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TRPV channels and vascular function

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Abstract

Transient receptor potential channels, of the vanilloid subtype (TRPV), act as sensory mediators, being activated by endogenous ligands, heat, mechanical and osmotic stress. Within the vasculature, TRPV channels are expressed in smooth muscle cells, endothelial cells, as well as in peri-vascular nerves. Their varied distribution and polymodal activation properties make them ideally suited to a role in modulating vascular function, perceiving and responding to local environmental changes. In endothelial cells, TRPV1 is activated by endocannabinoids, TRPV3 by dietary agonists, and TRPV4 by shear stress, epoxyeicosatrienoic acids (EETs), and downstream of Gq-coupled receptor activation. Upon activation, these channels contribute to vasodilation via nitric oxide (NO), prostacyclin (PGI_2), and intermediate/small conductance potassium channel (IK_{Ca}/SK_{Ca}) dependent pathways. In smooth muscle, TRPV4 is activated by endothelial derived EETs, leading to large conductance potassium channel (BK_{Ca}) activation and smooth muscle hyperpolarization. Conversely, smooth muscle TRPV2 channels contribute to global calcium entry and may aid constriction. TRPV1 and TRPV4 are expressed in sensory nerves and can cause vasodilation through CGRP and substance P release as well as mediating vascular function via the baroreceptor reflex (TRPV1) or via increasing sympathetic outflow during osmotic stress (TRPV4). Thus, TRPV channels play important roles in the regulation of normal and pathological cellular function in the vasculature.

Keywords

TRPV channels; smooth muscle; endothelium; neurovascular

Introduction

The Transient Receptor Potential (TRP) superfamily is diverse, encoded for by 28 TRP channel genes present in the mammalian genome and grouped into six subfamilies based on DNA and protein sequence homology: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPP (polycystin), and TRPML (mucolipin). TRP proteins form cation channels that can be gated by multiple stimuli, which include temperature, light, pressure, chemical ligands and mechanical and osmotic stress. All cells express multiple TRP channel proteins which often act as cellular sensors, regulating cellular function by responding to changes in extracellular stimuli. The focus of this review will be on the role of the TRPV subfamily in the vasculature. The TRPV subfamily can be further subdivided into 6 isoforms - TRPV1-6. However, at present only the TRPV isoforms 1–4 have clear roles in vascular function. This review will therefore start with a brief introduction to the structure,

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Conflict of Interest
The authors state that there is no conflict of interest.

distribution, and functional roles of TRPV channels followed by a summary of our current knowledge of TRPV channels in vascular endothelium, arterial myocytes, and perivascular neurons and their roles in the regulation of vascular tone. More detailed information concerning the biophysical properties, channel domains, membrane topology, pharmacology and functional roles of TRP channels can be found in several excellent reviews covering these topics (Clapham, 2003, Nilius et al., 2007, Venkatachalam and Montell, 2007, Appendino et al., 2008, Klausen et al., 2009, Vriens et al., 2009, Vriens et al., 2007, Flockerzi et al., 2007, Di and Malik, 2010, Earley, 2010).

TRPV channels - General

Structure

It is generally accepted that TRP channel proteins have seven hydrophobic domains, six of which span the membrane (S1–S6), whilst the seventh hydrophobic domain, together with the carboxy- and N- termini reside intracellularly (Adamian and Liang, 2006, Clapham et al., 2001, Vannier et al., 1998). TRPV subunits can form both hetero- and homotetramers, (Cheng et al., 2007, Hellwig et al., 2005, Hoenderop et al., 2003, Chang et al., 2004, Stewart et al., 2010, Kottgen et al., 2008, Rutter et al., 2005, Kedei et al., 2001) although it is unknown what complexes are present within the vasculature. The pore region is formed between S5 and S6 and is responsible for TRPV channel cation selectivity and the greater calcium selectivity of TRPV5 and TRPV6 (Chung et al., 2008, Dodier et al., 2007, Garcia-Martinez et al., 2000, Voets et al., 2003, Voets et al., 2002). The pore region may also modulate temperature activation (Grandl et al., 2008, Grandl et al., 2010, Yang et al., 2010b). The N-terminus of TRPV channels contains six ankyrin repeats, collectively referred to as the ankyrin repeat domain (Jin et al., 2006, Lishko et al., 2007, McCleverty et al., 2006, Phelps et al., 2008). This domain allows TRPV channels to adequately traffic to the membrane (Chang et al., 2004, Erler et al., 2004), is required for tetramerization (Arniges et al., 2006, Erler et al., 2004) and modulatory protein-protein interactions (Al-Ansary et al., 2010, Lishko et al., 2007, Phelps et al., 2007, Stokes et al., 2005). The intracellular C-terminus is also involved in tetramerization (Garcia-Sanz et al., 2004), membrane trafficking (Becker et al., 2008), regulation (Liu et al., 2004) and temperature sensitivity (Brauchi et al., 2006, Liu et al., 2004). The C-terminus also contains a conserved hydrophophic domain, referred to as the TRP domain, and contains the consensus sequence; WKFQR, where W is conserved between all members of the TRP family. TRPV members also have a Q at position 4 and positive residues at positions 2 and 5 (Latorre et al., 2009). The TRP domain has been associated with PIP_2 modulation (Rohacs, 2009, Rohacs et al., 2005), as well as tetramization (Chang et al., 2004, Garcia-Sanz et al., 2004) and lidocaine (Leffler et al., 2008), capsaicin and temperature sensitivity of TRPV1 (Garcia-Sanz et al., 2007).

Proposed TRPV channel functions

Thermosensation—TRPV1-4 channels are thermosensitive, but have different temperature activation profiles. Most evidence suggests that TRPV1 is activated by temperatures in the noxious range (>43 °C) with an activation threshold of 40 °C (Caterina et al., 1997, Tominaga et al., 1998). Heat shifts the voltage dependence of activation of TRPV1 expressed in HEK 293 cells, to more negative membrane potentials (V1/2 was approx. +70 mV at 25 °C compared to −50 mV at 42 °C), (Voets et al., 2004) and also sensitizes the channel by reducing the threshold for activation for repeated stimuli (Caterina et al., 1997). In vivo, TRPV1 is expressed in small/medium dorsal root ganglion (DRG) and trigeminal neurons. However, although TRPV1 ablation has illustrated that TRPV1 is important in thermal nociception (Caterina et al., 2000, Mishra and Hoon, 2010, Christoph et al., 2008), responses to noxious heat are not completely prevented after TRPV1

suppression (Caterina et al., 2000, Woodbury et al., 2004, Hiura, 2009), even after simultaneous TRPV1 and TRPV2 knockdown (Woodbury et al., 2004). The channel also does not seem to be important in thermoregulation (Iida et al., 2005, Mishra and Hoon, 2010, Romanovsky et al., 2009) but may be important in thermal hyperalgesia during inflammation or after tissue injury (Caterina et al., 2000, Davis et al., 2000, Vilceanu et al., 2010)

Cloned TRPV2 channels are activated by temperatures greater than 52 °C and are sensitized by repeated heat stimulation due to a reduction in the threshold for activation (from ~50 °C to 40 °C). As well, TRPV2 is expressed in medium and large diameter Aδ DRG neurons, which like TRPV2, are activated at temperatures greater that 52 °C (Caterina et al., 1999). It therefore seems likely then that TRPV2 channels in DRGs are involved in noxious heat sensation (Caterina et al., 2000), although responses to noxious heat (52 °C) still occurred in cultured C-fibers from TRPV1−/− mice following TRPV2 suppression (Woodbury et al., 2004).

TRPV3 has a lower temperature threshold (39 °C) than TRPV1 and TRPV2. Above this threshold, the channel displays steep temperature dependence up to the maximum temperature investigated (50 °C),(Smith et al., 2002). Repeated heat stimulation sensitizes the channel and is most effectively activated by rapid heating, producing a Q10 of 21.6 (for the steepest part of the Arrhenius curve) (Xu, 2002). *In vivo*, TRPV3 may not play a role in thermoregulation as TRPV3−/− mice have core body temperatures similar to wild type mice (Moqrich et al., 2005). However, its functionality in keratinocytes (Moqrich et al., 2005, Chung et al., 2003) and importance in environmental temperature sensing of warm and noxious heat (Moqrich et al., 2005) suggest that TRPV3 acts as a peripheral thermo sensor of noxious and non-noxious temperatures.

When TRPV4 is expressed in heterologous mammalian expression systems, an increase in temperature within the physiological range causes channel activation, increasing whole cell currents and/or calcium transients (Gao et al., 2003, Guler, 2002, Vriens et al., 2004, Wegierski et al., 2009, Watanabe, 2002). TRPV4 has a threshold temperature similar to TRPV3, ~25 °C, with a Q10 of 19.1 at temperatures greater than 24 °C. Unlike TRPV3, the channel desensitizes at temperatures outside the physiological range (>40 °C) (Watanabe, 2002b) and in response to repetitive heat stimulation (Chung et al., 2004, Watanabe, 2002b). Despite this desensitization, the channel still responds to changes in temperature, even during prolonged heat at 42 °C (Guler, 2002). However, when TRPV4 is expressed in a non-mammalian cell like yeast, the channel does not respond to heat (41 °C) (Loukin et al., 2009). Heat was also incapable of activating currents in inside-out patches of TRPV4 expressing HEK 293 cells (Watanabe, 2002b). Thus, In mammalian expression systems, as suggested by several recent studies, activation of TRPV4 by heat may occur downstream of various intracellular signaling cascades or require other molecular interactions. Specifically, TRPV4 activity is increased by TRPP2 (Kottgen et al., 2008), dependent on the ankyrin repeats in the N-terminus (Watanabe, 2002b), dependent on tyrosine residues Y555 (Vriens et al., 2004) and Y110 (Wegierski et al., 2009), and inhibited by PACSIN-3, a protein associated with endocytosis and trafficking (D'hoedt et al., 2008).

Although TRPV4 is found in the anterior hypothalamus, an area of the brain involved in thermoregulation (Guler, 2002), some evidence suggests that TRPV4 is not actually involved in thermal regulation. Core body temperatures of wild type and TRPV4 −/− mice, at different room temperatures, were similar (Liedtke and Friedman, 2003, Lee et al., 2005). Like TRPV3, TRPV4 is important in keratinocytes (Chung et al., 2003, Chung et al., 2004) and peripheral thermosensation in mice (Lee et al., 2005), however, TRPV4 does not seem to play a role in noxious heat sensation (Todaka et al., 2004, Suzuki et al., 2003a). Warmer

temperatures can sensitize the channel to activation by other stimuli, such as hypotonic stress (HTS), 4α-PDD, shear stress and PMA (Gao et al., 2003, Liedtke et al., 2000, Liedtke et al., 2003), which may also be important for channel regulation *in vivo*.

Osmoreception—TRPV isoforms demonstrate osmosensitivity and may play important roles as osmoreceptors. In isolated urothelial cells, a functional role for TRPV1 in osmotic sensation has been observed. Hypotonic solution (HTS) causes the release of ATP, which is blocked by capsazepine and absent in TRPV1−/− mice (Birder et al., 2002). However, TRPV1 mRNA can not be detected in mouse urothelium (Everaerts et al., 2010), rendering this observation somewhat inconclusive. TRPV1 is also expressed in areas of the brain involved in osmoregulation such as the organum vasculosum of the lamina terminalis (OVLT) and supraoptic nucleus (SON). Neurons from the OVLT and the SON displayed increased firing rates after treatment with a hypertonic solution which did not occur in neurons from TRPV1−/− mice (Ciura and Bourque, 2006, Naeini et al., 2006).

In TRPV2 expressing CHO cells, HTS increased currents in cells in the cell-attached configuration. In mouse aortic myocytes, HTS-induced increases in whole cell currents and cell calcium were reduced after cells had been treated with antisense targeted against TRPV2 (Muraki et al., 2003).

A large body of evidence indicates that TRPV4 can be modulated by osmotic stimuli in multiple cells types. When TRPV4 is expressed in heterologous expression systems, HTS can increase whole cell currents (Nilius et al., 2001) and induce calcium influx which is reversed upon the return to isotonic solution (Liedtke et al., 2000, Strotmann et al., 2000). Responses to osmotic stimuli in TRPV4 containing cells, are sensitive to PLA_2 and cytochrome P450 epoxygenase inhibition (Vriens et al., 2004, Loukin et al., 2010), although HTS responses still occur in yeast which lack endogenous EETs, a product of cytochrome P450 activity (Loukin et al., 2009). HTS-TRPV4 responses are also sensitive to PLC inhibition (Fernandes et al., 2008, Garcia-Elias et al., 2008), sensitized by IP_3 receptor binding (Garcia-Elias et al., 2008), heat (Grant et al., 2007, Liedtke et al., 2000, Gao et al., 2003), TRPP2 (Kottgen et al., 2008), PAR2 activation (Grant et al., 2007), and inhibited by PACSIN-3 (D'hoedt et al., 2008). Phosphorylation of Y253 may be important in HTS activation (Xu 2003) although this is debatable (Vriens et al., 2004). Phosphorylation of Y110 by PKC/PKA also occurs in response to HTS (Wegierski et al., 2009). *In vivo*, the channel is associated with a central role in sensing peripheral changes in osmolarity (Liedtke and Friedman, 2003), HTS mediated nociception after sensitization by PGE_2 (Alessandri-Haber 2003) and cell volume regulation (Becker et al 2008).

Mechanoreception—Mechanical stimulation, a condition that results in a physical perturbation of the membrane is usually achieved by membrane stretch, pressure or shear stress. The mechanosensitivity of TRP channels has been recently reviewed (Yin and Kuebler, 2010) and within the TRPV subfamily, mechanosensitivity has been mainly attributed to TRPV1, TRPV2 and TRPV4.

The majority of evidence implicating TRPV1 in mechanosensation has come from the use of TRPV1−/− mice. In excised bladders from TRPV1−/− mice, stretch-induced ATP release was less compared to controls (Birder et al., 2002). Intraluminal pressure in mouse jejunum increases the discharge of wide dynamic range afferent nerves, which is reduced in TRPV1 −/− mice (Rong et al., 2004). Intra-pelvic pressure in rats increases afferent renal nerve activity, and this response is reduced by capsazepine (Feng et al., 2008). However, in mutants of C.elgans that do not produce avoidance responses to mechanical nose touch, expression of TRPV1 was not sufficient to restore mechanical behavior (Liedtke et al., 2003) and mechanosensation is unaltered in TRPV1−/− mice (Caterina et al., 2000,

Christoph et al., 2008, Mishra and Hoon, 2010). Nevertheless, TRPV1 may be involved in mechanical hypersensitivity and allodynia following spinal nerve ligation (Christoph et al., 2008).

The only convincing evidence that TRPV2 is mechanosensitive is from TRPV2 expressing CHO cells, where membrane stretch applied using negative pressure via the patch pipette stimulated inward currents at −40 mV (Muraki et al., 2003).

In TRPV4 expressing cells, whole cell currents were increased by cell inflation (Suzuki et al., 2003a), and cell suction (Loukin et al., 2010). Shear stress can also evoke responses in TRPV4 containing cells (Mendoza et al., Fernandes et al., 2008, Wegierski et al., 2009, Kottgen et al., 2008) that are reduced following TRPV4 suppression (Kottgen et al., 2008, Mendoza et al., 2010). In mutant *C. elegans*, TRPV4 expression was required to restore nose touch avoidance behaviors (Liedtke et al., 2003). Mechanical responses mediated by TRPV4 are greater at physiological temperatures (Gao et al., 2003, Liedtke et al., 2003), dependent on Y110 (Wegierski et al., 2009), enhanced following IP_3 stimulation in cells co-expressing the IP_3 receptor (Fernandes et al., 2008), yet do not require PLA_2 or cytochrome P450 epoxygenase signaling (Loukin et al., 2010). Physiologically, mechanical activation of the channel is important in endothelium-dependent vasodilations (Mendoza et al.), sensitivity to acoustic injury (Liedtke and Friedman, 2003), stretch induced endothelial cell remodeling (Thodeti et al., 2009) and peripheral mechanosensation and mechanical hyperalgesia (Grant et al., 2007, Liedtke and Friedman, 2003, Suzuki et al., 2003b).

TRPV channels and vasomotor function

TRPV expression in vascular tissues

TRPV channels are differentially expressed throughout vascular endothelium, smooth muscle and innervating peri-vascular nerves (Table 1). In endothelial cells, investigations surrounding TRPV4 have been most numerous (Hartmannsgruber et al., 2007,Kohler et al., 2006,Loot et al., 2008,Mendoza et al., 2010,Watanabe, 2002b,Willette et al., 2008,Zhang et al., 2009), collectively suggesting that is also the most dominant isoform in this cell type. There is only one report for TRPV3 in endothelial cells (Earley et al., 2010) and TRPV2 has only been found in cultured vascular endothelial cells (Fantozzi et al., 2003). There are some reports that suggest that TRPV1 is present in vascular endothelium, however of these, three studies were conducted using cultured endothelial cells (Fantozzi et al., 2003,Golech et al., 2004,Yang et al., 2010) and another solely used immunohistochemistry (Bratz et al., 2008) with an antibody that produces non-specific staining (Everaerts et al., 2009). The final observation was made using RT-PCR in whole, chronically denervated arteries, with and without endothelium and although the endothelium was not isolated and tested specifically, TRPV1 mRNA expression was greater in vessels where the endothelium was kept intact, suggesting that TRPV1 was present in the endothelium (Bratz et al., 2008). However, TRPV1 mRNA was not found in the endothelium of rat middle cerebral arteries (Marrelli et al., 2007). In vascular smooth muscle, TRPV2 and TRPV4 appear to be the most abundantly expressed (Inoue et al., 2006,Yang et al., 2006); however, interestingly, TRPV4 mRNA was not present in mouse mesenteric arteries (Mendoza et al., 2010). TRPV3 expression in smooth muscle was the weakest (Inoue et al., 2006b,Yang et al., 2006) and was absent in mouse aorta (Muraki et al., 2003). TRPV1 may be expressed in vascular smooth muscle as mRNA for TRPV1 was found in de-endothelialized rat aorta and intralobular arteriers, although contamination of the sample by perivascular nerves or the endothelium was not examined (Yang et al., 2006). Other studies that have illustrated TRPV1 expression in vascular smooth muscle have done so using solely immunohistochemistry (Bratz et al., 2008,Kark et al., 2008), or by using cultured and not native cells (Wang et al., 2008). One study has shown that TRPV1 is not present in rat cerebral arteries (Marrelli et al., 2007).

Only TRPV1 and TRPV4 have been associated with peri-vascular innervation, although these observations were mainly based on immunohistochemistry (Benfenati et al., 2007,Gao and Wang, 2010,Scotland et al., 2004,Tanaka et al., 2008,Toth et al., 2005) and would have been more beneficial if they had been confirmed with PCR. Evidence for TRPV5 and TRPV6 expression is limited, but these TRPV isoforms were not expressed in rat mesenteric, cerebral and aortic smooth muscle (Inoue et al., 2006), rat intralobular pulmonary artery and aorta (Yang et al., 2006) or human pulmonary artery smooth muscle cells (Wang et al., 2008).

TRPV channels and endothelial cell function

TRPV expression has been found in various vascular endothelial cells (Table 1), however functional roles have only been demonstrated for TRPV1, TRPV3 and TRPV4, all of which result in vasoldilation. Upon stimulation, the endothelium produces many vasoactive substances, but the main vasodilators are NO, prostacyclin (PGI_2) and Endothelium-Derived Hyperpolarising Factor (EDHF) (Vanhoutte et al., 2009). EDHF has many identities and can differ between vascular beds (Edwards et al., 2010,Feletou and Vanhoutte, 2009), but for the purpose of this review, K^+ efflux from endothelial intermediate and small conductance potassium channels (IK_{Ca} and SK_{Ca} respectively) is an important EDHF mechanism that follows TRPV stimulation. The importance of IK_{Ca}/SK_{Ca} channel function has been highlighted in transgenic mice (Kohler and Ruth, 2010). As NO, PGI_2 and EDHF mechanisms can be calcium dependent, it is assumed that calcium influx through TRPV channels sufficiently activates these pathways.

TRPV1—As TRPV1 is expressed on endothelial cells, smooth muscle cells and peri-vascular nerves (Table 1), it is hard to distinguish true endothelial TRPV1 mediated responses. Further, endocannabinoids, which may also activate TRPV1, are known to activate many other receptors (CB1, CB2 and TRPV4), again making results difficult to interpret. Despite this, there is some evidence that shows capsaicin, a selective TRPV1 agonist, and endocannabinoids can affect vascular function through endothelial TRPV1 channels.

In rat skeletal muscle arteries, perivascular innervation appears to be absent, making skeletal muscle arteries an ideal preparation to study TRPV1 in vascular function (Kark et al., 2008). In this preparation, capsaicin (10 nM) produced endothelium-dependent vasodilations that were inhibited by L-NAME, an inhibitor of endothelial nitric oxide synthase (eNOS). Endothelial TRPV1 activation is therefore capable of causing vasodilation via a NO-dependent pathway. In porcine coronary artery endothelial cells, capsaicin (100 μM) induced calcium influx and relaxed arterial ring segments in a dose-dependent fashion. Capsaicin-induced relaxations were absent in endothelial denuded preparations and inhibited by the TRPV1 antagonist, capsazepine. Inhibition of eNOS with L-NAME, large conductance potassium channels (BK_{Ca}) with iberiotoxin and potassium channels in general with TEA (10 mM) reduced capsaicin-induced relaxations, suggesting that both NO and potassium channel activation is responsible for capsaicin-induced relaxations. Capsaicin, at concentrations above 1 μM, still produced a small relaxation in the presence of the inhibitors. Whether these effects were non-specific or due to an endothelium independent mechanism was not discussed (Bratz et al., 2008). In rat mesenteric arteries, Hoi et al., (2007) have shown that the effects of VSN16, a synthetic, cannabinoid-like compound, caused endothelium-dependent relaxations that were reduced by capsazepine. Responses were not completely TRPV1 dependent, as total inhibition only occurred in the presence of both capsazepine and O-1918, an antagonist of the novel endothelial ‘abnormal-cannabidiol receptor’ (see (Offertaler et al., 2003)). Relaxations were also reduced by inhibitors of eNOS (L-NNA), IK_{Ca}/BK_{Ca} (charybdotoxin) and SK_{Ca} (apamin), but not by cyclooxygenase

(COX) inhibition with indomethacin (Hoi et al., 2007). It is unknown whether VSN16 activates TRPV1 directly or downstream of the abnormal-cannabidiol receptor, but the study highlights that activation of endothelial TRPV1 is part of a VSN16-dependent vasodilatory pathway that is reliant on NOS and IK_{Ca}/SK_{Ca} activation. Work by Poblete et al., (2005) has also shown that TRPV1 activation can result in endothelial-dependent NO release. In a chronically denervated mesenteric vascular bed, capsaicin and anandamide perfusion through the lumen, led to endothelium-dependent NO production (measured from the perfusate) that was completely inhibited by capsazepine and L-NNA. A selective TRPV1 antagonist, SB366791 also reduced endothelium-dependent, capsaicin and anandamide-induced, NO release in non-denervated arteries, confirming that TRPV1 activation was partially responsible. Although the concentration of anandamide used (100 nM, to mimic physiological concentrations) was not high enough to elicit a vasodilation, the study illustrates that activation of the TRPV1 channel, either by anandamide or capsaicin, can lead to endothelial-dependent NO release (Poblete et al., 2005). As anandamide and its metabolites can activate TRPV4 (Watanabe et al., 2003), the effects of anandaminde on TRPV4 were ruled out by showing that 4α-PDD infusion did not cause significant NO production (Poblete et al., 2005). Perhaps the most convincing evidence for a functional role of TRPV1 in endothelial cells has been presented by Yang et al., (2010) who have shown that in cultured mouse aortic endothelial cells, capsaicin can increase intracellular calcium, increase the phosphorylation of eNOS and activate PKA. Capsaicin was also capable of producing endothelium-dependent vasodilation and NO production (measured using DAF-2DA fluorescence) in isolated mouse mesenteric arteries, both of which were inhibited by L-NAME and reduced in vessels from TRPV1−/− mice. This study is also the first to demonstrate that the blood pressure of spontaneously hypertensive (SHR) rats could be reduced by chronic dietary capsaicin treatment. The chronic capsaicin diet also seemed to improve endothelium function as mesenteric arteries form these animals and from mice that had also been chronically fed a capsaicin diet, responded more robustly to acetylcholine (ACh), a muscarinic receptor agonist, compared to normally fed controls. Improved ACh-mediated dilations did not occur in chronically capsaicin fed TRPV1−/−mice and were also inhibited by L-NAME. TRPV1, PKA and eNOS expression was also increased in tissue from chronically capsaicin fed animals. Although it can not be certain that endothelial TRPV1 is responsible for capsaicin-dependent reductions in blood pressure *in vivo*, as the channel is expressed throughout the body, the data from isolated cells and mesenteric arteries clearly show that TRPV1 is present and functional within the endothelium and that activation leads to calcium influx, NO production, PKA activation and dilation (Yang et al., 2010). TRPV1 channel activation may also lead to calcitonin gene-related peptide (CGRP) release, which has been demonstrated in HUVECs (Luo et al., 2008, Ye et al., 2007). Thermal activation of TRPV1 causes a capsazepine-sensitive increases CGRP expression in rat mesenteric and aortic endothelial cells (Ye et al., 2007). Whether endothelial-TRPV1 mediated CGRP release can regulate arterial tone is not known, but it can dilate vessels when released from sensory nerves (Scotland et al., 2004), see below. CGRP may help protect against cell death (Ye et al., 2007). One study has shown that in rat carotid endothelium there does not appear to be a role for TRPV1 in endothelium-dependent dilation as capsaicin failed to activate currents in isolated endothelial cell and dilate isolated vessels (Kohler et al., 2006). Collectively, these *in vitro* studies suggest that TRPV1 channels are present and functional in vascular endothelial cells and may modulate vascular function via the production of NO, CGRP and/or IK_{Ca}/SK_{Ca} channel activation in response to capsaicin, temperature or locally produced endocannabinoids.

It should be noted that TRPV1 may not be required for normal blood pressure regulation as there is no statistical difference between the baseline mean arterial pressures of TRPV1−/− and wild type mice (Pacher et al., 2004, Wang and Wang, 2009). Further, TRPV1 expression does not help to reduce deoxycorticosterone acetate-salt induced hypertension, as

blood pressures before and after deoxycorticosterone acetate-salt treatment were the same in wild type and TRPV1−/− mice, albeit TRPV1 might protect against deoxycorticosterone acetate-salt induced renal damage (Wang and Wang, 2009). Despite these observations, a role for TRPV1 in *in vivo* vascular control can not be completely ruled out as cinnamaldehyde (320 μmol/kg, i.v.), a TRPA1 agonist, caused a greater pressor effect on mean arterial pressure in TRPV1−/− mice compared to controls, which suggests that TRPV1 activation may oppose some vasoconstrictor mechanisms (Pozsgai et al., 2010).

TRPV3—To date there is only one investigation into TRPV3 and the endothelial control of vascular function (Earley et al., 2010). Earley and colleagues provide convincing evidence for a role of endothelial TRPV3 channels in rat cerebral arteries. Carvacrol, an activator of TRPV3 (and TRPA1) produced sensitizing whole cell currents in isolated endothelial cells. Currents were insensitive to the TRPA1 inhibitor, HC-030031, implicating TRPV3 as the source. In pressurized cerebral arteries (70 mmHg), carvacrol caused endothelium-dependent dilations, smooth muscle hyperpolarization, and reduced arterial wall calcium. The sensitivity of the TRPV3-induced dilations to TRAM-34, a selective IK_{Ca} blocker (Wulff et al., 2000), as well as to apamin and barium, inhibitors of SK_{Ca}, and smooth muscle inwardly rectifying potassium channels (Kir) respectively, suggests that TRPV3 activation increases cell calcium, activating IK_{Ca} and SK_{Ca}. This results in K^+ efflux, which activates smooth muscle Kir. Kir activation hyperpolarizes the smooth muscle cells and causes relaxation. There was no significant difference between carvacrol elicited dilations in the presence and absence of NOS and COX blockade, suggesting that NO and prostacyclin do not contribute to TRPV3 mediated dilations (Earley et al., 2010).

TRPV4—Endothelium-dependent TRPV4 channels regulate vascular function by stimulating NO, PGI_2 and EDHF production. The polymodal nature of TRPV4 allows activation by many endogenous stimuli, namely, shear stress, heat, hypotonic environments, Gq signaling, and directly via EETs.

Heterologously expressed TRPV4 channels can be activated fairly selectively by 4αPDD (EC_{50}; 0.2 μM mTRPV4) (Watanabe, 2002a), GSK1016790A (EC_{50}; 18 nM mTRPV4) (Thorneloe et al., 2008), RN-1747 (EC_{50}; 4 μM r/mTRPV4) (Vincent et al., 2009), arachidonic acid metabolites (EC_{50} of 5,6-EETs; 130 nM mTRPV4) (Watanabe et al., 2003) and bisandrographolides (EC_{50}; 950 nM mTRPV4)(Smith et al., 2006). Regarding specificity, it should be noted that GSK1016790A and RN-1747 can also activate TRPV1 (Vincent et al., 2009, Willette et al., 2008) and 4αPDD can activate a non-specific current in non-transfected TRPV4 cells (Thorneloe et al., 2008). Pharmacological activation of TRPV4 leads to profound dilations in mouse mesenteric arteries (Kohler et al., 2006, Mendoza et al., Earley et al., 2009) mouse carotid artery (Loot et al., 2008), rat aortic rings (Willette et al., 2008) and rat carotid artery (Kohler et al., 2006). TRPV4 mediated dilations are absent in TRPV4 −/− mice (Mendoza et al., Earley et al., 2009, Hartmannsgruber et al., 2007). Activation of TRPV4 increases whole cell currents (Kohler et al., 2006, Hartmannsgruber et al., 2007) and calcium influx (Zhang et al., 2009, Mendoza et al., Watanabe, 2002b, Wu et al., 2009, Kohler et al., 2006).in isolated endothelial cells; these responses are blocked by ruthenium red.

NO, PGI_2 and EDHF are vasodilatory pathways that are activated following TRPV4 stimulation (Earley et al., 2009, Kohler et al., 2006, Mendoza et al., 2010, Willette et al., 2008). In mouse mesenteric arteries, GSK1016790A -induced dilations were reduced from 67 % to 27 % following eNOS inhibition. The residual dilation was completely abolished upon the subsequent addition of high K^+ containing solutions (Mendoza et al., 2010). GSK1016790A also failed to cause vasodilation of mouse aortic rings from eNOS −/− mice (Willette et al., 2008). In rat carotid artery, joint NOS and COX inhibition reduced 4α-PDD

induced dilations from 70 to 10 %, but was surprisingly ineffective in the resistance artery, A. gracilis, where a robust 4α-PPD induced dilation still remained in the presence of L-NNA and indomethacin (Kohler et al., 2006). As well, perfusion of 4α-PDD through the rat mesenteric vasculature did not lead to NO production (Poblete et al., 2005). Earley et al., (2009) showed that 11, 12-EET induced dilations still remained after NOS and COX inhibition in mouse mesenteric arteries and required inhibition of endothelial IK_{Ca} and SK_{Ca} channels to reduce dilations by 50 % (Earley et al., 2009). The remaining 50 % of the response was due to TRPV4 mediated effects on the smooth muscle. TRPV4 responses to 4αPDD (and HTS) may also require PLA_2 signaling as inhibitors of PLA_2 and cytochrome P450 epoxygenase dramatically reduced 4αPDD-induced currents in TRPV4 expressing oocytes. It was speculated that threshold calcium entry through TRPV4 is needed to bind to and activate PLA_2, which produces EETs to activate/augment TRPV4 activity (Loukin et al., 2010).

Endothelial cells are in constant contact with the shear forces induced by the circulating blood. Increases in shear stress, arising from increases in blood flow or blood viscosity, are sensed by the endothelium, which then modulates vascular tone accordingly, dilating vessels to reduce intra-luminal shear force. Endothelial TRPV4 channels are involved in shear stress induced vasodilation. In gracilis muscle arterioles, shear stress (an increase of 2 dyn/cm2) causes a ruthenium red sensitive dilation (Kohler et al., 2006). In the mouse carotid artery, an increase in the shear stress, from 3 to 7 dyn/cm^2, induces a 40 % dilation which does not occur in TRPV4 −/− mice (Hartmannsgruber et al., 2007). Flow induces a 30 % dilation in mouse carotid arteries, that is much lower in TRPV4−/− mice (15.5 %) (Hartmannsgruber et al., 2007) and a 49 % dilation in mouse mesenteric arteries that is reduced to 24 % in TRPV4−/− vessels (Loot et al., 2008).

Shear stress-induced activation of TRPV4 dilates vessels via the subsequent production of NO, PGI_2 and EDHF, however the relative contribution of each differs between vessel types: Shear stress induced dilations were completely dependent on NO in rat carotid artery. Inhibition of NOS with L-NNA reduced dilations to less than 5 % (Kohler et al., 2006). In gracilis muscle arterioles, shear stress (10 dyn/cm^2) induced dilation was again, almost completely inhibited by NOS blockade (Kohler et al., 2006). Mendoza et al., (2010) showed that both NOS and high K^+ solutions were required to block the shear stress mediated dilation in mouse mesenteric arteries, whereas COX inhibition had no effect (Figure 1). In the mouse carotid artery, inhibitors of IK_{Ca} and SK_{Ca} channels were required to inhibit the remaining shear stress induced vasodilation after NOS and COX inhibition (Hartmannsgruber et al., 2007). Loot et al., (2008) found that EDHF was the primary mechanism by which shear stress activation of TRPV4 caused dilation. In mouse carotid arteries, in the absence of NOS and COX inhibition, flow induced dilations were similar between WT and TRPV4−/− mice. After the inhibition of NOS and COX, the remaining dilation, presumably due to EDHF, was absent in TRPV4 −/− mice. Thus, shear stress leads to endothelium-dependent, TRPV4 mediated vasodilation, mainly via NO and EDHF production.

Shear stress-TRPV4 mediated dilations appear to occur downstream of PLA_2 and are not due to direct channel activation. Inhibition of PLA_2, inhibited shear stress responses in gracilis muscle arterioles and in mouse carotid artery (Hartmannsgruber et al., 2007, Kohler et al., 2006) and were independent of PKC and tyrosine kinase (Kohler et al., 2006). Within the cell membrane, PLA_2 converts membrane phospholipids to arachidonic acid, which in turn is metabolized by Cytochrome P450 epoxygenase activity to various epoxyeicosatrienoic acid products (5,6-EET, 8,9-EET, 11,12-EET). Vriens et al., (2004) showed that inhibition of PLA_2 and cytochrome P450 epoxygenase prevents hypotonic-induced calcium responses in TRPV4 expressing HEK cells. In mouse carotid arteries, Loot

et al., (2008) also showed that inhibition of CYP epoxygenase with MS-PPOH, reduced the EDHF mediated dilation that only occurred in wild type mice. No further dilation could be elicited by the sequential addition of ruthenium red; suggesting that CYP epoxygenase and TRPV4 are part of the same pathway for EDHF mediated dilation.

Activation of endothelial muscarinic receptors is an effective vasodilatory pathway resulting in NO, PGI_2 and EDHF release. Downstream activation of TRPV4 and subsequent calcium influx mediates this response in some tissues. Although there was no difference between ACh mediated dilations of mouse carotid arteries from WT and TRPV4 −/− mice (Hartmannsgruber et al., 2007, Loot et al., 2008), and ruthenium red had no affect on ACh responses in gracilis muscle arterioles (Kohler et al., 2006), ACh-induced responses were partially inhibited by ruthenium red in mouse carotid artery (Kohler et al., 2006). Furthermore, ACh-mediated dilations were blunted in TRPV4 −/−mouse mesenteric arteries, reduced from an 88 % dilation in wild-type arteries to 49 % in TRPV4 −/− arteries. In TRPV4 −/− arteries the remainder of the dilation was inhibited by L-NAME implying that ACh-TRPV4 activation led to EDHF release in WT. The NO component of the ACh response, measured using DAF fluorescence, in carotid arteries was also less in the TRPV4−/− mice (Zhang et al., 2009). Earley et al., (2009) also concluded that EDHF was responsible for ACh-TRPV4 mediated dilations in mouse mesenteric arteries. In vessels incubated with L-NAME and indomethacin, ACh still induced endothelium-dependent dilation and smooth muscle hyperpolarization. This residual EDHF-mediated dilation was reduced by 75% in vessels from TRPV4 −/− mice (Earley et al., 2009). EDHF and NO components of a carbachol induced relaxation were both reduced in mouse mesenteric arteries from TRPV4−/− mice compared to WT (Saliez et al., 2008). Thus, muscarinic receptor activation leads to a downstream activation of TRPV4 that is responsible for the release of NO and EDHF.

Similar to shear-stress effects, muscarinic-dependent activation of TRPV4 may require PLA_2. Marrelli et al., (2007) suggest that UTP mediated vasodilation is a result of activating TRPV4 via PLA_2 signaling. UTP caused calcium influx in the endothelial cells of pressurized rat middle cerebral arteries, which was partially reduced by the individual application of ruthenium red and a PLA_2 inhibitor. However, since the compounds were never added together, which would be necessary to assess whether the effect was additive, it is not clear whether they were part of the same mechanism.

It is becoming increasingly apparent that endogenous TRPV4 agonists exist, including various EETs and anandamide (Watanabe et al., 2003). Earley et al., (2009) showed that 11,12-EET dependent dilation and smooth muscle cell hyperpolarization, in mouse mesenteric arteries, is partially the result of endothelium-dependent TRPV4 activation. Responses were absent in vessels from TRPV4 −/− mice and blocked by inhibitors of TRPV4 and IK_{Ca}/SK_{Ca} channels (Earley et al., 2009). 5,6-EET increased ruthenium red sensitive currents in whole cell and inside out patches of mouse aorta endothelial cells (Watanabe et al., 2003). Arachidonic acid, 5,6-EET and 8,9-EET increased cell calcium in native mouse carotid artery endothelial cells (Vriens et al., 2005). Responses to 5,6-EET and 8,9-EET were completely TRPV4 dependent as there was no effect in cells from TRPV4 −/− mice, and the effect of arachidonic acid was reduced by ~50 % in TRPV4 −/− mice. As would be predicted, modulation of CYP2C9 also altered the response to arachidonic acid - CYP2C9 inhibition with sulfaphenazole reduced the response, whereas increased CYP2C expression led to enhanced arachidonic acid responses. Responses to arachidonic acid and EETs were also increased upon inhibition of EET degradation. Arachidonic acid also increased endothelial cell calcium and dilated pressurized MCAs from rats (Marrelli et al., 2007).

In accordance with the temperature and osmotic sensitivity of TRPV4, endothelial cells respond to changes in temperature and HTS. In native mouse carotid endothelial cells, HTS produced whole-cell currents that were reduced by ~70 % in TRPV4−/−animals (Hartmannsgruber et al., 2007). HTS also stimulated TRPV4-like whole-cell currents in freshly isolated endothelial cells from rat carotid artery which were inhibited by PLA_2 inhibition (Kohler et al., 2006), which in mammalian cells (albeit, not in yeast) is indicative of TRPV4 ((Loukin et al., 2009), Loukin et al., 2010, Vriens et al., 2004),. HTS also increased intracellular calcium levels in isolated mouse aortic endothelial cells, a response that was much reduced in TRPV4 −/− mice (Vriens et al., 2005). TRPV4-like responses to heat have also been observed in native mouse aortic endothelial cells (Watanabe, 2002b, Vriens et al., 2005) which were reduced in TRPV4 −/− mice (Vriens et al., 2005). Kohler et al., (2006) showed that raising temperature to 37 °C increased cell calcium in rat carotid artery endothelial cells and attributed the phenomena, based on pharmacological experiments in the intact vessel, to TRPV4. Although *in vivo* temperature and tonicity are maintained relatively constant, TRPV4-mediated responses to changes in these parameters may be important during times of extreme heat or osmotic stress.

Evidence by Marrelli et al. (2007) shows that calcium influx in endothelial cells occurs mainly at the side of the endothelial cell closest to the smooth muscle, and not the luminal side. Only abluminally applied Mn^{2+}, which would presumably be able to reach the side of the endothelial cell closest to the smooth muscle, could quench the Fura-2 signal in the endothelial cells of pressurized rat middle cerebral arteries. Although it can not be concluded definitively that TRPV4 was responsible, as effects of TRPV4 inhibitors on Mn^{2+} quench were not studied, the response was reduced by PLA_2 inhibition. TRPV4 was also co-localized with caveolae, which are cholesterol rich domains that attract many signaling molecules and ion channels. In HUVECS, TRPV4 and Cav-1 (a structural protein of caveolae) co-immunoprecipitate (Saliez et al., 2008). The Cav-1 −/−mouse also showed reduced gap junction protein (connexins 37, 40 and 43) expression. As these proteins can form myoendothelial gap junctions, the data suggest that TRPV4 channels that are associated with Cav-1 are also located close to myoendothelial gap junctions. The localization of TRPV4 at the smooth muscle face of endothelial cells is in accordance with its role as a vasodilatory mediator. The close proximity to the smooth muscle would allow easy access of TRPV4-associated, endothelial derived vasodilators to the neighboring smooth muscle, either via the gap junctions or by diffusion.

Evidence summarized above indicates that production of endothelial-dependent vasodilators can be TRPV4-dependent. Therefore, it may be assumed that the channel is of substantial importance for normal vascular function *in vivo*. However, TRPV4−/−mice actually have similar resting blood pressures when compared to wild-type mice (Zhang et al., 2009, Earley et al., 2009) and a number of studies investigating gain of function mutations in humans do not report any cardiovascular complications (Landoure et al., 2010, Rock et al., 2008, Zimon et al., 2010). However the channel is functional since activation of TRPV4 *in vivo*, reduces blood pressure (Gao et al., 2009, Gao and Wang, 2010, Willette et al., 2008), possibly via NO and IK_{Ca}/SK_{Ca}-dependent pathways (Gao and Wang, 2010, Willette et al., 2008), as well as leading to circulatory collapse (Willette et al., 2008). Interestingly, L-NNA induced hypertension is greater in TRPV4−/−versus wild-type mice (Earley et al., 2009) and salt-induced hypertensive rats are more sensitive to ruthenium red treatment compared to normotensive controls (Gao et al., 2009). Thus, it seems plausible that TRPV4 function becomes necessary during times of excessive vasoconstricting stimuli, allowing the vasculature to respond appropriately during times of stress.

TRPV channels and vascular smooth muscle function

TRPV channels also play key roles in vascular smooth muscle cell function. Amongst the TRPV channels, TRPV2 and TRPV4 are the most likely candidates for vasomotor roles in the vascular smooth muscle cells per se.

TRPV2—TRPV2 channels, as indicated by measurement of message and protein, are present in rat (Inoue et al., 2006) and mouse (Muraki et al., 2003) mesenteric, cerebral, and aortic myocytes. Muraki et al., (2003) demonstrated that cell swelling following exposure to hypotonic bath solution activates a nonselective cation current and increases intracellular Ca^{2+} levels in mouse aortic myocytes. These responses are suppressed by ruthenium red, a TRPV channel inhibitor, and by treatment of isolated myocytes with TRPV2 antisense oligonucleotides. TRPV2-like currents, membrane depolarization, and increased intracellular [Ca^{2+}] were induced by membrane stretch in CHO cells transfected with TRPV2. These findings are consistent with the general observation that TRPV2 channels are mechanosensitive (O'Neil and Heller, 2005). Muraki et al. (Muraki et al., 2003) speculate that TRPV2 channel activation and Ca^{2+} entry following myoctye stretch could contribute to the myogenic vasoconstrictor responses of resistance arteries. However, functional evidence of a vasoconstrictor role for TRPV2 channels in intact blood vessels has not been reported.

TRPV2 channels in mesenteric artery myocytes are activated by endogenous cannabinoids and may contribute to the vasodilator response evoked by these substances (Qin et al., 2008). Further, TRPV2 channels in vascular smooth muscle cells appear to be activated by growth factors and contribute to vascular smooth muscle migration (Stokes et al., 2005).

TRPV4—Recent studies support the hypothesis that TRPV4 channels located in vascular smooth muscle are important mediators of vasodilation induced by epoxyeicosatrienoic acids, which are one type of endothelium-derived hyperpolarizing factor (Campbell et al., 1996). TRPV4 is clearly present in smooth muscle cells in the mesenteric and cerebral vascular beds (Marrelli et al., 2007, Earley et al., 2005) and in aortic myocytes (Tanaka et al., 2008). Evidence for the functional role of TRPV4 in vascular smooth muscle comes from studies of cerebral (Earley et al., 2005) and mesenteric (Earley et al., 2009) resistance arteries. EETs activate TRPV4 channels in myocytes isolated from these arteries (Figure 2) (Earley et al., 2005, Earley et al., 2009). Subsequent Ca^{2+} influx through the EETs-activated TRPV4 channels (which have substantial permeability to Ca^{2+}; (Voets et al., 2002)) induces the release of Ca^{2+} through ryanodine receptors located on the sarcoplasmic reticulum. These Ca^{2+} release events, termed Ca^{2+} sparks (Nelson et al., 1995), then activate nearby voltage-activated, BK_{Ca} channels in the sarcolemma and increase the frequency of macroscopic outward K^+ currents (STOCs). The increased BK_{Ca} current activity causes smooth muscle hyperpolarization and vasodilation. These studies support the hypothesis that TRPV4 is part of a Ca^{2+} signaling complex that includes ryanodine receptors and BK_{Ca} channels and also suggest that the TRPV4 channel serves as a receptor molecule for EETs in vascular smooth muscle. TRPV4 activity in vascular myocytes also contributes to regulation of peripheral vascular resistance *in vivo* (Earley et al., 2009). This study demonstrated that elevations in blood pressure to a hypertensive challenge are exaggerated in TRPV4−/− mice, indicating that TRPV4 channels are involved in a negative feedback mechanism that limits the response to hypertensive stimuli. The proposal that vascular TRPV4 channels may play significant roles in blood pressure regulation is supported by a recent study demonstrating the substantial hypotensive responses to activators of TRPV4 in normotensive and salt-loaded, hypertensive rats (Gao et al., 2009).

TRPV channels and neurovascular control

TRPV1—TRPV1 channels are involved in neurally mediated vasodilation. Stimulation of perivascular sensory nerves dilates isolated mesenteric arteries and this response is mediated by CGRP (Han et al., 1990). TRPV1 channels in the perivascular nerves are involved in this response as evidenced by an absence of neurally-evoked CGRP release and dilation of mesenteric arteries from TRPV1 null mice. (Wang et al., 2006). Similarly, hindlimb cutaneous vasodilation *in vivo* following spinal cord stimulation is absent in rats treated with resiniferotoxin, which desensitizes TRPV1 receptors, suggesting that TRPV1 channels in sensory nerves are important mediators of neurogenic vasodilation (Wu et al., 2006). TRPV1 channels are also expressed in afferent neuronal baroreceptor pathways. Recent results indicate that baroreflex signaling is compromised when TRPV1 channels are inhibited (Sun et al., 2009). In this study resiniferotoxin eliminated TRPV1 immunoreactivity in barroreceptor signaling pathways. This apparent ablation of TRPV1 channels in the barroreceptor afferents was associated with substantial impairment of the normal barroreflex in response to increases in mean arterial pressure. These results suggest that TRPV1 channels are potential mechanoreceptors that sense changes in arterial pressure and play a key role in blood pressure homeostasis. A study by Scotland and colleagues (Scotland et al., 2004) suggests that TRPV1 channels in mesenteric perivascular sensory nerves contribute to a mechanism of myogenic tone that involves release of Substance P. Ablation of TRPV1 in vascular sensory fibers, block of 20-HETE production, or block of NK1 receptors inhibited myogenic tone. These findings lead the authors to propose that increased intravascular pressure enhances 20-HETE release from vascular smooth muscle cells which then activates sensory TRPV1 channels, triggering neuronal Ca^{2+} entry and release of Substance P, which then causes smooth muscle contraction. However, although the data in this study are provocative, they are inconsistent with numerous previous reports indicating no direct role of periarterial nerves in vascular myogenic behavior (Bayliss, 1902, Johansson, 1989).

TRPV4—As reviewed above, TRPV4 channels are widely distributed in the cardiovascular system, found both in endothelial cells and vascular smooth muscle cells. Like TRPV1, TRPV4 channels are also found in perivascular sensory nerves and may contribute to the hypotensive response to activation of TRPV4 channels *in vivo* (Gao et al., 2009, Gao and Wang, 2010). Similar to the situation involving TRPV1 channels in mesenteric periarterial sensory nerve fibers, activation of neuronal TRPV4 channels appears to be coupled to release of CGRP and subsequent mesenteric artery dilation.

TRPV4 channels may also contribute to increased sympathetic nervous activity and blood pressure associated with hyposmotic conditions in the hepatic portal circulation (McHugh et al., 2010). These studies indicate that TRPV4 channels play a role as a hepatic portal osmoreceptor. The authors found that increased blood pressure after water ingestion in wild type mice is absent in TRPV4 null mice. Administration of isosmotic saline did not elicit an increase in BP, suggesting osmolality as the stimulus. The authors propose the osmopressor response to water, and involvement of TRPV4 represent new factors to be considered in the physiology of blood pressure regulation.

Conclusion

The available evidence strongly supports the proposal that TRPV channels modulate vascular endothelial and smooth muscle cell function, and perivascular neuronal activity (Figure 3). The presence of TRPV1, TRPV3 and TRPV4 in the vascular endothelium permits vasodilatory responses to a variety of stimuli including endocannabinoids (TRPV1), dietary agonists (TRPV3), shear stress, Gq-protein coupled receptor activation and EETs

(TRPV4). Endothelial TRPV-mediated Ca^{2+}-entry causes dilations by increasing NO, PGI2, or IK_{Ca}/SK_{Ca} channel activity. TRPV2 and TRPV4 are present in smooth muscle cells in numerous vascular beds. TRPV2 channels in aortic smooth muscle may be activated by mechanical stimulation, and Ca^{2+}-entry through smooth muscle TRPV4 channels activates ryanodine receptors and BK channels, leading to vasodilation. Activation of TRPV1 or TRPV4 channels in perivascular sensory nerves induces release of CGRP which causes vasodilation. Although, as alterations in TRPV1 and TRPV4 expression do not affect resting blood pressure (Earley et al., 2009,Landoure et al., 2010,Pacher et al., 2004,Rock et al., 2008,Wang and Wang, 2009,Zhang et al., 2009,Zimon et al., 2010) these channels may not be required for day-today regulation of blood pressure, but may provide useful pathways to counteract vasoactive stimuli (Earley et al., 2009,Gao et al., 2009,Pozsgai et al., 2010). While disturbances in TRPV channel expression, structure, or activity in cells that encompass the vascular wall would be predicted to be associated with cardiovascular pathology, to date the only known TRPV channelopathies have been linked to bone disorders (Mizoguchi et al., 2008). Transient receptor potential vanilloid 4 deficiency suppresses unloading-induced bone loss (Mizoguchi et al., 2008) and neurodegenerative skeletal muscle dyscrasias (Auer-Grumbach et al., 2010). Mutations in the N-terminal ankyrin domain of TRPV4 cause congenital and scapuloperoneal spinal muscular atrophy, and hereditary motor and sensory neuropathy 2C (Auer-Grumbach et al., 2010).

Nevertheless, it seems likely that development of agents that selectively modulate vascular TRPV channel function will prove useful in treatment of vascular diseases, particularly those associated with elevated vasomotor tone such as hypertension and vasospasm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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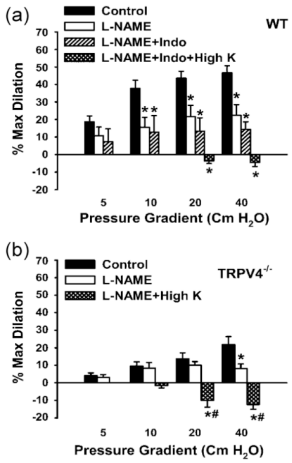

Figure 1.

Shear stress induced dilations are TRPV4 dependent and require NO and EDHF production. (a) In pressurized mouse mesenteric arteries, shear stress induced dilations were achieved by increasing luminal flow (by increasing pressure). L-NAME (100 μM) inhibited responses. Indomethacin (10 μM) produced no additional inhibition, but the addition of high K+ successfully prevented dilations. (b) In TRPV4−/− mice, shear stress induced dilations were less than wildtype (A) and also inhibited by L-NAME and high K+. (Data are from Mendoza et al., 2010. Am J Physiol Heart Circ Physiol. “Am Physiol Soc, with permission”).

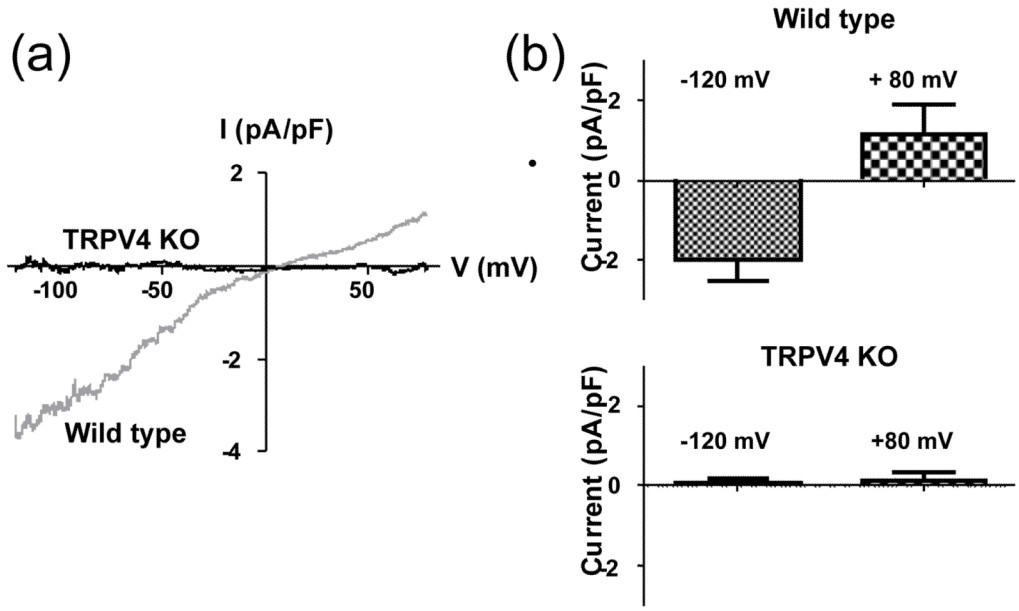

Figure 2.

Ionic currents activated by 11–12 EET are absent In vascular smooth muscle cells from TRPV4 null mice. (a) Examples of whole-cell currents (difference currents) activated by 11,12 EET (3 μmol/L) in mesenteric arterial smooth muscle cells isolated from WT and TRPV4 KO mice. (b) Summary data for 11,12 EET-activated currents in WT (n=5) and TRPV4 KO (n=3) mouse mesenteric artery myocytes. *:P<0.05 vs. WT. (Data are from Earley et al., 2009. Am J Physiol Heart Circ Physiol. “Am Physiol Soc, with permission”)

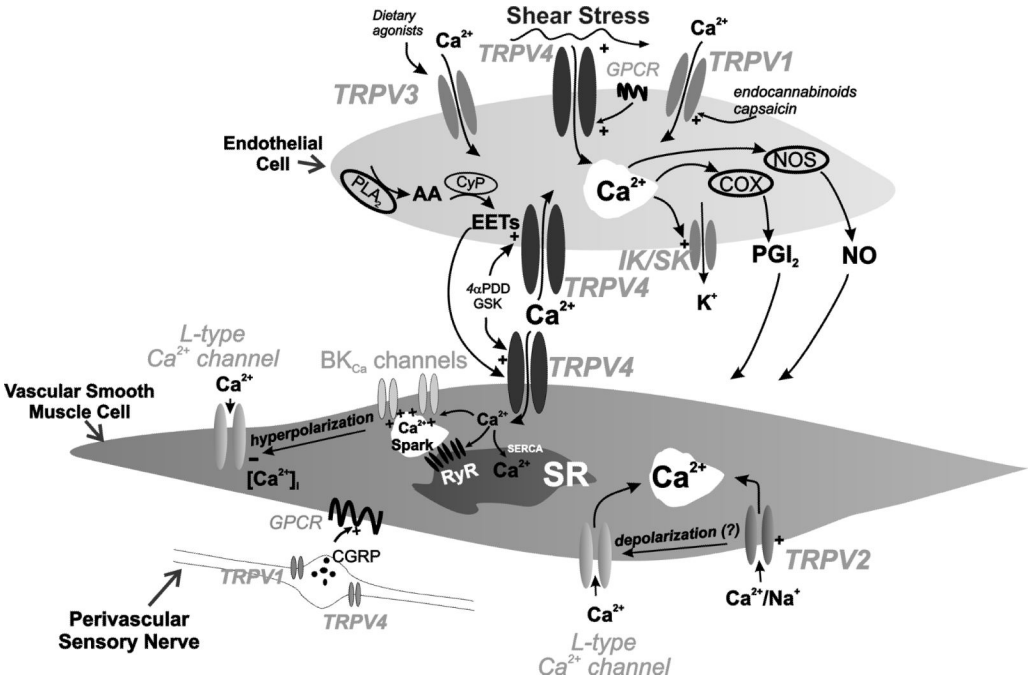

Figure 3.

Diversity of mechanisms by which vascular TRPV channels are regulated. In endothelial cells, TRPV2 can be activated by endocanabinoids and pharmacologically by capsaicin, TRPV3 by the dietary agonist, carvacrol, and TRPV4 by shear stress, GPCR activation, EETs and pharmacologically by GSK1016790A (GSK) and 4αPDD. Activation leads to calcium influx and the differential activation of NOS, COX and IKCa (IK)/SKCa (SK) channels to cause vasodilation. In the smooth muscle, activation of TRPV4 by endothelial derived EETs leads to calcium influx, stimulation of calcium sparks, subsequent activation of BKCa, K+ efflux and smooth muscle cell hyperpolarisation. TRPV2 channels in the smooth muscle mediate calcium entry and aid constriction. Activation of TRPV2 and TRPV4 in perivascular nerves leads to the release of CGRP (calcitonin-gene-related-peptide) and vasodilation. Abbreviations: AA (arachidonic acid), CyP (cytochrome P450 epoxygenase), PLA2 (phospholipase A2), EETs (epoxyeicosatrienoic acids), NO (nitric oxide), PGI2 (prostacyclin), eNOS (endothelial nitric oxide synthase), COX (cyclooxygenase), large/intermediate/small conductance potassium channel (BKCa/IKCa/SKCa respectively), GPCR (G-protein coupled receptor), SR (sarcoplasmic reticulum), RyR (ryanodine receptor), SERCA (sarco/endoplasmic reticulum Ca2+-ATPase).

Table 1

Expression of TRPV channels in peri-vascular nerves, vascular endothelial cells and smooth muscle cells.

Channel	Cell Type	Analysis	Reference
Peri-vascular nerves			
TRPV1	Mouse mesenteric arteries	IHC	(Scotland et al., 2004)
	Astrocytes surrounding brain capillaries and larger arteries	IHC	(Tóth et al., 2005)
TRPV4	Cultured rat cortical astroctyes	RT, W, IHC^{IP}	(Benfenati et al., 2007)
	Rat mesenteric arteries	IHC	(Gao and Wang, 2010)
Smooth muscle			
TRPV1	Rat intralobular artery and aorta	RT^{d}, W	(Yang et al., 2006)
	Rat skeletal muscle arteriole	IHC	(Kark et al., 2008)
	Cultured human pulmonary artery smooth muscle cells	RT, W	(Wang et al., 2008)
	Swine coronary artery	IHC	(Bratz et al., 2008)
TRPV2	Rat intralobular artery and aorta	RT^{d}	(Yang et al., 2006)
	Rat aorta, mesenteric and cerebral arteries	RT	(Inoue et al., 2006)
	Mouse aorta, mesenteric, basilar artery	RT^{i}, IHC^{IP}	(Muraki et al., 2003)
	Cultured human pulmonary artery smooth muscle cells	RT	(Wang et al., 2008)
TRPV3	Rat intralobular artery and aorta	RT^{d}	(Yang et al., 2006)
	Cultured human pulmonary artery smooth muscle cells	RT	(Wang et al., 2008)
TRPV4	Rat intralobular artery and aorta	RT^{d}, W^{d}	(Yang et al., 2006)
	Rat aorta, mesenteric and cerebral arteries	RT	(Inoue et al., 2006)
	Cultured human pulmonary artery smooth muscle cells	RT	(Wang et al., 2008)
	Mouse aorta artery	RT^{i}	(Muraki et al., 2003)
	Rat cerebral artery	RT^{AS}	Earley et al 2005
	Rat aortic freshly isolated and cultured myoctes	RT^{i}, IHC^{i}	(Tanaka et al., 2008)
	Rat middle cerebral artery	RT^{w}, IHC	(Marrelli et al., 2007)
	Mouse aorta	IHC*	(Zhang et al., 2009)
Endothelial cells			
TRPV1	Cultured cerebrovascular endothelial cells	RT, IHC	(Golech et al., 2004)
	Denervated rat mesenteric arteries	RT$^{\wedge}$	(Poblete et al., 2005)
	Swine coronary artery	IHC, RT	(Bratz et al., 2008)
	Cultured human pulmonary endothelial cell		(Fantozzi et al., 2003)
	Cultured mouse aortic endothelial cells	RT*, W*	(Yang et al., 2010)
TRPV2	Cultured human pulmonary endothelial cell	RT	(Fantozzi et al., 2003)
TRPV3	Rat cerebral arteries	RT^{w}, IHC	(Earley et al., 2010)
TRPV4	Rat mesenteric arteries	IHC	(Gao and Wang, 2010)
	Cultured human pulmonary endothelial cell	RT	(Fantozzi et al., 2003)
	Rat carotid artery	RT^{i}	(Kohler et al., 2006)
	Cultured human pulmonary endothelial cell	RT	(Fantozzi et al., 2003)

Channel	Cell Type	Analysis	Reference
	Rat middle cerebral artery	RT^{i}, IHC	(Marrelli et al., 2007)
	Mouse carotid artery	IHC*	(Hartmannsgruber et al., 2007)
	Mouse mesenteric	RT^{i}, HC*	(Mendoza et al., 2010)
	Mouse aorta	IHC*	(Zhang et al., 2009)

RT; RT-PCR, W; western blot, IHC; immunohistochemisty,

*knock-out control,

AS anti-sense oligonucleotides used as control,

IP immunizing peptide control,

^ denervated arteries, +/− endothelium,

w whole artery,

d deendothelialized,

i isolated cells.