Molecular expression and calcium signalling roles of native TRP channels in vascular cells

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List of abbreviations

AR, adrenergic receptor; BKCa, large-conductance Ca^{2+}-activated K^+ channel; EC, endothelial cell; EDHF, endothelium-derived hyperpolarising factors; EET, 11,12 epoxyeicosatrienoic acid; IK, intermediate conductance K^+ channels; MGJ, myo-endothelial gap junctions; NCX, Na^+/Ca^{2+} exchange; NO, nitric oxide; NSCC, non-selective cation channel; PE, phenylephrine; ROC, receptor-operated channel; RyR, ryanodine receptor; SAC, stretch-activated channel; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SK, small conductance K^+ channels; SR, sarcoplasmic reticulum; SOC, store-operated channel; STOC, spontaneous transient outward current; TM, transmembrane; TRP, transient receptor potential; TRPA, transient receptor potential ankyrin; TRPC, transient receptor potential canonical; TRPML, transient receptor potential mucolipin; TRPM, transient receptor potential melastatin; TRPP, transient receptor potential polycystin; TRPV, transient receptor potential vanilloid; VSM, vascular smooth muscle; VSMC, vascular smooth muscle cell; VGCC, voltage-gated Ca^{2+} channel.

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Abstract

In the vasculature, multiple members of the TRP-superfamily of non-selective cation channels (NSCCs) are expressed. These channels mediate diverse non-voltage-gated Ca^{2+}-entry pathways and functions, which involve both vascular myocytes and communicating endothelial cells. Here, we provide an overview of recent progress in this area of research and discuss several specific examples of the important roles of vascular TRP channels in Ca^{2+} signalling and electrophysiological responses. We especially focus on the recently discovered signal transduction mechanisms involving formation of specific complexes between TRP proteins and other better studied proteins that regulate cell calcium homeostasis, such as voltage-gated Ca^{2+} channels and ryanodine receptors. Finally, we provide an overview of the progress in our understanding of TRPM8, which is known as the principal neuronal cold receptor, expression, localisation and function in the vasculature. We conclude that this channel is likely involved in complex thermal behaviour of blood vessels, better understanding of which is relevant to hypothermic and cardiovascular surgery conditions, therefore further research in this area is needed.

Keywords

TRP channels, Vascular smooth muscle, Endothelium, Calcium signalling, Patch-clamp

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Introduction

The functional expression of a multitude of diverse ion channels in the cells that form our blood vessels regulate the physiological responses of the vasculature to tissue demands and likely underlie the abnormal responses observed in many disease states. The ion channels expressed in vascular smooth muscle cells (VSMCs) dynamically regulate changes in contractile state by altering intracellular free calcium concentration ($[Ca^{2+}]_i$), the critical signalling ion involved in the initiation and maintenance of VSMC contraction. Ca^{2+} influx from the extracellular environment and Ca2+ release from the sarcoplasmic reticulum (SR) ultimately regulate vascular tone by modifying excitation-contraction coupling. Moreover, Ca^{2+} signaling in endothelial cells (ECs), which are closely connected to VSMCs both structurally and functionally, plays an important role in the production and release of vasoactive compounds, such as nitric oxide (NO), endothelium-derived hyperpolarising factors (EDHF), prostacyclin and endothelins (Gryglewski et al., 1986, 1988; Ignarro et al., 1987; Palmer et al., 1987, 1988; Yanagisawa et al., 1988; Nakashima et al., 1993; Garland et al., 1995). These agents in turn modulate VSMC contractility.

While voltage-gated Ca^{2+} channel (VGCC)-mediated Ca^{2+} entry and receptor-mediated Ca^{2+} release are generally considered to be the two main pathways for the elevation of $[Ca^{2+}]_i$ in VSMCs and ECs, respectively, non-selective cation channels (NSCC) are equally recognised as important players in these cell-types (Benham & Tsien, 1987; Benham et al., 1987; Byrne & Large, 1988 and 1988b; Yao & Garland, 2005; Albert & Large, 2006). NSCCs regulate Ca^{2+} influx both directly and indirectly - via cation influx causing membrane depolarisation and VGCC opening. In addition, it has recently been shown that significant Na+ entry, and especially localised increases in $[Na^+]_i$, can cause plasmalemmal Na^+/Ca^{2+} exchange (NCX) reversal, causing Ca^{2+} entry coupled to Na^+ removal (Poburko et al., 2007, Liu et al., 2010).

In the vasculature, NSCCs mediate the primary response to numerous neurotransmitters and hormones, stretch, lipid messengers, and various metabolic/environmental factors (Beech, 2007; House et al., 2008 Inoue et al., 2009; Takahashi et al., 2011). Though the importance of NSCCs has been appreciated for more than 40 years, the molecular identities of most of these channels had remained unresolved until relatively recently. Since the discovery of the first human homologues of the Drosophila trp (Wes et al., 1995; Zhu et al., 1995), the majority of the members of the mammalian transient receptor potential (TRP) family have been detected in different vascular cells, such that currently TRP proteins are considered among the leading candidates for several vascular NSCCs (Xu & Beech, 2001; Beech et al., 2004; Beech, 2005; Yao & Garland, 2005; Albert & Large, 2006).

TRP proteins

In mammals there are at least 28 known members of the TRP superfamily of proteins separated into six subfamilies based on the similarity of their amino acid sequence and structures: TRPA ('ankyrin'), TRPC ('canonical'), TRPML ('mucolipin'), TRPM ('melastatin'), TRPP ('polycystin'), and TRPV ('vanilloid') (Montell et al., 2002; Clapham et al., 2005; Pederson et al., 2005). It is now well established that all TRPs form cation-selective channels in mammalian cells with each TRP protein forming one subunit consisting of six transmembrane (TM) domains (S1-S6) and cytosolic N^- and C^- termini. In native tissues these subunits are thought to form functional cation channels by assembling into homo- or hetero-tetrameric units with the pore-forming region expected to be between TM5 and TM6 (Pedersen et al. 2005; Firth et al., 2007).

Although TRPs form cation channels, their permeability to different mono- and divalent cations varies greatly from one isoform to the next. However, apart from the predominantly monovalent-selective channels, TRPM4 and TRPM5, all TRP channels are permeable to Ca^{2+} to some extent (Nilius et al., 2007). In most cell-types in which Ca^{2+}-permeable TRPs have been studied the localisation of these channels on the plasma membrane has indicated an involvement in Ca^{2+} influx. However, it is now well established that some TRPs can also be expressed intracellularly, potentially forming Ca^{2+}-release channels on the membranes of the internal Ca^{2+} stores, such as the endo/sarcoplasmic reticulum (Tsuzuki et al., 2004; Zhang & Barritt, 2004; Thebault et al, 2005; Gees et al., 2010).

Vascular TRPs and their role in Ca^{2+} signaling and electrophysiological responses

The first major breakthrough in the identification of a native TRP functionally expressed in the vasculature came with the identification of TRPC6 (then denoted TRP6) as the essential component of the receptor-operated channel (ROC) responsible for cation influx upon α1-adrenoceptor (α1-AR) activation in rabbit portal vein VSMCs (Inoue et al., 2001). It is now widely accepted that TRPs are ubiquitously expressed throughout the vasculature with

strong evidence for the involvement of TRPC1/4/5 as store-operated channels (SOC; Albert & Large, 2006), while members of the TRPV and TRPM subfamilies are now being implicated in diverse vascular functions. Among them TRPV4 and TRPM2/4/7/8 appear to be NSCCs of most notable importance in the myogenic response, oxidative stress, regulation of endothelial permeability, endothelium-induced VSMC hyperpolarisation, Mg^{2+} homeostasis and thermal behaviour of blood vessels (Earley et al., 2005; Yao & Garland, 2005; Inoue et al., 2006; Johnson et al., 2009; Sonkusare et al., 2012).

While the traditional functional roles proposed for vascular TRPs include SOC, ROC, and stretch-activated channels (SAC), new evidence suggests that TRPs can form important Ca^{2+} signalling pathways, interacting with other Ca^{2+}-dependent proteins to form loose complexes that can influence vascular function. Elementary Ca^{2+} events, such as Ca^{2+} sparks in VSMCs, which reflect the opening of several ryanodine receptor (RyR) channels clustered on the SR, are often associated with the activation of ion conductances on the plasma membrane, a consequence of the opening of Ca^{2+}-activated ion channels such as Ca^{2+}-activated K^+ channels (Nelson et al., 1995). Large-conductance Ca^{2+}-activated K^+ (BKCa) channels in VSMCs hyperpolarise the plasma membrane, which influences cell excitability, vascular tone, and arterial blood pressure. Earley et al. (2005) identified a mechanism in which a Ca^{2+} signalling complex involving TRPV4, RyR channels and BK_{Ca} channels in rat cerebral artery VSMCs could induce arterial vasodilatation upon TRPV4 activation by its endogenous activator, 11,12 epoxyeicosatrienoic acid (EET). In that study, the authors propose that activation of plasma membrane-bound TRPV4 in cerebral artery VSMCs could induce Ca^{2+} influx and localised elevations in $[Ca^{2+}]^i$ sufficient to activate RyR channels on the SR by Ca^{2+}-induced Ca^{2+}-release, increasing the frequency of Ca^{2+} spark events. The consequence of this elevation in Ca^{2+} sparks activity, according to Earley et al. (2005), was membrane hyperpolarisation due to an increased open probability of BK_{Ca} channels resulting in an increase in the frequency of spontaneous transient outward current (STOC) discharge. This proposed mechanism was further strengthened by the group when they observed similar responses in mouse mesenteric artery VSMCs, while EET had a much reduced effect on ionic currents, membrane potential or vessel diameter in tissues taken from TRPV4-null mice compared with wide-type (Earley et al., 2009a).

More recently, TRPV4 has been shown to play an important role in endothelial cells of mouse mesenteric artery. In an elegant study, Sonkusare et al. (2012) used a transgenic mouse which "expresses a genetically encoded Ca^{2+} biosensor (GCaMP2) exclusively in the endothelium". This ability to observe elementary Ca^{2+} events in the endothelium of intact vessels, without being obscured by the more robust signals from the VSM as would be the case using standard Ca^{2+} sensitive indicators, allowed the authors to record localised quantal Ca^{2+} signals ("sparklets") reflective of Ca^{2+} influx events through single TRPV4 channels when exposed to TRPV4 agonists (GSK1016790A, 4α-Phorbol 12,13-didecanoate and EET). The sparklets were observed at the ends of ECs and at positions within each EC representative of myo-endothelial gap junctions (MGJ), endothelial projections through the internal elastic lamina that come into close contact with the VSM. These sparklets increased the open probability of small (SK) and intermediate (IK) conductance K^+ channels resulting in hyperpolarisation and endothelial-dependent vasodilatation. This TRP-mediated endothelium-dependent mechanism for local control of vascular tone was previously proposed by Earley et al. (2009b) for TRPA1 in rat cerebral artery. Interestingly, Sonkusare et al. (2012) observed that activation of only three to eight TRPV4 channels per cell were sufficient to induce maximal vessel dilation. TRPV4 agonist-induced Ca^{2+} sparklets were blocked by the non-selective TRPV channel blocker, ruthenium red, selective TRPV4 antagonist, HC-067047, the absence of extracellular Ca^{2+} and were absent in TRPV4-null mice.

TRPM8

In our laboratory, the main channel of interest is a member of the melastatin TRP subfamily, TRPM8. Initially cloned from prostate tissue and found to be up-regulated in several tissue carcinomas, TRPM8 has now been shown to be widely expressed, with high expression in a subset of temperature-sensitive sensory neurons where it functions as the primary sensor of cold in the innocuous range (Tsavaler et al., 2001; McKemy et al., 2002; Peier et al., 2002; Bautista et al., 2007). In addition to its activation by cold, TRPM8 is also activated by menthol and other cooling compounds (Bodding et al., 2007).

TRPM8 in the vasculature

In our laboratory, our aim has been to determine a functional role for TRPM8 in the vasculature, building on previous findings by Yang et al. (2006). In their study, TRPM8 was shown to be present in rat aorta and pulmonary artery at both mRNA and protein level. The authors demonstrated a functional role for the channel, observing that the application of menthol (300 μM) to

isolated VSMCs from either vascular bed was sufficient to induce Ca^{2+} responses that were dependent on extracellular Ca^{2+} and independent of VGCC involvement (Yang et al., 2006). In a previous publication by our group, we further explored the TRPM8 expression in other vascular beds and tried to reveal the functional role played by the channel in the control of vascular tone. To do this we used a vascular ring organ bath tensiometric preparation along with TRPM8 pharmacological modulators and vasoconstrictive compounds to observe the consequences of its activation on vascular tone in different contractile states (Johnson et al., 2009). In that study, we were able to demonstrate, using conventional RT-PCR and Western blot analysis, the presence of TRPM8 at the mRNA and protein level in rat tail, femoral and mesenteric arteries and thoracic aortae. However, functional experiments indicated complex effects of TRPM8 agonists on vascular ring contractility, causing prominent vasodilatations when applied during phenylephrine (PE) and high-K^+-induced vasoconstrictions, although, in contrast, menthol consistently caused vasoconstrictions when applied to vascular rings at rest. Due to the structural differences of menthol and icilin, but their similar effects on pre-constricted vascular rings, these effects were interpreted as specific effects on vascular TRPM8, which was considered to mediate vasodilatation by some unknown mechanism. In Johnson et al. (2009), the $iPLA_2$ pathway was proposed to play a role in menthol-induced vasodilatations, since applications of the $iPLA_2$ blocker (S)-BEL caused significant inhibition of this effect, although used at concentrations in excess of those recommended by Jenkins et al. (2002). Recently, Baylie et al. (2010) have shown that menthol can inhibit L-type VGCCs at concentrations previously considered selective for TRPM8 activation. We currently use a more rigorous set of experiments to identify the specific effects of TRPM8 agonists on vascular tissues using a wide range of physiological techniques.

When organ bath tensiometric experiments were performed on tail artery rings in the presence of nifedipine, an L-type VGCC antagonist, TRPM8 agonist-induced vasodilatations were abolished indicating that previously observed TRPM8 agonist-induced vasodilatations were a consequence of a direct inhibition of L-type VGCC activity. Interestingly, when experiments were performed in the presence of nifedipine, we were able to observe prominent menthol-induced vasoconstrictions that had previously been masked by the inhibitory action of menthol on L-type Ca^{2+} currents. This menthol-induced potentiation of PE-induced vasoconstrictions, in the absence of a VGCC component, was found to be mediated predominantly by Ca^{2+}-release from the internal stores, since they were abolished by cyclopiazonic acid (10 μM). Furthermore, menthol-induced vasoconstrictions were significantly reduced in the presence of the selective TRPM8 antagonist AMTB (10 μM; 145.9±9.0 % to 79.5±8.7 % of the PE-induced amplitude, P<0.001; one-way ANOVA; n=9 cells, N=9 rats).

From these observations, it was hypothesised that TRPM8 is expressed in tail artery VSMCs, most likely intracellularly, and mediated vasoconstriction by a mechanism that involved Ca^{2+} release. RT-PCR on isolated tail artery VSMCs confirmed TRPM8 mRNA presence (figure 1A), while the presence of the TRPM8 protein

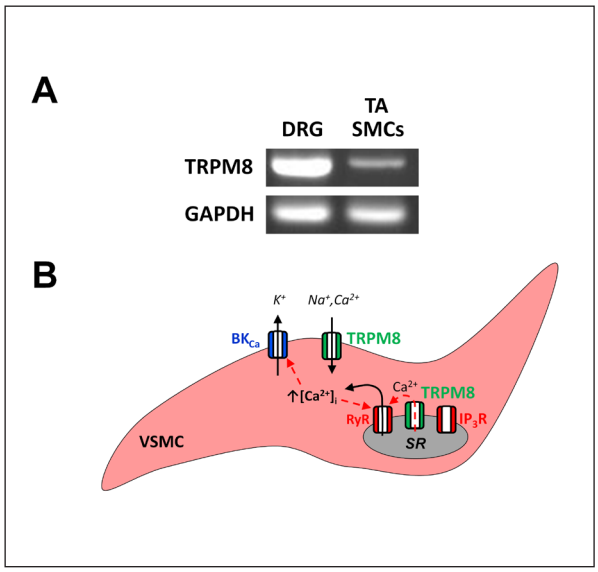

Figure 1. Detection of TRPM8 gene expression and schematics of sub-cellular localisation of TRPM8 proteins in rat tail artery VSMCs

A - RT-PCR analysis of TRPM8 channel expression of TRPM8 (amplicon size - 502 bp; Forward primer: 5'-GATCTTCACCAATGACCGCCG-3'; Reverse primer: 5'-CCCCAGCTGCGTTGATATCA-3') in isolated VSMCs from the rat tail artery using dorsal root ganglia as a positive control. GAPDH was used as the endogenous control (amplicon size - 237 bp; Forward primer: 5'-TTCACCACCATGGAGAAGGC-3'; Reverse primer: 5'- GGCATGGACTGTGGTCATGA-3').

B - Schematic diagram showing the proposed sub-cellular localisation of TRPM8 channels in rat tail artery VSMCs. Findings from immunocytochemistry experiments performed in our laboratory indicate that TRPM8 is localised on the perimeter of VSMCs and on the SR membranes, as evidenced by strong co-localisation of anti-TRPM8 (Alomone Lab, ACC-049; 1:200) antibody with antibodies for RyR1 (Sigma-Aldrich, R129; 1:100) and IP3R1 (Santa Cruz; SC-6093; 1:100) channels (data not shown).

was further strengthened by immunocytochemistry experiments on isolated rat tail artery VSMCs. In addition, co-localisation experiments performed using antibodies for RyR1 receptors and InsP31 receptors indicate that TRPM8 channels in these cells are localised predominantly on the SR and in close proximity to other important Ca^{2+}-release channels (figure 1B).

To determine the sub-cellular mechanisms by which TRPM8 activation could mediate the vascular responses we observed, we currently employ fast confocal Ca^{2+} imaging and patch-clamp electrophysiology to explore details of the intracellular Ca^{2+} signalling in response to menthol application and the membrane current responses associated with them. These approaches begin to reveal the previously unsuspected complexity and interplay between calcium signals and membrane current responses, such as a substantial increase in the frequency of Ca^{2+} sparks and STOCs induced by menthol. Intriguingly, under current-clamp conditions activation of these multiple ion channel types by menthol (e.g. TRPM8 and BK_{Ca}) translates into very complex, previously not documented membrane potential changes, namely sustained membrane depolarisation frequently interrupted by spontaneous large-amplitude hyperpolarisations.

Concluding remarks

TRP channels expressed in communicating VSMCs and ECs continue their emergence as a novel, diverse and remarkably multifunctional group of vascular ion channels. Our findings indicate that TRPM8, which is now firmly established as the principal neuronal cold receptor, is also expressed and functional in the rat arterial VSMCs, with the sub-cellular localisation of the protein appearing to be predominantly intracellular, in close proximity to important Ca^{2+} release channels. Our results also indicate that TRPM8 activation in the vasculature may also involve functional cross-talk with other Ca^{2+}-permeable (and Ca^{2+}-regulated) channels. Thus, it is possible that TRPM8 may form a Ca^{2+} signalling complex with RyR and BK_{Ca} channels in rat tail artery in much the same way as TRPV4 in rat cerebral artery VSM. Since TRPM8 is likely involved in complex thermal behaviour of blood vessels, better understanding of which is relevant to hypothermic and cardiovascular surgery conditions, further research in this, currently somewhat controversial area, is much needed.

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