not detected in these arteries. These findings suggest that capsaicin acts directly on smooth muscle TRPV1 channels to elicit vasoconstriction. Consistent with this possibility, TRPV1 was detected by immunostaining in smooth muscle cells in rat gracilis arteries (39,47).

A study by Wang et al. reports that TRPV1 mRNA and protein expression is increased in human pulmonary artery smooth muscle cells cultured under hypoxic (3% O₂, 72 hours) conditions (93). Expression levels of other TRPV channels were not altered by this treatment (93). In addition, this report shows that capsazepine inhibits hypoxia-induced cellular proliferation and partially reverses hypoxia-induced increases in capacitive (store-operated) Ca²⁺ entry in cultured pulmonary artery smooth muscle cells (93). The authors of this study conclude that TRPV1-dependent Ca²⁺ entry contributes to hypoxia-induced proliferation of pulmonary artery smooth muscle.

IV. TRPM Channels in Vascular Smooth Muscle

Expression of TRPM2–8 mRNA was detected by RT-PCR in de-endothelized rat intralobar pulmonary artery and aorta(105). Quantitative RT-PCR data suggests that TRPM8 is the most highly expressed TRPM mRNA in these tissues. TRPM4 and TRPM7 mRNAs are next most abundant (~40–50% of TRPM8 expression)(105). TRPM2 and TRPM3 mRNAs are present at lower levels (~10% of TRPM8) and TRPM5 and TRPM6 mRNA expression are barely detectable using quantitative RT-PCR (105). There is no evidence of TRPM1 expression in vascular smooth muscle.

TRPM4

Significant evidence suggests that TRPM4 channels are important for pressure-induced smooth muscle cell depolarization and vasoconstriction (19), and autoregulation of cerebral blood flow (76). TRPM4 mRNA is present in freshly-isolated rat cerebral artery smooth muscle cells and TRPM4 currents have been recorded from these cells in inside-out (19) and whole-cell (18) patch clamp experiments. Antisense-mediated downregulation of TRPM4 expression in intact cerebral arteries results in diminished pressure-induced smooth muscle cell depolarization and vasoconstriction, demonstrating that TRPM4 channel expression is essential for myogenic responsiveness (19). These findings were extended by Reading et al., who showed that introduction of TRPM4 antisense oligonucleotides to cerebral spinal fluid *in vivo* resulted in downregulation of TRPM4 expression in the cerebral arteries of intact rats (75). Diminished TRPM4 expression in the cerebral vasculature of these animals was associated with a loss of autoregulation of cerebral blood flow in response to changes in perfusion pressure (75). These findings demonstrate that TRPM4 is required for vascular smooth muscle membrane potential depolarization in response to elevations in intraluminal pressure, and this response is necessary for *in vivo* autoregulation of cerebral blood flow in response to pressure.

Mechanisms responsible for the regulation of smooth muscle TRPM4 activity in response to changes in intraluminal pressure are not fully understood. There is some evidence that the channel may be inherently mechanosensitive (55), but these findings have not been confirmed. It is also possible that TRPM4 channels may be indirectly activated by increases in intraluminal pressure. In support of this possibility, PKC activity enhances TRPM4 currents in HEK expression systems (62) and in native cerebral artery myocytes (18).

Furthermore, TRPM4 expression is required for smooth muscle cell depolarization and vasoconstriction in response to the phorbol PKC activator PMA (18). PKC activity is necessary for myogenic constriction (29,66), consistent with the hypothesis that pressure-induced PKC activation increases TRPM4 activity to depolarize smooth muscle.

TRPM7

TRPM7 is present in primary cultures of smooth muscle cells derived from rat aorta and rat and mouse mesenteric arteries (27). He et al. demonstrate that TRPM7 mRNA levels are elevated in rat vascular smooth muscle cells treated with angiotensin II (Ang II) and aldosterone. Furthermore, total and membrane-associated TRPM7 protein levels are increased in cells treated with Ang II (27). Downregulation of TRPM7 with siRNA impaired increases in intracellular $[Mg^{2+}]$ associated with elevated extracellular Mg^{2+} (27). In addition, this study shows that silencing of TRPM7 expression has no effect on acute (5 min) changes in intracellular $[Mg^{2+}]$ in response to Ang II administration, but increases in intracellular $[Mg^{2+}]$ in response to chronic (24 hour) exposure to Ang II were blunted in cells treated with TRPM7 siRNA (27). $^3[H]$ thymidine and $^3[H]$ leucine incorporation in response to Ang II stimulation were diminished following TRPM7 downregulation (27). These findings suggest that TRPM7-dependent Mg^{2+} influx is required for vascular smooth muscle cell proliferation in response to Ang II.

TRPM8

TRPM8 is highly expressed in a number of vascular beds, including rat aorta, mesenteric artery, femoral artery, and tail artery (37). The TRPM8 agonists menthol and icillin cause dilation of precontracted rat tail and mesenteric arteries and thoracic aorta (37). Aortic dilation in response to these agents is independent of nitric oxide synthase activity and endothelium removal (37), suggesting that menthol and icillin act directly on smooth muscle TRPM8 channels to elicit dilation. However, this study also reports that increases in forearm blood flow in response to menthol are blocked by atropine and blockade of nitric oxide synthase activity (37), suggesting that an endothelium-dependent mechanism mediates the response in this vascular bed.

V. Conclusions and Future Directions

The combined TRPV and TRPM subfamilies consist of 14 genes. Current evidence suggests that TRPV5, TRPV6, and TRPM1 channels are not expressed in smooth muscle. Message encoding TRPV3, TRPM2, TRPM3, and TRPM6 has been detected in arterial myocytes but the function of these channels in these cells is not current known. TRPV1, TRPV2, TRPV4, TRPM4, TRPM7 and TRPM8 channels are present in vascular smooth muscle and the evidence supporting potential functional roles for these channels has been reviewed. Consistent with the diversity that characterizes the TRP superfamily, these channels play a number of roles in smooth muscle cells. Much work remains to be done. Some of the burning issues requiring attention are discussed below.

Regulation of Intracellular $[Mg^{2+}]$

Clear evidence demonstrates that TRPM7 channels are important in the regulation of intracellular $[Mg^{2+}]$ in cultured vascular smooth muscle cells (27). However, it is unclear if TRPM6 channels participate in this response or if TRPM7/TRPM6 heteromultimeric channels are present in native vascular smooth muscle. A potential role for TRPM7 (and TRPM6) channels in the development of systemic hypertension was recently discussed in detail (85).

Agonist-Induced Vasomotor Responses

Capcasin, a selective agonist of TRPV1, causes constriction of isolated skeletal muscle arteries (39). Kark et al. propose that TRPV1 channels present in arterial myocytes mediate this response. However, electrophysiological characterization of TRPV1 activity in vascular smooth muscle cells, including the effects of capcasin on cation currents, is necessary to confirm this hypothesis. Furthermore, it is unlikely that TRPV1 channels in arterial myocytes are exposed to capcasin under physiological conditions. Unless endogenous TRPV1 agonists are present in the vascular wall, it is doubtful that the channel significantly contributes to vascular smooth muscle function *in vivo*.

The TRPM8 agonists menthol and icillin cause arterial dilation of endothelium-denuded arteries, suggesting that TRPM8 channels in smooth muscle cells mediate the response (37). Johnson et al. suggest the possibility that TRPM8 currents increase SR Ca^{2+} release from ryanodine receptors to BK_{Ca} -dependent STOC frequency, thereby hyperpolarizing the smooth muscle plasma membrane and causing arterial dilation (37). However, findings reported by Mahieu et al., suggest that menthol causes release of intracellular Ca^{2+} by a mechanism that is independent of TRPM8 (49). In addition, direct evidence for TRPM8 involvement in arterial dilation in response to menthol has not been reported. These data are critical because low concentrations of menthol activates TRPA1 (38), a channel that mediates endothelium-dependent dilation of cerebral arteries (14). Furthermore, TRPM8 currents have not been reported in smooth muscle cells and endogenous chemical activators of the channel are unknown. Because of these issues, the role of TRPM8 channels in the regulation of smooth muscle function and arterial tone remains unclear (48).

Two reports support the hypothesis that TRPV4 channels in smooth muscle cells mediate membrane hyperpolarization and vasodilation in response to EETs (15,17). However, other laboratories demonstrate involvement of endothelial TRPV4 channels in this response (26,42,50,89,95,107). Although current evidence suggests that TRPV4 channels in smooth muscle and endothelial cells may contribute equally to 11,12 EET-induced dilation of mesenteric arteries (17), further work is need to resolve the respective roles of TRPV4 channels in these cell types.

Pressure-Induced Membrane Depolarization and Vasoconstriction

Resistance arteries in many vascular beds constrict in response to increases in intraluminal pressure (3). This behavior, known as the vascular myogenic response, is a result of smooth muscle cell membrane depolarization (25) and Ca^{2+} influx via voltage dependent Ca^{2+} channels (41). A number of studies suggest that activation of mechanosensitive TRP channels is responsible for pressure-induced membrane depolarization, but evidence supporting at least three channels (TRPC6 (97), TRPV2 (56), and TRPM4 (18,19,55,75) in this response has been reported. The evidence supporting involvement of these channels in myogenic vasoconstriction is summarized in Table 3. Issues that require further study are discussed below.

TRPV2 channels are activated by hypotonicity-induced cell swelling in smooth muscle cells, prompting Muraki et al. to hypothesize that TRPV2 channels mediate pressure-induced vasomotive responses (56). Swelling-induced currents recorded from arterial myocytes were assigned to TRPV2 on the basis of antisense-mediated silencing experiments (56). However, the effects of antisense-mediated downregulation of TRPV2 on TRPV4 expression were not reported. These data are important because TRPV4 is also a swelling-activated, ruthenium-red sensitive cation channel. Agonists such as 4 α -PDD and EETs activate ruthenium red sensitive cation currents in cerebral (15) and mesenteric artery smooth muscle cells (17), suggesting that functional TRPV4 channels are present in vascular

smooth muscle cells. Thus, it is possible that TRPV4 could account for the cation currents reported by Muraki et al. In addition, the effects of TRPV2 silencing on smooth muscle cell membrane potential depolarization or pressure-induced vasoconstriction have not been reported. Until these issues are addressed, the importance of TRPV2 in regulation of smooth muscle function will remain unresolved.

A number of studies using antisense-mediated silencing demonstrate that TRPM4 channels regulate changes in membrane potential in response to increase in intraluminal pressure (19,76) and PKC activity (18). However, activation of TRPM4 channels in patch clamp experiments employing HEK expression systems (45,60) and native cerebral artery smooth muscle cells (18,19) requires levels of intracellular Ca^{2+} (~10 μM) that are greater than global Ca^{2+} levels reported for smooth muscle cells (100-300 nM) (41). The source of intracellular Ca^{2+} responsible for TRPM4 activity in arterial myocytes remains unknown. It is possible that TRPM4 channels, like BK_{Ca} channels (57), are regulated by localized, transient elevations of intracellular $[\text{Ca}^{2+}]$ resulting from Ca^{2+} influx or Ca^{2+} release from intracellular stores, but this hypothesis has not been tested.

Pressure-induced membrane depolarization and vasoconstriction requires expression of TRPC6 channels (97) in addition to TRPM4. It is not known if, or how, these channels cooperate to regulate pressure-induced membrane depolarization. It is possible that TRPC6 and TRPM4 subunits form heteromultimeric channels in native cells. However, there is little structural homology between the two channels and there is no evidence that TRPC6/TRPM4 heteromultimeric channels exists. A more likely possibility is that TRPC6 channels influence localized increases in intracellular Ca^{2+} required for TRPM4 activation.

Future Directions

The first reports of TRP channel involvement in smooth muscle cell physiology (36,97) appeared approximately nine years prior to this review and we now know that many TRP channels are critical for aspects of arterial function. In the next decade, the challenge will be to utilize this knowledge to improve human cardiovascular health. Studies examining changes in expression and/or regulation of TRP channel activity during common cardiovascular disease such as atherosclerosis, stroke, and pulmonary and systemic hypertension will be of particular interest. These studies, in conjunction with efforts to develop selective pharmacological agents that activate or inhibit TRP channels involved in arterial function may provide powerful new tools for the treatment of cardiovascular disease.

Acknowledgments

SOURCES OF FUNDING

NIH grant RO1HL091905 supported this work.

REFERENCES CITED

1. Abramowitz J, Birnbaumer L. Physiology and pathophysiology of canonical transient receptor potential channels. *FASEB J*. 2009; 23:297–328. [PubMed: 18940894]
2. Alvarez DF, King JA, Weber D, Addison E, Liedtke W, Townsley MI. Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. *Circ Res*. 2006; 99:988–995. [PubMed: 17008604]
3. Bayliss WM. On the local reactions of the arterial wall to changes of internal pressure. *J Physiol (London)*. 1902; 28:220–231. [PubMed: 16992618]
4. Brauchi S, Orio P, Latorre R. Clues to understanding cold sensation: thermodynamics and electrophysiological analysis of the cold receptor TRPM8. *Proc Natl Acad Sci U S A*. 2004; 101:15494–15499. [PubMed: 15492228]

5. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ Res.* 1996; 78:415–423. [PubMed: 8593700]
6. Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D. A capsaicin-receptor homologue with a high threshold for noxious heat. *Nature.* 1999; 398:436–441. [PubMed: 10201375]
7. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature.* 1997; 389:816–824. [PubMed: 9349813]
8. Chubanov V, Waldegger S, Mederos y Schnitzler M, Vitzthum H, Sassen MC, Seyberth HW, Konrad M, Gudermann T. Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesemia with secondary hypocalcemia. *Proc Natl Acad Sci U S A.* 2004; 101:2894–2899. [PubMed: 14976260]
9. Chung MK, Lee H, Mizuno A, Suzuki M, Caterina MJ. 2-aminoethoxydiphenyl borate activates and sensitizes the heat-gated ion channel TRPV3. *J Neurosci.* 2004; 24:5177–5182. [PubMed: 15175387]
10. Clapham, DE.; Nilius, B.; Owsianik, G. Transient Receptor Potential Channels: IUPHAR database. 2009.
11. Colbert HA, Smith TL, Bargmann CI. OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. *J Neurosci.* 1997; 17:8259–8269. [PubMed: 9334401]
12. Damann N, Voets T, Nilius B. TRPs in our senses. *Curr Biol.* 2008; 18:R880–889. [PubMed: 18812089]
13. Duncan LM, Deeds J, Hunter J, Shao J, Holmgren LM, Woolf EA, Tepper RI, Shyjan AW. Down-regulation of the novel gene melastatin correlates with potential for melanoma metastasis. *Cancer Res.* 1998; 58:1515–1520. [PubMed: 9537257]
14. Earley S, Gonzales AL, Crnich R. Endothelium-dependent cerebral artery dilation mediated by TRPA1 and Ca²⁺-Activated K⁺ channels. *Circ Res.* 2009; 104:987–994. [PubMed: 19299646]
15. Earley S, Heppner TJ, Nelson MT, Brayden JE. TRPV4 forms a novel Ca²⁺ signaling complex with ryanodine receptors and BKCa channels. *Circ Res.* 2005; 97:1270–1279. [PubMed: 16269659]
16. Earley S, Pastuszyn A, Walker BR. Cytochrome p-450 epoxygenase products contribute to attenuated vasoconstriction after chronic hypoxia. *Am J Physiol Heart Circ Physiol.* 2003; 285:H127–136. [PubMed: 12623785]
17. Earley S, Pauyo T, Drapp R, Tavares MJ, Liedtke W, Brayden JE. TRPV4-dependent dilation of peripheral resistance arteries influences arterial pressure. *Am J Physiol Heart Circ Physiol.* 2009; 297:H1096–1102. [PubMed: 19617407]
18. Earley S, Straub SV, Brayden JE. Protein kinase C regulates vascular myogenic tone through activation of TRPM4. *Am J Physiol Heart Circ Physiol.* 2007; 292:H2613–2622. [PubMed: 17293488]
19. Earley S, Waldron BJ, Brayden JE. Critical role for transient receptor potential channel TRPM4 in myogenic constriction of cerebral arteries. *Circ Res.* 2004; 95:922–929. [PubMed: 15472118]
20. Grand T, Demion M, Norez C, Mettey Y, Launay P, Becq F, Bois P, Guinamard R. 9-phenanthrol inhibits human TRPM4 but not TRPM5 cationic channels. *Br J Pharmacol.* 2008; 153:1697–1705. [PubMed: 18297105]
21. Grimm C, Kraft R, Sauerbruch S, Schultz G, Harteneck C. Molecular and functional characterization of the melastatin-related cation channel TRPM3. *J Biol Chem.* 2003; 278:21493–21501. [PubMed: 12672799]
22. Grimm C, Kraft R, Schultz G, Harteneck C. Activation of the melastatin-related cation channel TRPM3 by D-erythro-sphingosine [corrected]. *Mol Pharmacol.* 2005; 67:798–805. [PubMed: 15550678]
23. Guler AD, Lee H, Iida T, Shimizu I, Tominaga M, Caterina M. Heat-evoked activation of the ion channel, TRPV4. *J Neurosci.* 2002; 22:6408–6414. [PubMed: 12151520]
24. Hara Y, Wakamori M, Ishii M, Maeno E, Nishida M, Yoshida T, Yamada H, Shimizu S, Mori E, Kudoh J, Shimizu N, Kurose H, Okada Y, Imoto K, Mori Y. LTRPC2 Ca²⁺-permeable channel

- activated by changes in redox status confers susceptibility to cell death. *Mol Cell*. 2002; 9:163–173. [PubMed: 11804595]
25. Harder DR. Pressure-dependent membrane depolarization in cat middle cerebral artery. *Circ Res*. 1984; 55:197–202. [PubMed: 6744529]
 26. Hartmannsgruber V, Heyken WT, Kacik M, Kaistha A, Grgic I, Harteneck C, Liedtke W, Hoyer J, Kohler R. Arterial response to shear stress critically depends on endothelial TRPV4 expression. *PLoS One*. 2007; 2:e827. [PubMed: 17786199]
 27. He Y, Yao G, Savoia C, Touyz RM. Transient receptor potential melastatin 7 ion channels regulate magnesium homeostasis in vascular smooth muscle cells: role of angiotensin II. *Circ Res*. 2005; 96:207–215. [PubMed: 15591230]
 28. Hill K, McNulty S, Randall AD. Inhibition of TRPM2 channels by the antifungal agents clotrimazole and econazole. *Naunyn Schmiedeberg Arch Pharmacol*. 2004; 370:227–237. [PubMed: 15549272]
 29. Hill MA, Falcone JC, Meininger GA. Evidence for protein kinase C involvement in arteriolar myogenic reactivity. *Am J Physiol*. 1990; 259:H1586–1594. [PubMed: 2240255]
 30. Hoenderop JG, van der Kemp AW, Hartog A, van de Graaf SF, van Os CH, Willems PH, Bindels RJ. Molecular identification of the apical Ca²⁺ channel in 1, 25-dihydroxyvitamin D₃-responsive epithelia. *J Biol Chem*. 1999; 274:8375–8378. [PubMed: 10085067]
 31. Hoenderop JG, Vennekens R, Muller D, Prenen J, Droogmans G, Bindels RJ, Nilius B. Function and expression of the epithelial Ca(2+) channel family: comparison of mammalian ECaC1 and 2. *J Physiol*. 2001; 537:747–761. [PubMed: 11744752]
 32. Hoenderop JG, Voets T, Hoefs S, Weidema F, Prenen J, Nilius B, Bindels RJ. Homo- and heterotetrameric architecture of the epithelial Ca²⁺ channels TRPV5 and TRPV6. *Embo J*. 2003; 22:776–785. [PubMed: 12574114]
 33. Hofmann T, Chubakov V, Gudermann T, Montell C. TRPM5 is a voltage-modulated and Ca(2+)-activated monovalent selective cation channel. *Curr Biol*. 2003; 13:1153–1158. [PubMed: 12842017]
 34. Hotta Y, Benzer S. Genetic dissection of the *Drosophila* nervous system by means of mosaics. *Proc Natl Acad Sci U S A*. 1970; 67:1156–1163. [PubMed: 5274445]
 35. Hu HZ, Gu Q, Wang C, Colton CK, Tang J, Kinoshita-Kawada M, Lee LY, Wood JD, Zhu MX. 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. *J Biol Chem*. 2004; 279:35741–35748. [PubMed: 15194687]
 36. Inoue R, Okada T, Onoue H, Hara Y, Shimizu S, Naitoh S, Ito Y, Mori Y. The transient receptor potential protein homologue TRP6 is the essential component of vascular alpha(1)-adrenoceptor-activated Ca(2+)-permeable cation channel. *Circ Res*. 2001; 88:325–332. [PubMed: 11179201]
 37. Johnson CD, Melanaphy D, Purse A, Stokesberry SA, Dickson P, Zholos AV. Transient receptor potential melastatin 8 channel involvement in the regulation of vascular tone. *Am J Physiol Heart Circ Physiol*. 2009; 296:H1868–1877. [PubMed: 19363131]
 38. Karashima Y, Damann N, Prenen J, Talavera K, Segal A, Voets T, Nilius B. Bimodal action of menthol on the transient receptor potential channel TRPA1. *J Neurosci*. 2007; 27:9874–9884. [PubMed: 17855602]
 39. Kark T, Bagi Z, Lizanecz E, Pasztor ET, Erdei N, Czikora A, Papp Z, Edes I, Porszasz R, Toth A. Tissue-specific regulation of microvascular diameter: opposite functional roles of neuronal and smooth muscle located vanilloid receptor-1. *Mol Pharmacol*. 2008; 73:1405–1412. [PubMed: 18256211]
 40. Kerschbaum HH, Kozak JA, Cahalan MD. Polyvalent cations as permeant probes of MIC and TRPM7 pores. *Biophys J*. 2003; 84:2293–2305. [PubMed: 12668438]
 41. Knot HJ, Nelson MT. Regulation of arterial diameter and wall [Ca²⁺] in cerebral arteries of rat by membrane potential and intravascular pressure. *J Physiol*. 1998; 508 (Pt 1):199–209. [PubMed: 9490839]
 42. Kohler R, Heyken WT, Heinau P, Schubert R, Si H, Kacik M, Busch C, Grgic I, Maier T, Hoyer J. Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation. *Arterioscler Thromb Vasc Biol*. 2006; 26:1495–1502. [PubMed: 16675722]

43. Kolisek M, Beck A, Fleig A, Penner R. Cyclic ADP-ribose and hydrogen peroxide synergize with ADP-ribose in the activation of TRPM2 channels. *Mol Cell*. 2005; 18:61–69. [PubMed: 15808509]
44. Kotlikoff MJ. EDHF redux: EETs, TRPV4, and Ca²⁺ sparks. *Circ Res*. 2005; 97:1209–1210. [PubMed: 16339490]
45. Launay P, Fleig A, Perraud AL, Scharenberg AM, Penner R, Kinet JP. TRPM4 is a Ca²⁺-activated nonselective cation channel mediating cell membrane depolarization. *Cell*. 2002; 109:397–407. [PubMed: 12015988]
46. Li M, Jiang J, Yue L. Functional characterization of homo- and heteromeric channel kinases TRPM6 and TRPM7. *J Gen Physiol*. 2006; 127:525–537. [PubMed: 16636202]
47. Lizanec Z, Bagi Z, Pasztor ET, Papp Z, Edes I, Kedei N, Blumberg PM, Toth A. Phosphorylation-dependent desensitization by anandamide of vanilloid receptor-1 (TRPV1) function in rat skeletal muscle arterioles and in Chinese hamster ovary cells expressing TRPV1. *Mol Pharmacol*. 2006; 69:1015–1023. [PubMed: 16338989]
48. Ma S. Letter to the editor: “Is menthol- or icilin-induced vasodilation mediated by the activation of TRPM8?”. *Am J Physiol Heart Circ Physiol*. 2009; 297:H887. author reply H888. [PubMed: 19635848]
49. Mahieu F, Owsianik G, Verbert L, Janssens A, De Smedt H, Nilius B, Voets T. TRPM8-independent menthol-induced Ca²⁺ release from endoplasmic reticulum and Golgi. *J Biol Chem*. 2007; 282:3325–3336. [PubMed: 17142461]
50. Marrelli SP, O’Neil RG, Brown RC, Bryan RM Jr. PLA2 and TRPV4 channels regulate endothelial calcium in cerebral arteries. *Am J Physiol Heart Circ Physiol*. 2007; 292:H1390–1397. [PubMed: 17071727]
51. Maruyama Y, Nakanishi Y, Walsh EJ, Wilson DP, Welsh DG, Cole WC. Heteromultimeric TRPC6-TRPC7 Channels Contribute to Arginine Vasopressin-Induced Cation Current of A7r5 Vascular Smooth Muscle Cells. *Circ Res*. 2006
52. McIntyre P, McLatchie LM, Chambers A, Phillips E, Clarke M, Savidge J, Toms C, Peacock M, Shah K, Winter J, Weerasakera N, Webb M, Rang HP, Bevan S, James IF. Pharmacological differences between the human and rat vanilloid receptor 1 (VR1). *Br J Pharmacol*. 2001; 132:1084–1094. [PubMed: 11226139]
53. McKemy DD, Neuhauser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature*. 2002; 416:52–58. [PubMed: 11882888]
54. Montell C, Rubin GM. Molecular characterization of the *Drosophila* trp locus: a putative integral membrane protein required for phototransduction. *Neuron*. 1989; 2:1313–1323. [PubMed: 2516726]
55. Morita H, Honda A, Inoue R, Ito Y, Abe K, Nelson MT, Brayden JE. Membrane stretch-induced activation of a TRPM4-like nonselective cation channel in cerebral artery myocytes. *J Pharmacol Sci*. 2007; 103:417–426. [PubMed: 17420615]
56. Muraki K, Iwata Y, Katanosaka Y, Ito T, Ohya S, Shigekawa M, Imaizumi Y. TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. *Circ Res*. 2003; 93:829–838. [PubMed: 14512441]
57. Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, Lederer WJ. Relaxation of arterial smooth muscle by calcium sparks. *Science*. 1995; 270:633–637. [PubMed: 7570021]
58. Nilius B, Mahieu F, Prenen J, Janssens A, Owsianik G, Vennekens R, Voets T. The Ca(2+)-activated cation channel TRPM4 is regulated by phosphatidylinositol 4,5-bisphosphate. *Embo J*. 2006
59. Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. *Physiol Rev*. 2007; 87:165–217. [PubMed: 17237345]
60. Nilius B, Prenen J, Droogmans G, Voets T, Vennekens R, Freichel M, Wissenbach U, Flockerzi V. Voltage dependence of the Ca²⁺-activated cation channel TRPM4. *J Biol Chem*. 2003; 278:30813–30820. [PubMed: 12799367]
61. Nilius B, Prenen J, Janssens A, Voets T, Droogmans G. Decavanadate modulates gating of TRPM4 cation channels. *J Physiol*. 2004; 560:753–765. [PubMed: 15331675]

62. Nilius B, Prenen J, Tang J, Wang C, Owsianik G, Janssens A, Voets T, Zhu MX. Regulation of the Ca^{2+} sensitivity of the nonselective cation channel TRPM4. *J Biol Chem*. 2005; 280:6423–6433. [PubMed: 15590641]
63. Nilius B, Prenen J, Voets T, Droogmans G. Intracellular nucleotides and polyamines inhibit the Ca^{2+} -activated cation channel TRPM4b. *Pflugers Arch*. 2004; 448:70–75. [PubMed: 14758478]
64. Oancea E, Vriens J, Brauchi S, Jun J, Splawski I, Clapham DE. TRPM1 forms ion channels associated with melanin content in melanocytes. *Sci Signal*. 2009; 2:ra21. [PubMed: 19436059]
65. Oberwinkler J, Lis A, Giehl KM, Flockerzi V, Philipp SE. Alternative splicing switches the divalent cation selectivity of TRPM3 channels. *J Biol Chem*. 2005; 280:22540–22548. [PubMed: 15824111]
66. Osol G, Laher I, Cipolla M. Protein kinase C modulates basal myogenic tone in resistance arteries from the cerebral circulation. *Circ Res*. 1991; 68:359–367. [PubMed: 1991343]
67. Pak WL, Grossfield J, Arnold KS. Mutants of the visual pathway of *Drosophila melanogaster*. *Nature*. 1970; 227:518–520. [PubMed: 5428475]
68. Palmer CP, Zhou XL, Lin J, Loukin SH, Kung C, Saimi Y. A TRP homolog in *Saccharomyces cerevisiae* forms an intracellular Ca^{2+} -permeable channel in the yeast vacuolar membrane. *Proc Natl Acad Sci U S A*. 2001; 98:7801–7805. [PubMed: 11427713]
69. Patapoutian A, Tate S, Woolf CJ. Transient receptor potential channels: targeting pain at the source. *Nat Rev Drug Discov*. 2009; 8:55–68. [PubMed: 19116627]
70. Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A. A TRP channel that senses cold stimuli and menthol. *Cell*. 2002; 108:705–715. [PubMed: 11893340]
71. Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, Hergarden AC, Story GM, Colley S, Hogenesch JB, McIntyre P, Bevan S, Patapoutian A. A heat-sensitive TRP channel expressed in keratinocytes. *Science*. 2002; 296:2046–2049. [PubMed: 12016205]
72. Peng JB, Chen XZ, Berger UV, Vassilev PM, Tsukaguchi H, Brown EM, Hediger MA. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J Biol Chem*. 1999; 274:22739–22746. [PubMed: 10428857]
73. Perraud AL, Fleig A, Dunn CA, Bagley LA, Launay P, Schmitz C, Stokes AJ, Zhu Q, Bessman MJ, Penner R, Kinet JP, Scharenberg AM. ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. *Nature*. 2001; 411:595–599. [PubMed: 11385575]
74. Prawitt D, Monteilh-Zoller MK, Brixel L, Spangenberg C, Zabel B, Fleig A, Penner R. TRPM5 is a transient Ca^{2+} -activated cation channel responding to rapid changes in $[\text{Ca}^{2+}]_i$. *Proc Natl Acad Sci U S A*. 2003; 100:15166–15171. [PubMed: 14634208]
75. Reading SA, Brayden JE. Central role of TRPM4 channels in cerebral blood flow regulation. *Stroke*. 2007; 38:2322–2328. [PubMed: 17585083]
76. Reading SA, Earley S, Waldron BJ, Welsh DG, Brayden JE. TRPC3 mediates pyrimidine receptor-induced depolarization of cerebral arteries. *Am J Physiol Heart Circ Physiol*. 2005; 288:H2055–2061. [PubMed: 15604128]
77. Runnels LW, Yue L, Clapham DE. TRP-PLIK, a bifunctional protein with kinase and ion channel activities. *Science*. 2001; 291:1043–1047. [PubMed: 11161216]
78. Schlingmann KP, Weber S, Peters M, Niemann Nejsun L, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E, Dinour D, Syrou M, Nielsen S, Sassen M, Waldegger S, Seyberth HW, Konrad M. Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat Genet*. 2002; 31:166–170. [PubMed: 12032568]
79. Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, Penner R, Kurosaki T, Fleig A, Scharenberg AM. Regulation of vertebrate cellular Mg^{2+} homeostasis by TRPM7. *Cell*. 2003; 114:191–200. [PubMed: 12887921]
80. Smith GD, Gunthorpe MJ, Kelsell RE, Hayes PD, Reilly P, Facer P, Wright JE, Jerman JC, Walhin JP, Ooi L, Egerton J, Charles KJ, Smart D, Randall AD, Anand P, Davis JB. TRPV3 is a temperature-sensitive vanilloid receptor-like protein. *Nature*. 2002; 418:186–190. [PubMed: 12077606]

81. Spassova MA, Hewavitharana T, Xu W, Soboloff J, Gill DL. A common mechanism underlies stretch activation and receptor activation of TRPC6 channels. *Proc Natl Acad Sci U S A*. 2006; 103:16586–16591. [PubMed: 17056714]
82. Strotmann R, Harteneck C, Nunnenmacher K, Schultz G, Plant TD. OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. *Nat Cell Biol*. 2000; 2:695–702. [PubMed: 11025659]
83. Thorneloe KS, Sulpizio AC, Lin Z, Figueroa DJ, Clouse AK, McCafferty GP, Chendrimada TP, Lashinger ES, Gordon E, Evans L, Misajet BA, Demarini DJ, Nation JH, Casillas LN, Marquis RW, Votta BJ, Sheardown SA, Xu X, Brooks DP, Laping NJ, Westfall TD. N-((1S)-1-([4-((2S)-2-((2,4-dichlorophenyl)sulfonyl)amino)-3-hydroxypropyl)-1-piperazinyl]carbonyl)-3-methylbutyl)-1-benzothiophene-2-carboxamide (GSK1016790A), a novel and potent transient receptor potential vanilloid 4 channel agonist induces urinary bladder contraction and hyperactivity: Part I. *J Pharmacol Exp Ther*. 2008; 326:432–442. [PubMed: 18499743]
84. Togashi K, Inada H, Tominaga M. Inhibition of the transient receptor potential cation channel TRPM2 by 2-aminoethoxydiphenyl borate (2-APB). *Br J Pharmacol*. 2008; 153:1324–1330. [PubMed: 18204483]
85. Touyz RM. Transient receptor potential melastatin 6 and 7 channels, magnesium transport, and vascular biology: implications in hypertension. *Am J Physiol Heart Circ Physiol*. 2008; 294:H1103–1118. [PubMed: 18192217]
86. Ullrich ND, Voets T, Prenen J, Vennekens R, Talavera K, Droogmans G, Nilius B. Comparison of functional properties of the Ca²⁺-activated cation channels TRPM4 and TRPM5 from mice. *Cell Calcium*. 2005; 37:267–278. [PubMed: 15670874]
87. Vennekens R, Hoenderop JG, Prenen J, Stuijver M, Willems PH, Droogmans G, Nilius B, Bindels RJ. Permeation and gating properties of the novel epithelial Ca(2+) channel. *J Biol Chem*. 2000; 275:3963–3969. [PubMed: 10660551]
88. Voets T, Nilius B, Hoefs S, van der Kemp AW, Droogmans G, Bindels RJ, Hoenderop JG. TRPM6 forms the Mg²⁺ influx channel involved in intestinal and renal Mg²⁺ absorption. *J Biol Chem*. 2004; 279:19–25. [PubMed: 14576148]
89. Vriens J, Owsianik G, Fisslthaler B, Suzuki M, Janssens A, Voets T, Morisseau C, Hammock BD, Fleming I, Busse R, Nilius B. Modulation of the Ca²⁺ permeable cation channel TRPV4 by cytochrome P450 epoxygenases in vascular endothelium. *Circ Res*. 2005; 97:908–915. [PubMed: 16179585]
90. Wagner TF, Loch S, Lambert S, Straub I, Mannebach S, Mathar I, Dufer M, Lis A, Flockerzi V, Philipp SE, Oberwinkler J. Transient receptor potential M3 channels are ionotropic steroid receptors in pancreatic beta cells. *Nat Cell Biol*. 2008; 10:1421–1430. [PubMed: 18978782]
91. Walder RY, Landau D, Meyer P, Shalev H, Tsoia M, Borochoy Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, Sheffield VC. Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. *Nat Genet*. 2002; 31:171–174. [PubMed: 12032570]
92. Walker RL, Hume JR, Horowitz B. Differential expression and alternative splicing of TRP channel genes in smooth muscles. *Am J Physiol Cell Physiol*. 2001; 280:C1184–1192. [PubMed: 11287332]
93. Wang YX, Wang J, Wang C, Liu J, Shi LP, Xu M, Wang C. Functional expression of transient receptor potential vanilloid-related channels in chronically hypoxic human pulmonary arterial smooth muscle cells. *J Membr Biol*. 2008; 223:151–159. [PubMed: 18787888]
94. Watanabe H, Davis JB, Smart D, Jerman JC, Smith GD, Hayes P, Vriens J, Cairns W, Wissenbach U, Prenen J, Flockerzi V, Droogmans G, Benham CD, Nilius B. Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. *J Biol Chem*. 2002; 277:13569–13577. [PubMed: 11827975]
95. Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature*. 2003; 424:434–438. [PubMed: 12879072]
96. Watanabe H, Vriens J, Suh SH, Benham CD, Droogmans G, Nilius B. Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. *J Biol Chem*. 2002; 277:47044–47051. [PubMed: 12354759]

97. Welsh DG, Morielli AD, Nelson MT, Brayden JE. Transient receptor potential channels regulate myogenic tone of resistance arteries. *Circ Res*. 2002; 90:248–250. [PubMed: 11861411]
98. Wes PD, Chevesich J, Jeromin A, Rosenberg C, Stetten G, Montell C. TRPC1, a human homolog of a *Drosophila* store-operated channel. *Proc Natl Acad Sci U S A*. 1995; 92:9652–9656. [PubMed: 7568191]
99. Willette RN, Bao W, Nerurkar S, Yue TL, Doe CP, Stankus G, Turner GH, Ju H, Thomas H, Fishman CE, Sulpizio A, Behm DJ, Hoffman S, Lin Z, Lozinskaya I, Casillas LN, Lin M, Trout RE, Votta BJ, Thorneloe K, Lashinger ES, Figueroa DJ, Marquis R, Xu X. Systemic activation of the transient receptor potential vanilloid subtype 4 channel causes endothelial failure and circulatory collapse: Part 2. *J Pharmacol Exp Ther*. 2008; 326:443–452. [PubMed: 18499744]
100. Wong PY, Lai PS, Falck JR. Mechanism and signal transduction of 14 (R), 15 (S)-epoxyeicosatrienoic acid (14,15-EET) binding in guinea pig monocytes. *Prostaglandins Other Lipid Mediat*. 2000; 62:321–333. [PubMed: 11060896]
101. Xu H, Delling M, Jun JC, Clapham DE. Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. *Nat Neurosci*. 2006; 9:628–635. [PubMed: 16617338]
102. Xu H, Ramsey IS, Kotecha SA, Moran MM, Chong JA, Lawson D, Ge P, Lilly J, Silos-Santiago I, Xie Y, DiStefano PS, Curtis R, Clapham DE. TRPV3 is a calcium-permeable temperature-sensitive cation channel. *Nature*. 2002; 418:181–186. [PubMed: 12077604]
103. Xu SZ, Zeng F, Boulay G, Grimm C, Harteneck C, Beech DJ. Block of TRPC5 channels by 2-aminoethoxydiphenyl borate: a differential, extracellular and voltage-dependent effect. *Br J Pharmacol*. 2005; 145:405–414. [PubMed: 15806115]
104. Yang W, Tuniki VR, Anjaiah S, Falck JR, Hillard CJ, Campbell WB. Characterization of epoxyeicosatrienoic acid binding site in U937 membranes using a novel radiolabeled agonist, 20–125i-14,15-epoxyeicosa-8(Z)-enoic acid. *J Pharmacol Exp Ther*. 2008; 324:1019–1027. [PubMed: 18171909]
105. Yang XR, Lin MJ, McIntosh LS, Sham JS. Functional expression of transient receptor potential melastatin- and vanilloid-related channels in pulmonary arterial and aortic smooth muscle. *Am J Physiol Lung Cell Mol Physiol*. 2006; 290:L1267–1276. [PubMed: 16399784]
106. Yue L, Peng JB, Hediger MA, Clapham DE. CaT1 manifests the pore properties of the calcium-release-activated calcium channel. *Nature*. 2001; 410:705–709. [PubMed: 11287959]
107. Zhang DX, Mendoza SA, Bubolz AH, Mizuno A, Ge ZD, Li R, Warltier DC, Suzuki M, Gutterman DD. Transient receptor potential vanilloid type 4-deficient mice exhibit impaired endothelium-dependent relaxation induced by acetylcholine in vitro and in vivo. *Hypertension*. 2009; 53:532–538. [PubMed: 19188524]
108. Zhang Z, Okawa H, Wang Y, Liman ER. Phosphatidylinositol 4,5-bisphosphate rescues TRPM4 channels from desensitization. *J Biol Chem*. 2005; 280:39185–39192. [PubMed: 16186107]

Figure 1. Proposed mechanism for TRPV4-dependent EETs-induced smooth muscle hyperpolarization
From (15).

Table 1

Properties of TRPV Channels

Channel	Unitary Conductance	Selectivity (pCa ²⁺ /pNa ⁺)	Activators	Inhibitors
TRPV1	~35–80 pS	~10:1	heat (>42°C) capsaicin	capsazepine ruthenium red
TRPV2	Not reported	~3:1	heat (>50°C) hypotonicity-induced cell swelling 2-APB	ruthenium red
TRPV3	~170 pS	~12:1	camphor carvacrol cinnamaldehyde eugenol menthol thymol heat (>30°C) 2-APB	ruthenium red
TRPV4	~30–60 pS (–60 mV) ~90–100pS (+ 60 mV)	~6:1	heat (>25–34°C) hypotonicity-induced cell swelling 4 α -PDD EETs GSK1016790A	ruthenium red
TRPV5	~80 pS	~100:1	constitutive	none reported
TRPV6	40–60 pS	~100:1	constitutive	ruthenium red

Table 2

Properties of TRPM Channels

Channel	Unitary Conductance	Selectivity (pCa ²⁺ /pNa ⁺)	Activators	Inhibitors
TRPM1	not reported	1:1	not reported	La ³⁺
TRPM2	~60–80 pS	1:1 (20°C) 6:1 (35°C)	ADP-ribose cyclic ADPR H ₂ O ₂	clotrimazole econazole miconazole flufenamic acid 2-APB
TRPM3	~73–133 pS	1:1	sphingosine nifedipine pregnenolone sulfate	2-APB Gd ³⁺ La ³⁺ [Mg ²⁺] _i
TRPM4	~25 pS	Ca ²⁺ impermeable	[Ca ²⁺] _i PKC activity PtdIns(4,5)P ₂ decavanadate	AMP ADP ATP flufenamic acid spermine 9-phenanthrol
TRPM5	~23–25 pS	Ca ²⁺ impermeable	[Ca ²⁺] _i	none reported
TRPM6	~84 pS (negative holding potentials)	not reported	2-APB	[Mg ²⁺] _i ruthenium red
TRPM7	40 – 105 pS	not reported	None reported	[Mg ²⁺] _i La ³⁺ polyvalent cations
TRPM8	60 pS (10°C) 75 pS (30°C)	1:1	Cold (max at 10°C) menthol icillin	2-APB

Table 3

TRP Channels Possibly Involved in Regulation of Myogenic Tone

Channel	Evidence Supporting Channel Mechanosensitivity	Evidence Demonstrating Involvement in Myogenic Vasoconstriction
TRPC6	Activation by hypotonicity-induced cell swelling (97) and membrane stretch (81)	Loss of pressure-induced membrane depolarization and vasoconstriction following antisense-mediated silencing (cerebral arteries) (97).
TRPM4	Activation by membrane stretch (55) and PKC activity (18)	Loss of pressure-induced membrane depolarization and vasoconstriction following antisense-mediated silencing (cerebral arteries) (19,75); Loss of cerebral blood flow autoregulation of following antisense-mediated silencing <i>in vivo</i> (75).
TRPV2	Activation by hypotonicity-induced cell swelling and membrane stretch (56)	None reported.