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TRP channel activation and endothelium-dependent dilation in the systemic circulation

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Abstract

The endothelium plays a crucial role in the regulation of vascular tone by releasing a number of vasodilator mediators, including nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factor(s) (EDHF). The production of these mediators is typically initiated by an increase in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in endothelial cells. An essential component of this Ca^{2+} signal is the entry of Ca^{2+} from the extracellular space through plasma membrane Ca^{2+} -permeable channels. Although the molecular identification of the potential Ca^{2+} entry channel(s) responsible for the release of endothelial relaxing factors is still evolving, accumulating evidence indicates that the transient receptor potential (TRP) channels, a superfamily of Ca^{2+} -permeable cation channels, serve as an important mechanism of Ca^{2+} entry in endothelial cells and other non-excitable cells. The activation of these channels has been implicated in diverse endothelial functions ranging from control of vascular tone, regulation of vascular permeability, to angiogenesis and vascular remodeling. This review summarizes the most recent evidence concerning TRP channels and endothelium-dependent dilation in several systemic vascular beds. In particular, we highlight the emerging roles of several TRP channels from the canonical and vanilloid subfamilies, including TRPV4, TRPC4 and TRPC6, in vasodilatory responses to shear stress and receptor agonists, and discuss potential signaling mechanisms linking TRP channel activation and the initiation of EDHF responses in endothelial cells.

Keywords

transient receptor potential; endothelium-derived hyperpolarizing factor; endothelium; calcium signaling

Introduction

The endothelium regulates vascular tone through the release of various vasoactive factors, including nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor(s) (EDHF).¹ These mediators are generated in response to receptor activation (e.g. bradykinin and acetylcholine) as well as to mechanical stimulation such as shear stress. A common pathway for these stimuli involves an increase in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$), which typically consists of an initial rapid Ca^{2+} transient due to Ca^{2+} release from internal

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Conflicts of interest None

IP₃ and/or ryanodine-sensitive stores, followed by a sustained elevation of [Ca²⁺]_i mediated by Ca²⁺ entry (presumably via Ca²⁺-permeable channels) from the extracellular space.³ The shape and duration of the Ca²⁺ entry may vary between different endothelial cell types and stimuli; however, it is thought that by prolonging [Ca²⁺]_i elevation, Ca²⁺ entry facilitates or is prerequisite for the release of vasodilator factors in endothelial cells.² Despite the importance of this Ca²⁺ entry in endothelial functions, the molecular identity of the responsible Ca²⁺ channel(s) remains elusive.

During the last 15 years, the discovery and study of the transient receptor potential (TRP) channels, a new superfamily of Ca²⁺-permeable cation channels, have provided exciting new insights into the mechanisms of Ca²⁺ entry in endothelial and various other non-excitable cell types. These TRP channels has been implicated in diverse endothelial functions such as control of vascular tone, regulation of vascular permeability, mechanosensing, oxidative stress response, angiogenesis, and vascular remodeling.^{3,4} The purpose of this review is to summarize our current knowledge regarding the role of TRP channels in endothelium-dependent dilation, with an emphasis on EDHF-mediated dilation. Several TRP channels from the canonical and vanilloid subfamilies, especially the vanilloid type 4 (TRPV4) channel, will be discussed.

Endothelial TRP channels

The mammalian TRP superfamily of cation channels, whose founding member is the *Drosophila* Trp, can be organized by their sequence homology into six subfamilies: TRPC ('Canonical'), TRPV ('Vanilloid'), TRPM ('Melastatin'), TRPA ('Ankyrin'), TRPML ('Mucolipin'), and TRPP ('Polycystin').⁵ The functional channels are assembled from four subunits as homo- or hetero-tetramers, with each subunit containing six putative transmembrane domains (S1–6) with a pore-forming loop between S5 and S6, and cytoplasmic NH₂- and COOH-terminus. All functionally characterized TRP channels are Ca²⁺ permeable with a few exceptions, although most of these channels are only poorly selective for Ca²⁺ over others cations such as Na⁺. Besides plasma membrane localization, several TRP channels may reside on intracellular membranes and function as Ca²⁺ release channels.⁵

To date, more than 20 mammalian TRP channels have been identified, and many of these channels, from all subfamilies except for TRPML (probably restricted to intracellular vesicles), are found in vascular endothelial cells.⁴ The expression profile of TRP channels seems to vary between different endothelial cells from different species and vascular beds. It is important to note that many early studies were performed on cultured endothelial cells, which may not express the same profile of TRP channels as intact vessels.⁴ Many TRP channels, especially from the TRPC, TRPV and TRPM subfamilies, are also expressed in vascular smooth muscle cells.⁶ This may create difficulties in data interpretation when modulating TRP channels in blood vessels if the same TRP channel is present in both endothelial and smooth muscle cells.

As a well characterized TRP channel in endothelial cells, the expression and channel activity of TRPV4 have been examined by several groups in multiple species and vascular beds. Using immunohistochemistry and/or RT-PCR analysis of freshly isolated vascular cells, several studies have shown that TRPV4 proteins and/or mRNA are predominantly expressed in endothelial cells of mouse aorta, carotid and mesenteric arteries,^{7,8,9} and rat carotid arteries.^{10,11} In additional, removal of the endothelium abolished TRPV4-mediated vasodilation in mouse mesenteric arteries and rat aorta, further indicating that the potential contribution of smooth muscle TRPV4 to the overall vascular responses is minimal in these vascular beds.^{9,11} The electrophysiological properties of TRPV4 in native endothelial cells

are similar to those of cloned human and murine TRPV4.^{10,12,13} However, a recent study has described a TRPV4-like current activated by 11,12- epoxyeicosatrienoic acid (EET) in freshly isolated mesenteric artery myocytes from wild-type but not TRPV4 knockout (TRPV4^{-/-}) mice.¹⁴ Given that detailed pharmacological characterizations have not been performed, further studies are needed to identify this TRPV4-like current.

TRPV4 has also been detected in smooth muscle cells of several vascular beds such as rat cerebral arteries and aorta,^{15,16,17} although it is more prominently expressed in endothelial cells of these tissues.^{11,16} Whether TRPV4 channels are present in human vessels have not been reported. Our unpublished data indicate that these channels are expressed in human coronary small arteries and arterioles and primarily localized in endothelial cells with much less expression in smooth muscle cells. These coronary myocytes may express other isoforms of TRPV4, most likely group II channels (TRPV4-B, -C, and -E). Intriguingly, these group II TRPV4 channels, which lack some ankyrin domains, are unable to oligomerize and thus are retained intracellularly, mainly in the endoplasmic reticulum.¹⁸

TRPV4 is activated by diverse chemical and physical stimuli, including the synthetic phorbol derivative 4 α -phorbol-12,13-didecanoate (4 α -PDD),¹⁹ arachidonic acid and its cytochrome P450 metabolites (e.g. 5,6- and 8,9-EETs),²⁰ moderate warmth (>27°C),^{21,22} hypotonic cell swelling,^{23,24} pressure,²⁵ membrane stretch,²⁶ and shear stress.²⁷ Two selective TRPV4 agonists are currently available: 4 α -PDD and GSK1016790A (300–1000-fold more potent than 4 α -PDD).^{11,19} The specificities of these two compounds for TRPV4 have been confirmed by the lack of vascular responses to stimulation using vascular tissues from TRPV4^{-/-} mice.^{7,8,9,11,12,13,14} In contrast, several commonly used TRPV4 blockers, such as ruthenium red, Gd³⁺ and La³⁺, also inhibit other TRP or non-TRP channels. It is therefore important to use other more specific approaches, such as short interfering RNA (siRNA) or genetic knockout animals, to confirm the results obtained with these pharmacological inhibitors.

Endothelial Ca²⁺ and EDHF pathways

The acronym 'EDHF' was originally introduced to describe a hypothetical factor responsible for agonist-induced smooth muscle hyperpolarization that is not associated with either NO or prostacyclin. It is now realized that EDHF is not a single factor but probably involves many mechanisms that lead to myocyte hyperpolarization and relaxation. Although the nature of the specific mediators of EDHF phenomenon remains controversial, EDHF responses have been grouped into two broad categories: the classical pathway and the second pathway.¹ The former is associated with endothelial cell hyperpolarization through the activation of two Ca²⁺-activated K⁺ (K_{Ca}) channels expressed in endothelial cells, namely the small-conductance K_{Ca} (SK_{Ca}) and intermediate-conductance K_{Ca} (IK_{Ca}) channels. This hyperpolarization is then directly transmitted to myocytes through myoendothelial gap junctions, or it can cause myocyte hyperpolarization via K⁺ ions that efflux through endothelial K_{Ca} channels and subsequently activate smooth muscle inwardly rectifying K⁺ (Kir) channels and Na⁺/K⁺-ATPases. The second pathway involves the release of diffusible endothelial factors such as EETs and hydrogen peroxide (H₂O₂), which hyperpolarize myocytes by opening various K⁺ channels, e.g. the large-conductance K_{Ca} (BK_{Ca}) channels. The two EDHF pathways can coexist in some vascular beds, as have been shown for porcine coronary arteries and rabbit small mesenteric arteries.^{28,29}

Activation of both EDHF pathways depends on an increase in endothelial [Ca²⁺]_i.¹ In the classic pathway, opening of endothelial SK_{Ca} and IK_{Ca} channels requires an increase in [Ca²⁺]_i, because these channels are not voltage-gated but are stimulated by [Ca²⁺]_i elevation through an association with calmodulin. For the second pathway, the release of diffusible

endothelial factors, especially EETs, is also thought to be initiated by a Ca^{2+} -dependent process. The synthesis of EETs by cytochrome P450 requires the Ca^{2+} -dependent release of arachidonic acid by phospholipase $\text{A}_2/30$ although 2-arachidonoylglycerol (2-AG) formed from the sequential action of phospholipase C and diglyceride lipase may also serve as a source of free arachidonic acid in some endothelial cells.³¹ In this context, TRP channel-mediated Ca^{2+} entry could provide an important source of the Ca^{2+} signal required for EDHF responses. It remains largely unexplored whether the second pathway also requires the opening of endothelial SK_{Ca} and IK_{Ca} channels, although it has been suggested that endothelial hyperpolarization is typically not required for the release of endothelial factors.¹

There is some debate on whether plasma membrane potential conversely regulates endothelial Ca^{2+} entry. Studies on cultured endothelial cells showed that hyperpolarization enhances Ca^{2+} entry by increasing the electrochemical gradient for Ca^{2+} , whereas membrane depolarization has the opposite effect.^{32,33} This is thought to be important for endothelial cells, which do not express functional voltage-gated Ca^{2+} channels as smooth muscle cells. In contrast, others studies using fresh dissociated endothelial cells or intact vessels found that although a rise in endothelial cell $[\text{Ca}^{2+}]_i$ stimulates plasma membrane hyperpolarization, agonist-induced Ca^{2+} influx is not significantly altered by this hyperpolarization.^{34,35,36} It remains unknown whether TRP channel-mediated endothelial Ca^{2+} entry is regulated by membrane potential. Therefore, as emphasized in an earlier Editorial article by Cohen,³⁷ more extensive studies are needed to have a deeper understanding of the mechanisms by which intracellular Ca^{2+} and membrane potential are regulated in endothelial cells and by which these changes are transmitted to the smooth muscle resulting in hyperpolarization and relaxation. This will require new integrated methodologies to measure the flux of major ions such as Ca^{2+} , K^+ , Na^+ and Cl^- , along with membrane potential and vessel diameter.

TRP channels in flow-mediated dilation

Shear stress generated by blood flow is one of the most important physiological regulators of vascular tone. In most vascular beds, flow stimulates endothelial cell to release vasodilator factors that subsequently relax underlying smooth muscle, a response often known as flow-induced dilation.³⁸ Similar to receptor agonist-induced dilation, flow-induced dilation also involves Ca^{2+} signaling.³⁹ Shear increases $[\text{Ca}^{2+}]_i$ in endothelial cells from a variety of vascular beds.^{40,41,42,43,44} This increase in $[\text{Ca}^{2+}]_i$ is thought to involve Ca^{2+} influx through mechanosensitive Ca^{2+} -permeable channels on the plasma membrane, in addition to Ca^{2+} release from intracellular Ca^{2+} stores.

Until now, TRPV4 is the only TRP channel that has been proposed as a potential candidate for the mechanosensitive channel(s) in the endothelium through which shear stimulus is transduced into Ca^{2+} signaling and then flow-mediated dilation. Earlier studies indicated that TRPV4 is a Ca^{2+} -entry channel sensitive to mechanical stimuli such as hypotonic cell swelling.^{23,24} Later, it was found that shear stress also increased $[\text{Ca}^{2+}]_i$ in HEK-293 and CHO cells transiently or stably transfected with TRPV4 cDNA but not in control untransfected cells, indicating that TRPV4 is also sensitive to shear stress.²⁷ In a more recent study, Koehler and colleagues provided the first pharmacological evidence for the role of endothelial TRPV4 in flow-mediated dilation by showing that, in rat carotid arteries and small gracilis arteries, the TRPV4 agonist 4α -PDD increased endothelial $[\text{Ca}^{2+}]_i$ and elicited potent vasodilation, and that the TRPV4 blocker ruthenium red significantly inhibited flow-mediated dilation.¹⁰ Using two independently-generated knockout mouse lines, several laboratories have subsequently demonstrated that flow-induced dilation was markedly reduced in both conduit and small arteries from TRPV4^{-/-} mice compared with wild-type controls.^{7,9,13} Furthermore, shear induced a rapid Ca^{2+} influx in endothelial cells

with a time course similar to TRPV4-transfected HEK-293 cells, and this Ca^{2+} influx was inhibited by the TRPV4 channel blocker and TRPV4-specific siRNA.⁹ Similar to endothelial cells, flow-induced Ca^{2+} entry via TRPV4 has been shown in renal tubular epithelial cells.^{45,46} Together, these findings strongly support that endothelial TRPV4 is importantly involved in shear-induced Ca^{2+} response and vasodilation.

It is of note that shear-induced Ca^{2+} increase^{40,41,42,43,44} and Ca^{2+} -dependent vasodilation^{47,48} have not been observed in all endothelial cultures or isolated arteries.^{49,50} For example, shear induced only a small $[\text{Ca}^{2+}]_i$ increase in the endothelium of rabbit coronary arterioles, and clamping of endothelial $[\text{Ca}^{2+}]_i$ using BAPTA-AM inhibited dilation to acetylcholine and substance P but had no significantly effect on shear-induced dilation.⁵⁰ These results indicate that a substantial component of shear-induced dilation occurs through a Ca^{2+} -insensitive mechanism. The reasons for the differences pertaining to Ca^{2+} dependency of flow-induced dilation remain unresolved but may involve methodological or species differences. In particular, fura-2 has often been used to measure shear-induced Ca^{2+} responses in endothelial cells but this probe is less sensitive to localized Ca^{2+} influx, which most likely occurs during shear, at least in some vascular beds. A more sensitive method (e.g., Mn^{2+} quenching assay) may be needed to detect this physiologically important Ca^{2+} event.⁹ Additionally, BAPTA-AM at commonly used concentrations (10–20 μM) seems less effective in buffering near membrane Ca^{2+} entry from the extracellular space compared with Ca^{2+} release from intracellular stores,^{51,52} although the precise mechanisms are unknown. Finally, shear-induced Ca^{2+} entry may play a more important role in those vascular beds with a more prominent EDHF component, because shear can induce NO release via some Ca^{2+} -independent mechanisms such as protein phosphorylation,⁵³ whereas the EDHF response is more tightly linked with Ca^{2+} signaling.

Several studies have examined the potential mechanisms of TRPV4 activation in response to shear stress. In TRPV4-transfected HEK-293 cells attached to a patch pipette, Strotmann et al reported that changes in pipette pressure (both negative and positive) had no effect on the activity of this channel, and therefore concluded that TRPV4 is probably not directly gated by mechanical force such as membrane stretch.²⁴ In endothelial cells as well as TRPV4-transfected cells, TRPV4-mediated Ca^{2+} entry in response to shear^{9,27} exhibited a delay with the maximal response within 1–2 min, further indicating that an intermediate signaling event is probably involved prior to TRPV4 activation. There is evidence that this potential intermediate signaling may include the arachidonic acid-EET pathway. In mouse carotid arteries, flow-induced dilation was abolished by the phospholipase A_2 inhibitor AACOCF₃, as well as by the TRPV4 inhibitor ruthenium red or TRPV4 knockout.¹³ In another study, MS-PPOH, an inhibitor of cytochrome P450 epoxygenase, and ruthenium red attenuated to a similar extent the flow-induced EDHF responses in carotid arteries of wild-type mice, but these two inhibitors had no cumulative effects.⁷ MS-PPOH had no effect on EDHF-related dilation in TRPV4 knockout animals. Similarly ruthenium red failed to affect dilatory responses when cytochrome P450 epoxygenase was downregulated. These studies indicate that flow-induced dilation involves both TRPV4 and EET, likely in a sequential or facilitative manner, although a more specific approach for modulating TRPV4 function is needed for confirmation of data obtained with the non-specific inhibitor ruthenium red. In a more recent study, 11,12-EET, although not a typical EET isomer that activates TRPV4,^{12,20} elicited an endothelium-dependent vasodilation in wild-type mice but not in TRPV4^{-/-} animals, suggesting that the EET acts as an upstream signal to induce TRPV4 activation and subsequent vasodilation.¹⁴

Besides EET-mediated activation, other mechanisms such as tyrosine phosphorylation, protein translocation, and caveolar association may also contribute to TRPV4 activation, as have been reported for this or other TRP channels in response to shear or other mechanical

stimuli.^{7,54,55,56} These mechanisms may not be mutually exclusive because an important feature of TRPV4 lies in its polymodal regulation by different factors that can exert synergistic effects on channel activity.⁵⁷ In addition to vessel reactivity studies using isolated vessel preparations, further detailed analysis of shear-induced Ca^{2+} entry and channel currents in native endothelial cells and TRPV4-transfected cells may also provide valuable insights into the potential mechanisms by which TRPV4 is activated by shear or other mechanical stimuli.

Several stretch-activated TRP channels such as TRPC1⁵⁸ and TRPV2⁵⁹ are also present in vascular endothelial cells. The TRPP1/2 channel complex is also mechanosensitive and mediates flow-induced Ca^{2+} influx in renal epithelial cells.⁶⁰ TRPM7 rapidly translocates to the plasma membrane of vascular smooth muscle cells in response to fluid flow.⁵⁵ It will be of interest to determine whether these mechanosensitive TRP channels that are expressed in endothelial cells are also involved in endothelial shear sensing and flow-mediated dilation.

TRP channels in agonist-induced dilation

Similar to shear-induced Ca^{2+} responses, a sustained increase in $[\text{Ca}^{2+}]_i$ upon stimulation with many receptor agonists requires Ca^{2+} entry from the extracellular space. This agonist-stimulated Ca^{2+} entry can be categorized into two general mechanisms: the store-operated Ca^{2+} (SOC) entry activated upon Ca^{2+} store depletion, and receptor-activated Ca^{2+} (ROC) entry dependent on the generation of second messengers but not the filling state of Ca^{2+} stores.² Recent studies indicate that TRP channels may function as either a ROC or SOC channel contributing to receptor agonist-induced Ca^{2+} entry in endothelial and various other cell types.³ In line with these findings, several TRP channels have been implicated in agonist-induced release of endothelial relaxing factors and vasodilation.^{8,14,56,61,62}

Using a TRPC4^{-/-} mouse model, Freichel et al showed that the store-operated Ca^{2+} current was absent in endothelial cells deficient in this channel and as a consequence Ca^{2+} entry and vasodilation in response to agonists such as acetylcholine and ATP were markedly reduced. These results indicate that TRPC4 is an essential component of SOC channels in native endothelial cells and that this channel provides a Ca^{2+} -entry pathway directly linked to the regulation of blood vessel tone.⁶² In another recent study, Fleming et al reported that bradykinin induced a Ca^{2+} influx into endothelial cells and subsequent K^+ channel activation and membrane hyperpolarization and that these effects were associated with a rapid translocation of TRPC6 to the plasma membrane, therefore providing evidence that links TRPC channel activation to EDHF responses.⁵⁶ The translocation of TRPC6 was prevented by the inhibitors of protein kinase A (PKA) and mimicked by 11,12-EET, suggesting participation of the EET/PKA-mediated signaling pathway. Thus, TRPC6 may also function as a ROC in endothelial cells.

Recent studies indicate that TRPV4 is also important in mediating agonist-induced dilation. Using TRPV4^{-/-} mice, several studies have found that cholinergic receptor agonist-induced dilation was significantly reduced in knockout mice.^{8,14,61} In particular, acetylcholine-induced vasodilator responses in vivo and in isolated small mesenteric arteries were markedly attenuated in TRPV4^{-/-} mice, and were accompanied by blunted Ca^{2+} entry into endothelial cells compared with wild-type mice which express high level of endothelial TRPV4.⁸ It remains unclear how receptor agonists activate TRPV4 in endothelial cells. TRPV4 has been shown to act as a ROC channel in epithelial cells.⁶³

Several other TRP channels may also contribute to the regulation of agonist-induced vasodilation, including TRPV1, TRPV3 and TRPA1.^{64,65,66,67} Although their specific roles in agonist-induced dilation have not been tested, activation of each induces potent endothelium-dependent dilation in isolated arteries. The findings that multiple TRP channels

are involved in endothelial Ca^{2+} entry could relate to the formation of heteromeric channels, 3 as have been reported for several TRPC channels as well as a recently described TRPC1-TRPV4 complex.⁶⁸ The formation of these heteromeric channels is thought to contribute to various modes of Ca^{2+} entry and thus various functional features of different types of endothelial cells.³ Thus the specific expression profile of TRP channels in the endothelium could alter the pattern of the Ca^{2+} response to extracellular stimuli. In addition, the expression of different TRP channels could also be important for cellular functions since upregulation of certain TRP channels compensates for the loss of others, as has been shown for the TRPC family in vascular smooth muscle cells.⁶⁹

TRP channel activation and EDHFs

A central theme of Ca^{2+} signaling, as well as many other cellular processes including the production of NO and EDHFs, is compartmentation. By organizing Ca^{2+} channels in specialized membrane or intracellular subdomains, cells can control the Ca^{2+} signal spatially and temporally to initiate appropriate downstream signaling, a feature that appears essential for cellular function.⁷⁰ There is evidence that TRPV4 channel-mediated Ca^{2+} entry may be more important for EDHF-dependent dilation. For example, in mouse superior and small mesenteric arteries stimulated with cholinergic agonists, the K^+ -sensitive dilation was reduced to a greater extent than NO-mediated dilation in TRPV4^{-/-} mice compared with wild-type controls.^{8,14,61} In mouse carotid arteries, TRPV4-mediated Ca^{2+} entry in response to shear is preferentially associated with EDHF-dependent dilation, specifically the dilation mediated by EETs.⁷ The underlying mechanisms responsible for these selective effects remain unclear but could be due to signaling compartmentation. Indeed, TRPV4 has been reported to localize in caveolae, a specialized membrane subdomain in endothelial and other cell types.⁶¹

Besides changing $[\text{Ca}^{2+}]_i$, opening of TRP channels may also alter plasma membrane potential. Because TRP channels are permeable to Na^+ ions, activation of these channels would be expected to cause membrane depolarization. However, activation of TRPV4, for example, elicited an endothelium-dependent dilation sensitive to endothelial K^+ channel blockers, indicating that TRP channel opening induces membrane hyperpolarization in endothelial cells.¹³ This TRP channel-mediated endothelial hyperpolarization may also explain part of EDHF-dependent dilation in response to receptor agonists and shear stress. The underlying mechanisms remain to be determined but may involve a close association of these channels with other signaling molecules such as endothelial SK_{Ca} or IK_{Ca} channels. Indeed, TRPV4 channels form a Ca^{2+} signaling complex with ryanodine receptors and large-conductance K_{Ca} (BK_{Ca}) channels in vascular smooth muscle cells.¹⁵

EETs, the cytochrome P450-derived metabolites of arachidonic acid, function as an important class of EDHFs in many species, including humans.^{1,71} As soluble and diffusible factors released from endothelial cells, EETs relax smooth muscle cells through membrane hyperpolarization via the activation of BK_{Ca} channels.³¹ Recent evidence indicates that EETs may also function as endothelium-derived hyperpolarizing factors within endothelial cells by activating TRP channels (e.g. TRPV4 and TRPC6).^{7,56} As described previously, stimulation of endothelial cells with receptor agonists (e.g. bradykinin) activates K_{Ca} channels through a mechanism that involves EET-dependent intracellular translocation of TRP channels.⁵⁶ Therefore, EETs may act as a link for two otherwise distinct mechanisms of EDHF-dependent relaxation (see the section on endothelial Ca^{2+} and EDHF pathways). It remains unclear whether the activation of TRP channels leads to further production of EETs through Ca^{2+} entry and resulting phospholipase A_2 activation. Such an effect would form a feed-forward mechanism to enhance EDHF production.

Endothelial TRP channels in vivo

Several studies have examined the cardiovascular phenotypes associated with TRPV4 using in vivo animal models. Recently, Earley and colleagues reported that elevations in mean arterial pressure (MAP) to a hypertensive challenge (the L-NNA infusion) were significantly greater in TRPV4^{-/-} mice compared with wild-type animals.¹⁴ They proposed that TRPV4 may be involved in a negative feedback mechanism that limits the response to hypertensive insults. This vascular protective effect of endothelial TRPV4 has also been suggested by studies showing that TRPV4 agonists reduce MAP in mice and several other animal models^{8,11,72,73} and this depressor effect is significantly enhanced in rats fed with a high-salt diet, which induces protein expression of vascular TRPV4.^{72,73} Surprisingly, there are no significant differences in baseline blood pressure between TRPV4^{-/-} and WT mice, ^{8,11,14,61} or between normal and high-salt diet rats.^{72,73} This observation of an enhanced TRPV4 response in disease is consistent with the findings that TRPV4 is more closely linked to EDHF release than NO formation. EDHF commonly serves as a back-up mechanism in the absence of NO during disease or in the presence of cardiovascular risk factors. It remains to be determined whether the expression and channel activity of TRPV4 or other TRP channels are modulated by vascular diseases such as hypertension, diabetes, and coronary artery disease.

Summary and perspectives

In summary, there is accumulating evidence that endothelial TRP channels, by serving as an essential component of Ca²⁺ signaling in endothelial cells, are importantly involved in the regulation of endothelium-dependent dilation (Fig. 1). In particular, TRPV4 channels are predominantly expressed in endothelial cells and, in response to channel activation by receptor agonists and mechanical shear stress, induce potent stimulation of endothelial relaxing factor (especially EDHF) release in both resistance and conduit arteries from a variety of species and vascular beds. These findings, together with the observation of a potential enhancement of TRPV4 responses in diseased states, indicate that TRPV4 may represent a conserved mechanism contributing to the regulation of vascular tone and possibly other endothelial functions both in health and in disease.

Investigation of the role of endothelial TRP channels in regulation of vascular tone is still at an early stage, and many questions remain. For example, the precise roles of TRPV4 or other TRP channels in vasodilator responses to receptor agonists or shear remain to be established. The subcellular localization of these channels and their potential associations with other proteins (e.g. SK_{Ca} and IK_{Ca}) are still poorly understood. It is also unclear whether the functions of TRP channels are altered in vascular diseases, especially in humans, where pharmacological manipulation of channel function might have beneficial effects. Nevertheless, the study of these channels will continue to be an exciting area of research in vascular biology, and the results of this research will not only promote our understanding of basic cellular mechanisms involved in endothelial functions but also help to identify new therapeutic targets for the prevention and treatment of vascular diseases.

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Figure 1.

Proposed roles for endothelial transient receptor potential (TRP) channels in the activation of endothelium-derived hyperpolarizing factor (EDHF) pathways in response to shear stress and receptor agonists. In endothelial cells, shear stress or receptor agonists (e.g. bradykinin and acetylcholine), by acting on a potential mechanosensor or corresponding receptors respectively, stimulate PLA_2 and PLC and subsequently cause the release of intermediate signaling molecules such as AA and its derivatives, and IP_3 . These mediators then activate several signaling pathways as follows. (1) EETs (CYP metabolites of AA) may activate plasma membrane TRP channels to induce Ca^{2+} entry from the extracellular space, (2) whereas IP_3 causes Ca^{2+} release from intracellular IP_3 -sensitive stores. A subsequent elevation in endothelial $[Ca^{2+}]_i$, consisting of this Ca^{2+} release (initial transient) followed by the Ca^{2+} entry (plateau phase), may activate endothelial IK_{Ca}/SK_{Ca} channels resulting in membrane hyperpolarization in endothelial cells. This hyperpolarization may be further transmitted to underlying smooth muscle cells, leading to smooth muscle hyperpolarization and relaxation. (3) Endothelial EETs also function themselves as diffusible EDHF factors to directly hyperpolarize and relax smooth muscle cells. Plausible parallel/alternative TRP channel-associated signaling events (marked in light colored arrows) may also occur in endothelial cells. These include the activation of TRP channels by other potential mechanisms such as shear stress itself (4) or Ca^{2+} -store depletion (5), regulation of TRP channel activity by endothelial membrane hyperpolarization as the result of IK_{Ca}/SK_{Ca} opening (6), and stimulation of PLA_2 by TRP channel-mediated Ca^{2+} entry (7). Ach, acetylcholine; AA, arachidonic acid; BK, bradykinin; CYP, cytochrome P450; EETs, epoxyeicosatrienoic acids; ER, endoplasmic reticulum; IP_3 , inositol 1,4,5-triphosphate; IK_{Ca}/SK_{Ca} , intermediate/small conductance Ca^{2+} -activated K^+ channel; PLA_2 , phospholipase A_2 ; PLC, phospholipase C.