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REVIEW

Pharmacology of transient receptor potential melastatin channels in the vasculature

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Mammalian transient receptor potential melastatin (TRPM) non-selective cation channels, the largest TRP subfamily, are widely expressed in excitable and non-excitable cells where they perform diverse functions ranging from detection of cold, taste, osmolarity, redox state and pH to control of Mg^{2+} homeostasis and cell proliferation or death. Recently, TRPM gene expression has been identified in vascular smooth muscles with dominance of the TRPM8 channel. There has been in parallel considerable progress in decoding the functional roles of several TRPMs in the vasculature. This research on native cells is aided by the knowledge of the activation mechanisms and pharmacological properties of heterologously expressed TRPM subtypes. This paper summarizes the present state of knowledge of vascular TRPM channels and outlines several anticipated directions of future research in this area.
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Keywords: TRPM; vascular smooth muscle; cardiovascular disease; vascular tone; TRPM agonist; TRPM inhibitor

Abbreviations: ACA, *N*-(*p*-amylcinnamoyl)anthranilic acid; AMTB, *N*-(3-aminopropyl)-2-([(3-methylphenyl) methyl]oxy)-*N*-(2-thienylmethyl)benzamide hydrochloride salt; BTP2, 4-methy-4′-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide; CaM, calmodulin; CAN, Ca^{2+}-activated non-selective channels; GPCR, G protein-coupled receptor; LPL, lysophospholipids; PIP_2, phosphatidylinositol-4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; PUFA, polyunsaturated fatty acids; ROC, receptor-operated channel; ROS, reactive oxygen species; S1P, sphingosine-1-phosphate; SOC, store-operated channel; TM, transmembrane domain; VSMC, vascular smooth muscle cells

Introduction

Adequate blood supply is critical for the proper function of all tissues and organs in the body, and cardiovascular diseases remain the leading cause of disability or death in industrial countries. Vascular smooth muscle cells (VSMC) regulate blood pressure and blood flow by adjusting their contractile state according to tissue metabolic or environmental needs (e.g. in thermoregulation). Ca^{2+}-permeable ion channels initiate and maintain VSMC contraction, and hence the identification of their genes is important for our understanding of vascular function and, ultimately, for the treatment of human cardiovascular diseases. Evidence is also being accumulated for the important role of cation entry in slow phenotypic remodelling processes in the vascular system leading to hypertension, atherosclerosis, neointimal hyperplasia and other proliferative disorders.

Vascular smooth muscle cells are enriched with a multitude of ion channels of different types that act in concert to regulate cell membrane potential and smooth muscle excitability and excitation process. Calcium influx via various non-selective cation channels and voltage-dependent Ca^{2+} channels, an ultimate determinant of VSMC contractile state, is thus regulated in a very complex manner. As an added layer of complexity, same calcium signals that initiate VSMC contraction also often couple to 'inhibitory' Ca^{2+}-dependent channels, such as Ca^{2+}-activated potassium and, under certain conditions, chloride channels. For many channels, there also exist tight positive or negative feedback loops that control their activity, usually employing membrane potential and calcium level for activating or deactivating them. Microdomain organization of signal proteins and local efficient coupling between Ca^{2+} entry, Ca^{2+} release and activation of Ca^{2+}-dependent channels (Bolton, 2006) makes identification of the roles of individual channel types an even more challenging task.

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Standard abbreviations conform to *BJP*'s Guide to Receptors & Channels (Alexander *et al.*, 2008) and to the IUPHAR guidelines, as published in *Pharmacological Reviews*.
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Transient receptor potential (TRP) cation channels (Montell, 2005; Nilius *et al.*, 2007; Venkatachalam and Montell, 2007) are currently considered as the leading candidate proteins mediating diverse non-voltage-gated Ca^{2+} entry pathways in vascular and communicating endothelial cells (Beech, 2005; Yao and Garland, 2005; Albert and Large, 2006; Inoue *et al.*, 2006; Simard *et al.*, 2007; Saleh *et al.*, 2008). However, in contrast to cells overexpressing a certain type of TRP channel, it is far more difficult to reveal its role in native cells such as VSMC. It also follows from these general considerations that even the net functional outcome of channel activation (e.g. VSMC contraction or relaxation) can be sometimes difficult to predict. For example, Ca^{2+}-permeable non-selective cation channels, by elevating Ca^{2+} in the above mentioned signalling microdomains, can induce not only membrane depolarization but also spontaneous hyperpolarizations caused by the Ca^{2+} 'sparks'/BK_{Ca}/STOCs mechanism as was shown for TRPV4 activation resulting in membrane hyperpolarization (and vasodilation) of cerebral artery mediated by the TRPV4/RyR/BK_{Ca} complex (Earley *et al.*, 2005). Nevertheless, there has been considerable recent progress in decoding the function of several TRPs in VSMC, mostly from the family of 'canonical' TRP channels (TRPC), as reviewed elsewhere (Beech, 2005; Albert and Large, 2006; Inoue *et al.*, 2006; Dietrich *et al.*, 2007; Firth *et al.*, 2007; Saleh *et al.*, 2008). The purpose of this review is to outline the present state of knowledge of vascular 'melastatin' or long TRPs (TRPM), one of the most novel, largest and diverse TRP subfamilies, while emphasizing the usefulness and limitations of current TRPM pharmacological modulators that help reveal their roles in native cells.

TRPM channels

Based on structural homology, TRPMs belong to TRP group 1 that includes three other mammalian subfamilies (TRPC, TRPV and TRPA), as well as TRPN channels found only in invertebrates and zebrafish (Montell, 2005; Venkatachalam and Montell, 2007). The other two mammalian subfamilies, TRPP and TRPML, in group 2 are their much more distant relatives. Eight mammalian members of the TRPM subfamily are structurally and functionally diverse non-selective cation channels, which are involved in processes ranging from detection of cold, taste, osmolarity, redox state and pH to control of Mg^{2+} homeostasis and cell proliferation or death. Their phylogenetic analysis suggests subdivision into four groups, TRPM1/3, TRPM6/7, TRPM4/5 and TRPM2/8 (Harteneck, 2005). Remarkably, three out of eight TRPM members have connections with cancer development, but their role in vascular angiogenesis has not been investigated. The founding member melastatin (TRPM1) as well as TRPM5 and TRPM8 were identified by analysis of gene expression in several carcinomas (Kraft and Harteneck, 2005).

Transient receptor potential channels in native cells sometimes do not faithfully reproduce properties of their heterologously expressed counterparts, likely due to differences in the cellular milieu or heteromultimerization between different TRP subtypes. However, the knowledge of biophysical properties, interacting molecules and activation mechanisms of recombinant TRPs together with the analysis of the corresponding gene expression in native cells has significant predictive power in decoding native channel protein functions. Thus, Table 1 summarizes some key features of the individual members of the TRPM subfamily that have been reviewed in detail elsewhere (Fleig and Penner, 2004; Harteneck, 2005; Kraft and Harteneck, 2005; Ramsey *et al.*, 2006; Nilius *et al.*, 2007; Venkatachalam and Montell, 2007). Furthermore, pharmacological modulators of heterologously expressed TRP channels together with more selective molecular biology approaches such as gene silencing or gene knockout are invaluable tools for probing the functional roles of native channels. Therefore, Table 2 provides a summary of the pharmacological properties of TRPM channels.

Structure

In common with other TRP channels, TRPMs have cytoplasmic N- and C-terminals separated by six putative transmembrane (TM) domains with the pore-forming region found in the loop between TM5 and TM6 (Figure 1); the TRPM4 selectivity filter is also located in this region (Nilius *et al.*, 2005a). TM4 and the TM4–TM5 linker in TRPM8 determines its sensitivity to voltage, temperature and menthol (Voets *et al.*, 2007), while the distal part of TM6 determines cation versus anion selectivity, at least in TRPM2 and TRPM8 channels (Kuhn *et al.*, 2007).

Similarly to TRPC channels, they have a TRP box in the C-terminal. Their N-terminus lacks ankyrin repeats found in TRPCs and TRPVs, but instead has a common large TRPM homology domain. Functional TRP channels are most likely homo- or hetero-tetramers, and the C-terminus coiled-coil domain is necessary for TRPM channel assembly and sufficient for tetrameric formation (Tsuruda *et al.*, 2006). There are also additional domains specific to TRPM subtypes (Table 1 and Figure 1) that contribute to large differences in protein length – from 1104-amino-acid residues in hTRPM8 to 2022 residues in hTRPM6 (Birnbaumer *et al.*, 2003). These will be discussed in more detail later in connection with interacting proteins and activation mechanisms of individual TRPM channels.

Biophysical properties

Although TRPM proteins demonstrate high diversity in their electrophysiological behaviour they can be divided into two major groups in each case. All TRPMs can form functional cation channels either as homo- or heteromultimers. This was shown by patch-clamp measurements of cation currents specifically arising in mammalian cells lines such as human embryonic kidney (HEK)293 or Chinese hamster ovary cells transfected with TRPM plasmid DNAs. Single channel measurements have also been performed as the gold standard for studying ion channels. This provides valuable clues for the identification of TRPM channels in native cells as single channel conductance and channel kinetics are unique 'signature' properties of the channel. However, in TRP research single channel analysis in native cells is generally lacking. Also notable, in some cases (TRPM3/7) the reported single channel conductance values differ by a factor of 2–2.5, likely

Table 1 Expression and properties of melastatin TRPM channels

Subtype	*TRPM1*	*TRPM2*	*TRPM3*	*TRPM4*	*TRPM5*	*TRPM6*	*TRPM7*	*TRPM8*
Structure and interacting proteins								
Specialized domains		C – Nudix		C – PIP_2 N,C – 5 CaM	C – PIP_2	C – atypical protein kinase	C – atypical protein kinase	C – PIP_2
Interacting proteins	Short TRPM1 (MLSN-S)	CaM Sir2	CaM	CaM TRPM5 SUR1	TRPM4	TRPM7	TRPM6, PLC-β, snapin, myosin IIA heavy chain	
Biophysical properties								
g (pS)	Not determined	52–80	65–133	25	16–25	84	40–105	74–83
I–V relationship	Outwardly rectifying	Linear	Linear	Outwardly rectifying	Outwardly rectifying	Outwardly rectifying	Outwardly rectifying	Outwardly rectifying
Selectivity (P_{Ca}/P_{Na})	<1	0.5–1.6	1.6–2.0	<0.05	<0.05	6.9	0.3–3	1–3.3
Activation mechanisms, expression profiles and functional roles								
Activation	Constitutively active	ADPR, NAD, oxidative stress, intracellular Ca^{2+}, heat (>35°C)	Constitutively active, hypo-osmolarity, sphingolipids	Intracellular Ca^{2+}, PIP_2, heat (15–35°C)	Intracellular Ca^{2+}, PIP_2, heat (15–35°C)	Acidity	Acidity, PIP_2	Cold, PIP_2, lysophospholipids
Major proposed functions	Tumour suppressor in melanocytes	Oxidant stress sensor, control of cell death	Volume- and mechanosensitive channel, renal osmo-homeostasis	Ca^{2+}-activated cation channel	Ca^{2+}-activated cation channel, taste sensation	Renal and GI Mg^{2+} absorption	Control of Mg^{2+} homeostasis	Cold sensation, up-regulated in cancer
GPCR regulation	No effect	No effect	Activate	Inhibit	Activate		Activate or inhibit	Inhibit
Store-operated regulation		No effect	Activate					Activate
Highest expression	Brain	Brain and bone marrow	Brain and pituitary	Intestine and prostate	Intestine, pancreas and prostate	Intestine and brain	Heart, pituitary, bone and adipose tissue	Prostate and liver
Vascular expression		Aorta, pulmonary artery	Aorta, pulmonary artery	Aorta, pulmonary and cerebral artery		Mesenteric artery	Aorta, A7r5, pulmonary, cerebral and mesenteric artery	Aorta, pulmonary, tail, femoral and mesenteric artery

See text for explanations and references.
CaM, calmodulin; GPCR, G protein-coupled receptor; I–V, current–voltage; PIP_2, phosphatidylinositol-4,5-bisphosphate; PLC, phospholipase C; TRPM, transient receptor potential melastatin.

Table 2 Pharmacological properties of TRPM channels

	Activators		*Inhibitors*	
TRPM1	Unknown		La^{3+}	80–100 μM
TRPM2	H_2O_2	$pD_2 = 4.3$	2-APB	$IC_{50} \sim 1$ μM
	Other oxidants		Miconazole	10 μM
	tert-butyl hydroperoxide (tBOOH)	4 mM	ACA	20 μM
	Dithionite ($Na_2S_2O_4$)	1 mM	Flufenamic acid	50 μM–1 mM
	Arachidonic acid	30 μM	Clotrimazole and econazole	3–30 μM
	β-NAD^+	$pD_2 \sim 3$	La^{3+}	Insensitive
	ADPR	$pD_2 \sim 4$		
	cADPR	$pD_2 = 3.2–4.9$		
	NAADP	$pD_2 = 5$		
	Intracellular Ca^{2+}	$pD_2 = 4.8–6.3$		
TRPM3	D-erythrosphingosine and SPH analogues	$pD_2 = 4.9$	Gd^{3+} and La^{3+}	100 μM
	Pregnelone sulphate	$pD_2 = 4.9$	2-APB	100 μM
	Nifedipine	$pD_2 = 4.5$	SKF-96365	Insensitive
	Ceramides, S1P, AA, DAG	Insensitive		
TRPM4	BTP2	$pD_2 = 8.1$	Gd^{3+} and La^{3+}	80 μM
	Intracellular Ca^{2+} (sensitivity regulated by ATP, PKC and CaM)	$pD_2 = 3.4–6.4$	ATP, ADP, AMP and AMP-PNP	$IC_{50} = 2–19$ μM
	PIP_2	$pD_2 = 5.3$	Spermine	$IC_{50} = 61$ μM
	Decavanadate	$pD_2 = 5.7$	Flufenamic acid	$IC_{50} = 2.5$ μM
			ATP^{4-}	$IC_{50} = 0.8$ μM
TRPM5	Intracellular Ca^{2+}	$pD_2 = 4.7–6.2$	Protons	IC_{50} at pH 6.2
	PIP_2 (restores rundown)	20 μM	Flufenamic acid	$IC_{50} = 24$ μM
			Spermine	$IC_{50} = 37$ μM
			ATP^{4-}	Insensitive
			ATP	Insensitive
TRPM6	2-APB	$pD_2 = 3.4–3.7$	Ruthenium red (voltage-dependent)	$IC_{50} = 0.1–10$ μM
	Protons	$pH_{0.5} = 4.3$	Mg^{2+}	$IC_{50} = 1.1$ μM
			Ca^{2+}	$IC_{50} = 4.8$ μM
TRPM7	PIP_2	20 μM	2-APB	$IC_{50} = 178$ μM
	2-APB	>1 mM	Mg^{2+}	$IC_{50} = 0.6$ mM
	Protons	$pH_{0.5} = 4.7$	La^{3+}	2 mM
			Spermine	0.2–20 μM
TRPM8	Cooling	$t_{0.5} = 25.5$°C	Clotrimazole (voltage-dependent)	$IC_{50} = 0.2$ μM
	WS-12	$pD_2 = 4.9–7.4$	AMTB	$IC_{50} = 0.6$ μM
	Icilin	$pD_2 = 5.3–6.9$	BCTC	$IC_{50} = 0.8$ μM
	CPS-113	$pD_2 = 5.9$	SKF-96365	$IC_{50} = 0.8–1$ μM
	FrescolatML	$pD_2 = 5.5$	Thio-BCTC	$IC_{50} = 3.5$ μM
	CPS-369	$pD_2 = 5.4$	Capsazepine	$IC_{50} = 18$ μM
	WS-3	$pD_2 = 5.4$	2-APB	$IC_{50} = 8–12$ μM
	WS-148	$pD_2 = 5.4$	Tetracaine	100 μM
	WS-30	$pD_2 = 5.3$	Chlorpromazine	1 μM
	FrescolatMAG	$pD_2 = 5.3$	PUFA	10 μM
	Cooling agent 10	$pD_2 = 5.2$	Spermine	$IC_{50} = \sim 50$ μM
	WS-11	$pD_2 = 5.2$	Ruthenium red	20 μM
	WS-14	$pD_2 = 4.7$	1,10-phenanthroline	$IC_{50} = 100$ μM
	Menthol	$pD_2 = 4–5$		
	PMD38	$pD_2 = 4.5$		
	WS-23	$pD_2 = 4.4$		
	Coolact P	$pD_2 = 4.2$		
	Geraniol	$pD_2 = 2.2$		
	Linalool	$pD_2 = 2.2$		
	Eucalyptol	$pD_2 = 2.1–2.5$		
	Hydroxycitronellal	$pD_2 = 1.7$		
	Lysophospholipids (LPC, LPI)	3–10 μM		
	PIP_2 and PI(4)P (directly and restores rundown)	20 μM		

References: TRPM1 (Xu *et al.*, 2001; Oancea *et al.*, 2009); TRPM2 (Perraud *et al.*, 2001; Hara *et al.*, 2002; Hill *et al.*, 2004a,b; Kolisek *et al.*, 2005; Beck *et al.*, 2006; Togashi *et al.*, 2008; Du *et al.*, 2009); TRPM3 (Grimm *et al.*, 2003; 2005; Lee *et al.*, 2003; Xu *et al.*, 2005; Wagner *et al.*, 2008); TRPM4 (Xu *et al.*, 2001; Launay *et al.*, 2002; Nilius *et al.*, 2003; 2004a,b; 2005b; 2006; Ullrich *et al.*, 2005; Takezawa *et al.*, 2006); TRPM5 (Liu and Liman, 2003; Liu *et al.*, 2005; Ullrich *et al.*, 2005); TRPM6 (Voets *et al.*, 2004b; Li *et al.*, 2006); TRPM7 (Runnels *et al.*, 2001; 2002; Kerschbaum *et al.*, 2003; Li *et al.*, 2006); TRPM8 (McKemy *et al.*, 2002; Andersson *et al.*, 2004; Behrendt *et al.*, 2004; Hu *et al.*, 2004; Voets *et al.*, 2004a; Liu and Qin, 2005; Abeele *et al.*, 2006; Andersson *et al.*, 2007; Beck *et al.*, 2007; Bodding *et al.*, 2007; Malkia *et al.*, 2007; Lashinger *et al.*, 2008; Meseguer *et al.*, 2008; Sherkheli *et al.*, 2008).

ACA, *N*-(*p*-amylcinnamoyl)anthranilic acid; AMTB, *N*-(3-aminopropyl)-2-([(3-methylphenyl) methyl]oxy)-*N*-(2-thienylmethyl)benzamide hydrochloride salt; BTP2, 4-methy-4′-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide; CaM, calmodulin; LPC, lysophosphatidylcholine; LPI, lysophosphatidylinositol; PKC, protein kinase C; PUFA, polyunsaturated fatty acids; S1P, sphingosine-1-phosphate; TRPM, transient receptor potential melastatin.

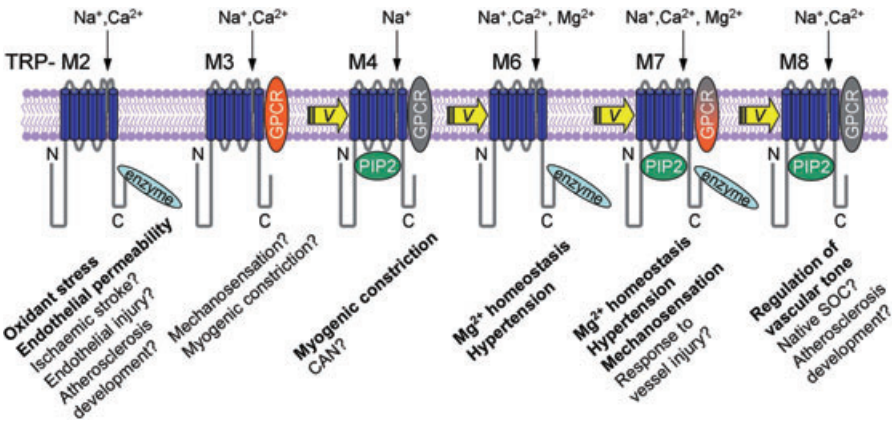

Figure 1 TRPM subtypes expressed in vascular smooth muscle cells and their established (bold) as well as hypothetical physiological roles in the vasculature. The diagram highlights some structural features of TRPM channels as well as their subtype-specific regulation by membrane potential (V – activation by membrane depolarization), G protein-coupled receptors (GPCR: orange – activation; grey – inhibition; mixed colour – dual effect) and PIP_2 (potentiation). CAN, Ca^{2+}-activated non-selective channels; PIP_2, phosphatidylinositol-4,5-bisphosphate; SOC, store-operated channel; TRPM, transient receptor potential melastatin.

due to differences in the recording conditions (e.g. ion composition of the solutions used).

Based on the shape of their current–voltage (I–V) relationships TRPM channels also fall into two groups: TRPM2/3 that show almost linear relation, and TRPM1/4–8 that show outward rectification. The voltage dependence in some cases is very strong (e.g. TRPM8 compared with other voltage-sensitive TRPs) but not as strong as in other classical voltage-gated channels, such as K_V channels. Based on the ion selectivity, TRPMs can be again subdivided in practically impermeable (TRPM4/5) and moderately permeable to Ca^{2+} (TRPM1/2/3/6/7/8) channels. Thus, under physiological conditions TRPMs can induce membrane depolarization due to Na^+ influx and Ca^{2+} influx via TRPM2/3/6/7/8 or voltage-gated Ca^{2+} channels if these are expressed, such as in arterial VSMC.

Activation mechanisms and functional roles of TRPM channels

TRPM1 and TRPM3. Although the TRPM1 channel is the founding member of the TRPM subfamily, very little is known about its activation properties and function. Its tissue expression also seems to be limited compared with other TRPMs (see below). Its expression is inversely correlated with potential for melanoma metastasis (Duncan *et al.*, 1998). The main function of TRPM1 was suggested to be intracellular and critical to normal melanocyte pigmentation (Oancea *et al.*, 2009). TRPM3 shows constitutive activity that can be increased by hypotonic solution (Grimm *et al.*, 2003). Therefore, from this function and expression in the human kidney its role in renal Ca^{2+} homeostasis has been postulated. This is also the first TRP channel found to be activated by D-erythro-sphingosine (but not by sphingosine-1-phosphate, S1P) (Grimm *et al.*, 2005). The effects of hypotonicity on TRPM3 is likely mediated by cell swelling, thus TRPM3 can also function as a volume- and mechanosensor.

TRPM2 and TRPM8. These are the closest relatives within the TRPM subfamily, which share 42% of identical residues (Peier *et al.*, 2002). Their activation mechanisms are entirely different and well investigated for each protein. TRPM2 is activated by reactive oxygen species (ROS, such as H_2O_2), ADP ribose (ADPR), NAD^+ and intracellular Ca^{2+} making it truly a multifunctional channel with a central role in oxidative/nitrosative stress and cell death (Harteneck, 2005; Kraft and Harteneck, 2005; Perraud *et al.*, 2005; Kaneko *et al.*, 2006; Zhang *et al.*, 2006; Hecquet and Malik, 2009). TRPM2 has C-terminal domain with enzymatic activity similar to Nudix hydrolases with ADPR hydrolase function. A model of oxidative and nitrosative stress has been proposed according to which mitochondria produce ADPR that activates TRPM2 via binding cleft in this domain (Perraud *et al.*, 2005). Further interaction of TRPM2 with the silent information regulator 2 (Sir2) contributes to its role in cell death (Grubisha *et al.*, 2006). TRPM8, one of the best studied TRP channel, has a major role in the cold sensation, which has been firmly established through its initial cloning strategy from cold-sensing trigeminal and DRG neurons (McKemy *et al.*, 2002; Peier *et al.*, 2002), extensive biophysical and pharmacological investigation (McKemy *et al.*, 2002; Peier *et al.*, 2002; Reid *et al.*, 2002; Behrendt *et al.*, 2004; Brauchi *et al.*, 2004; Voets *et al.*, 2004a; Hui *et al.*, 2005; Bandell *et al.*, 2006; Bodding *et al.*, 2007) and confirmed, more recently, by using mouse TRPM8 knockout models (Bautista *et al.*, 2007; Colburn *et al.*, 2007; Dhaka *et al.*, 2007). Interestingly, this same channel is clearly involved in cancer development (Zhang and Barritt, 2004; Bidaux *et al.*, 2005; Thebault *et al.*, 2005; Beck *et al.*, 2007; Bidaux *et al.*, 2007) whereby it shows increased expression that helped its initial cloning (Tsavaler *et al.*, 2001).

TRPM4 and TRPM5. Ca^{2+}-activated cation (CAN) channels are widely expressed in various excitable and non-excitable cells, including VSMC, where they play important roles in resting membrane potential control, rhythmical electrical activity and regulation of Ca^{2+} oscillations, but their molecular identity remained a mystery for a long time (Petersen, 2002). Identification of TRPM4b with the distinct properties of a CAN channel opened up a significant prospect for resolving the composition of CANs (Launay *et al.*, 2002). TRPM4

sensitivity to intracellular Ca^{2+} is controlled by multiple signalling events that include membrane potential, ATP, PKC-dependent phosphorylation and calmodulin (CaM) binding to C-terminal CaM domains (Nilius *et al.*, 2005b). Membrane potential strongly modulates channel activity in a Ca^{2+}-dependent manner, but an important difference with other Ca^{2+}-activated channels is that membrane depolarization alone is insufficient to open TRPM4 (Nilius *et al.*, 2003). Studies of TRPM4-deficient mice are revealing important roles of this channel in Ca^{2+}-dependent cell functions, such as regulation of cytoskeletal rearrangements in mast cell migration (Shimizu *et al.*, 2009). The related TRPM5 channel shows several important similarities as well as differences, including in pharmacological properties discussed later (Ullrich *et al.*, 2005). TRPM5 is highly expressed in the taste buds of the tongue where it has a key role in taste transduction (Talavera *et al.*, 2005; Liman, 2007; Zhang *et al.*, 2007), for example in sweet taste transduction as was recently revealed in TRPM5 knockout mice (Ohkuri *et al.*, 2009). TRPM5 also shows interesting sensing properties of the rate, rather than steady-state levels, of $[Ca^{2+}]_i$ change, which emphasizes its role in coupling Ca^{2+} release events to electrical activity (Prawitt *et al.*, 2003). Phosphatidylinositol-4,5-bisphosphate (PIP_2)- and CaM-binding domains regulate Ca^{2+} and voltage sensitivity of these channels (Nilius *et al.*, 2005c), while temperature increase in the range 15–35°C additionally shifts the voltage dependence towards more negative potentials (Talavera *et al.*, 2005).

TRPM6 and TRPM7. These are two other 'chanzymes' in the TRPM subfamily, which share a C-terminal atypical protein kinase domain. This domain is essential for channel function, at least in TRPM6 (Runnels *et al.*, 2001). Both channels are critically important for normal Mg^{2+} homeostasis. TRPM6 is tightly regulated by Mg^{2+} (fivefold higher affinity for Mg^{2+} compared with Ca^{2+}) and is Mg^{2+}-permeable (Voets *et al.*, 2004b). TRPM6 mutations cause hypomagnesemia with secondary hypocalcaemia (HSH) as a result of impaired renal and/or intestinal Mg^{2+} homeostasis. The naturally occurring TRPM6 mutation disrupts its assembly with TRPM7 to form functional TRPM6/7 complexes, providing cellular mechanism for HSH (Chubanov *et al.*, 2004). TRPM6 activity is uniquely regulated by a receptor for activated C-kinase 1 (RACK1) that associates with the alpha-kinase domain (Cao *et al.*, 2008). Homomeric TRPM6 and TRPM7 channels can be also activated at reduced pH with similar $pH_{1/2}$ values of about 4.5 while the $pH_{1/2}$ of heteromeric TRPM6/7 was shifted to 5.5 (Li *et al.*, 2006).

Receptor- and store-operated regulation of TRPM channels

Functional importance of TRPM channels is further highlighted by their regulation via pathways employing G protein-coupled receptors (GPCR) and Ca^{2+} store depletion. These mechanisms are especially relevant to our understanding of TRPM functions in the vasculature where Ca^{2+} homeostasis and hence vascular tone are strongly influenced by diverse and not yet completely understood receptor- and store-operated (ROC and SOC respectively) cation channels (Smani *et al.*, 2004; Beech, 2005; Albert and Large, 2006; Inoue *et al.*, 2006; Dietrich *et al.*, 2007; Firth *et al.*, 2007; Saleh *et al.*, 2008). TRPC channels are well known to be commonly activated by GPCR engaging phospholipase C (PLC), PIP_2 hydrolysis and $DAG/InsP_3$ production (Venkatachalam and Montell, 2007), but research on the relevance of these or related signal transduction pathways to TRPM function is at a very early stage. This deficiency is especially in contrast with the far better understood PIP_2-binding domains and PIP_2 roles in regulating TRPM4, TRPM5, TRPM7 and TRPM8 channels (Runnels *et al.*, 2002; Liu and Liman, 2003; Takezawa *et al.*, 2004; Liu and Qin, 2005; Rohacs *et al.*, 2005; Nilius *et al.*, 2006; Daniels *et al.*, 2009). Current consensus and controversies of PIP_2-dependent modulation of TRP channels have been recently reviewed (Rohacs, 2007; Voets and Nilius, 2007). In TRPM4/5/7/8 a rise in intracellular Ca^{2+} causes PLC activation, depletion of PIP_2 and channel desensitization that can be reversed by application of PIP_2. Thus, PIP_2 potentiates these channels (Figure 1). Specifically, in TRPM4 PIP_2 causes leftward shift of its voltage dependence and increases its Ca^{2+} sensitivity 100-fold (Nilius *et al.*, 2006). In addition, TRPM8 can be directly activated by exogenous PIP_2 (Liu and Qin, 2005), while cold, menthol and membrane depolarization increase the apparent affinity of TRPM8 for PIP_2 (Rohacs *et al.*, 2005). This is an important mechanism of adaptation to cold that can be regulated by receptor agonists (Daniels *et al.*, 2009).

While there is generally an agreement on the roles of PIP_2 in regulation of at least four members of the TRPM family, relative importance of PIP_2 depletion and other receptor signalling pathways (e.g. involving PKC and PKA) in channel regulation remains less clear (reviewed by Rohacs, 2007). Several studies have addressed the role of GPCR receptors in the regulation of TRPM channels. None of the melanocyte receptor agonists affected TRPM1 currents (Oancea *et al.*, 2009). TRPM2 activation was also insensitive to the stimulation of endogenous muscarinic receptors in HEK293 cells (Hara *et al.*, 2002), but this activated TRPM3 (Lee *et al.*, 2003). Consistently, these studies showed no effect of Ca^{2+} store depletion in the case of TRPM2, but activation in the case of TRPM3 (Table 1). It should be noted that sphingosine-induced TRPM3 activation is not mediated by Ca^{2+} store depletion (Grimm *et al.*, 2005). Also in accord with the stimulatory role of PIP_2 activation of M_1 ($G_{q/11}$/PLC-coupled) muscarinic receptor subtype was shown to potently inhibit TRPM4 activity (Nilius *et al.*, 2006). This was also the case for negative regulation of TRPM8 by the NGF receptor trkA, which stimulates PLCγ and hence PIP_2 hydrolysis (Liu and Qin, 2005). However, TRPM5 channel was stimulated by acetylcholine in HEK293 M_1-expressing cells contransfected with the chimeric G protein G16z44. This activation was only observed without intracellular Ca^{2+} buffering showing that physiological rise in $[Ca^{2+}]_i$ can activate TRPM5 despite parallel PIP_2 hydrolysis (Liu and Liman, 2003).

The situation with TRPM7 is even more complex, as this channel can be either activated or inhibited by GPCR. Activation of the $G_{q/11}$-coupled M_1 muscarinic receptor or the epidermal growth factor receptor inhibited heterologously expressed TRPM7 via PIP_2 depletion, while TRPM7 currents in ventricular fibroblasts were not modulated by angiotensin II or bradykinin, but inhibited by another $G_{q/11}$-coupled receptor, the lysophosphatidic acid receptor (Runnels *et al.*, 2002).

In addition, Takezawa *et al.* (2004) showed that this same channel can be regulated by pertussis toxin-sensitive G proteins: activation of β-adrenoceptors coupled to G_s potentiated TRPM7 while stimulation of muscarinic receptors coupled to G_i inhibited TRPM7 currents. The authors concluded that TRPM7 activity is up- and down-regulated in a cAMP- and protein kinase A-dependent manner, and that this regulation also involves TRPM7 endogenous kinase. Importantly, several key vasoactive agonists, including angiotensin II, bradykinin and aldosterone, have been shown to influence TRPM6/7 expression and activity in primary rat, mouse and human VSMC (He *et al.*, 2005; Touyz *et al.*, 2006; Callera *et al.*, 2009; Yogi *et al.*, 2009).

TRPM8 presents another interesting and unusual case of 'dissociation' between PIP_2 and Ca^{2+} store depletion effects. While activation of receptors coupled to $G_{q/11}$ inhibits TRPM8 (Liu and Qin, 2005) the channel is strongly potentiated by Ca^{2+} store depletion (Abeele *et al.*, 2006; Bidaux *et al.*, 2007). This effect engages chemical signalling via lysophospholipids (LPL) that strongly potentiate TRPM8 (Abeele *et al.*, 2006; Andersson *et al.*, 2007). These are produced following Ca^{2+} store depletion and activation of the Ca^{2+}-independent phospholipase A_2 (GVI, or $iPLA_2$), a novel mechanism for the activation of SOC channels (Smani *et al.*, 2004). The recently discovered functional interaction of several TRPCs, which are currently considered as the main TRP components of this Ca^{2+} entry pathway, with STIM–Orai1 complexes suggests that SOC/CRAC channels are heteromeric complexes that include both TRPCs and Orai proteins (Liao *et al.*, 2008). Activation of TRPM3/8 by Ca^{2+} store depletion thus raises the possibility that some TRPMs can also function as molecular components of SOC channels.

Tissue expression profiles of TRPM channels

Functional roles of TRPM channels are further supported by the analysis of their expression profiles. These channels are widely expressed in both excitable and non-excitable cells, which suggests their physiological roles in various organs. Of 22 TRP channels (TRPC, TRPV, TRPM and TRPA) analysed in the mouse, TRPM7 and TRPC3 showed consistence dominance in most tissues, TRPM3/7 dominated in brain and TRPM3/7 as well as TRPC3/6 mRNAs were characteristically present in all tested muscle tissues (Kunert-keil *et al.*, 2006). Fonfria *et al.* (2006) have recently performed a similarly systematic comparative survey of TRPM mRNA expression both in human peripheral tissues and in the CNS using TaqMan and SYBR Green quantification. They found the highest expression of TRPM1/2/3 in the brain while other TRPM subtypes were predominantly expressed in the viscera, such as intestine (TRPM4/5/6), prostate (TRPM4/5/8), pancreas (TRPM5) and liver (TRPM8) (Table 1). Numerous studies detected TRPM channel expression in the skin, melanocytes (TRPM1), kidney (TRPM3/4/6), lung (TRPM2), endothelium (TRPM2/3/4), uterus (TRPM5), testis (TRPM5/8) and bladder (TRPM8), but further comparisons are difficult to make due to differences in the techniques used (Fleig and Penner, 2004; Harteneck, 2005; Kraft and Harteneck, 2005; Ramsey *et al.*, 2006; Nilius *et al.*, 2007; Venkatachalam and Montell, 2007).

Comparative analysis of TRPM expression specifically in blood vessels showed the highest expression of TRPM8 both in pulmonary artery and aorta while TRPM2/3/4/7 were also expressed in these vessels (Yang *et al.*, 2006). Moreover, TRPM2/8 protein expression was confirmed by Western blot analysis while functional responses to the TRPM8 agonist menthol showed dependence on external Ca^{2+} and nifedipine resistance suggesting the functional role of TRPM8 in pulmonary and systemic circulation. In addition, we showed TRPM8 expression in rat aorta, tail, femoral and mesenteric arteries by semiquantitative PCR, Western blotting and immunocytochemistry, the latter showing predominant TRPM8 expression on the cell boundary (Johnson *et al.*, 2009). TRPM4/6/7 are also expressed in cerebral and mesenteric arteries (Inoue *et al.*, 2006; Touyz *et al.*, 2006; Firth *et al.*, 2007; Inoue *et al.*, 2009; Yogi *et al.*, 2009).

The evidence for TRPM expression in the vasculature and the knowledge of the diverse mechanisms of activation of TRPM channels naturally leads us to consider their various functional roles in VSMC, which have been increasingly emerging during the last 5 years. This research has been aided by the available pharmacological tools for the study of TRPM channels as discussed below.

Pharmacology of TRPM channels

Both agonists and antagonists are available for most TRPM subtypes, as summarized in Table 2. For some TRPMs, such as TRPM2/4/8, the list of known channel ligands is very extensive, but for others (e.g. TRPM1) pharmacological modulators are still lacking. Table 2 summarizes data on the available activators and their affinities [expressed as pD_2, or $-\log(EC_{50})$], including references; when this was not established the concentration range used is shown. Apparent affinities of antagonists are given as the IC_{50} value; when single concentration is shown, this caused significant or complete inhibitory effect. Several pharmacological features of TRPM channels can be summarized as follows.

High-affinity selective and potent TRPM ligands are generally lacking, which is a common problem in TRP research. The effective concentrations are typically in the range of micromolar or even millimolar. For example, 2-APB, which is considered as a general inhibitor of TRP channels, commonly inhibits TRPM2/3/7/8 channels, although between individual members its apparent affinity varies from 1 to 200 μM. Several other blockers, such as SKF-96365, flufenamic acid, tetracaine, ruthenium red and spermine also inhibit other TRP and non-TRP channels, often with much higher potency (e.g. ruthenium red inhibits ryanodine receptors at nanomolar concentrations and it is a potent blocker of several TRPVs; flufenamic acid inhibits various chloride and potassium channels and SKF-96365 inhibits various ROC and, especially, SOC channels). However, there are also some exceptional examples, for example TRPM2 is insensitive to La^{3+} although lanthanides commonly inhibit TRP channels including TRPM3/4/7. ATP^{4-} allows pharmacological differentiation between TRPM4 and TRPM5. In the case of TRPM8 its most potent known antagonist clotrimazole also shows characteristic voltage dependency (e.g. higher potency at negative potentials explained by a positive shift of the activation curve caused by the blocker). Clotrimazole can be an especially

useful pharmacological tool to discriminate between TRPM8- and TRPA1-mediated responses as it has the opposite, activating effect on TRPA1 (Meseguer *et al.*, 2008). Another recently discovered antagonist AMTB [*N*-(3-aminopropyl)-2-([(3-methylphenyl) methyl]oxy)-*N*-(2-thienylmethyl)benzamide hydrochloride salt] also shows high affinity among other TRPM8 blockers; these include BCTC, thio-BCTC and capsazepine that are well-known TRPV1 antagonists. TRPM6/7 channels are inhibited by Mg^{2+} or Ca^{2+}; in contrast, Ca^{2+} potentiates TRM2/4/5. Inhibition of the ubiquitously expressed TRPM7 by intracellular Mg^{2+} can be very useful for isolation of currents mediated by other TRPMs (e.g. Oancea *et al.*, 2009).

TRPM agonists also offer several unique properties for characterization of individual channel subtypes. Oxidants, such as H_2O_2, β-NAD^+, ADPR, cADPR and $NAADP^+$ activate TRPM2. Interestingly, the activation by cADPR shows strong temperature sensitivity: cADPR does not activate TRPM2 at 25°C, but heat dramatically potentiates TRPM2 activation by this ligand (Togashi *et al.*, 2006). TRPM3 is activated by D-erythrosphingosine and SPH analogues, but not by other lipids, including ceramides, S1P, AA and DAG. TRPM3 activation by the neurosteroid pregnelone sulphate allowed its recent identification as an essential component of an ionotropic steroid receptor in pancreatic β-cells (Wagner *et al.*, 2008). The action of pregnelone sulphate and closely related substances (e.g. pregnenolone) indeed seems very specific to TRPM3 as other TRPs (TRPM2/7/8 and TRPV1/4/6) are insensitive to these steroids. TRPM4 can be activated by BTP2 (4-methy-4′-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]-1,2,3-thiadiazole-5-carboxanilide) in a voltage-dependent manner while decavanadate activates TRPM4 but not TRPM5. Interestingly, the related TRPM6 and TRPM7 channels are activated, rather than inhibited, by 2-APB that acts more potently on TRPM6. TRPM8 displays perhaps the most impressive array of known activators and their discovery was often guided by the analysis of compounds that are well known to produce cooling sensation, such as icilin, menthol and its derivatives. Interestingly, chemicals with diverse structures can act as TRPM8 agonists. Among them the carboxamides WS-12, CPS-369 and CPS-113 activate TRPM8 with EC_{50} in sub- to low micromolar range. These new pharmacological tools to study TRPM8 show specificity as they do not activate TRPM3 and TRPV6. TRPM8 channel is also strongly activated by LPL, such as lysophosphatidylcholine and lysophosphatidylinositol but it is inhibited by polyunsaturated fatty acids such as arachidonic acid (Abeele *et al.*, 2006; Andersson *et al.*, 2007). Lysophosphatidylcholine is also known to activate a distant TRPC5 channel (Flemming *et al.*, 2006). Moreover, two widely used TRPM8 agonists, menthol and icilin, can also activate another cold-sensitive TRPA1 channel, although for menthol the modulation can be bimodal or even species-specific (Story *et al.*, 2003; Karashima *et al.*, 2007; Xiao *et al.*, 2008).

Both intracellular Ca^{2+} measurements and more direct patch-clamp measurements of ion currents have been extensively used in evaluating affinity and potency of pharmacological modulators of TRPM channels. In some cases, large, up to 2–3 orders, differences in the apparent ligand affinity are also notable, which may arise from the differences in the techniques or cell expression system used in different studies. WS-12 well exemplifies such differences in pharmacological properties: its apparent affinity (EC_{50}) for TRPM8 varies from 39 nM in current measurements in TRPM8 expressing HEK293 cells (Beck *et al.*, 2007) to 193 nM in Fura-2 calcium measurements or 680 nM in current measurements in the same study using TRPM8 expressing HEK293 cells (Bodding *et al.*, 2007). A much higher value (12 μM) was reported in TRPM8 current measurements when the channel was expressed in *Xenopus laevis* oocytes (Sherkheli *et al.*, 2008). Such possible differences in channel pharmacological properties depending on cell environment are further highlighted by findings that indicate substantial differences in the biophysical properties of native and recombinant channels (e.g. about 140 mV difference in the potential of half-maximal activation of TRPM8) and their stronger modulation by the same drug in native cells (Malkia *et al.*, 2007).

TRPM channel in vascular function and disease

The above-discussed diverse mechanisms of channel activation are paralleled by diverse vascular functions of TRPM channels, as is increasingly recognized during the last 5 years. These range from mechanosensory transduction and regulation of the arterial myogenic response to magnesium transport in hypertension, and from cold sensitivity to vascular inflammation (Figure 1).

TRPM2 channels are likely to be involved in a range of pathophysiological processes in oxidant-induced vascular injury, cerebral ischemia and stroke (Simard *et al.*, 2007; Hecquet and Malik, 2009). In endothelial cells, TRPM2 activation by H_2O_2 causes Ca^{2+} entry thus increasing endothelial permeability (Hecquet *et al.*, 2008). In monocytes, ROS-evoked Ca^{2+} entry via TRPM2 is a key trigger of chemokine production in inflammation. These processes are attenuated by the *Trpm2* gene disruption in mice (Yamamoto *et al.*, 2008). The functional roles of TRPM2 channels expressed in VSMCs are not known, but they may contribute to atherosclerosis development, which is associated with mitochondrial dysfunction, ROS production and inflammation. The related TRPM8 channel is the most highly expressed TRPM member in blood vessels (Yang *et al.*, 2006). Notably, the heat-sensitive TRPV4 is the most highly expressed TRPV channel and the presence of these thermoTRPs in blood vessels raises intriguing questions as to their functional roles. Although most blood vessels are not exposed to any essential temperature variations, thermal control of cutaneous vessels is physiologically important, but not completely understood (Roosterman *et al.*, 2006). Cooling of peripheral blood vessels causes vasoconstriction that is important for heat conservation, but excessive or prolonged cooling causes vasodilatation [this includes a poorly understood non-neurogenic component (Johnson *et al.*, 2005)] and rapid heat loss. We found that TRPM8 channels are both expressed and functional in several rat arteries. Both in isolated cells and in blood vessels *in situ* menthol produced Ca^{2+} transients that consisted of an initial 'phasic' component, followed by a sustained component. The 'phasic' component appeared as asynchronous intracellular propagating Ca^{2+} waves associated with asynchronous mechanical oscillations that integrated into a small

contraction of the vessel segment. Both components were resistant to nifedipine suggesting little role for voltage-gated Ca^{2+} channels (Borisova *et al.*, 2008). In contractile studies, the major effect of TRPM8 activation was seen in precontracted vessels, where TRPM agonists, menthol and icilin, caused a profound vasodilation and similar effects were also observed in human forearm cutaneous vessels (Johnson *et al.*, 2009). Interestingly, in different microvessels TRPV1 activation can also cause vasoconstriction or vasodilation (Kark *et al.*, 2008) while TRPV4 activation, especially in endothelial cells, causes vasodilatation (Earley *et al.*, 2005; Zhang *et al.*, 2009). TRPM8 can also be activated by Ca^{2+} store depletion (Thebault *et al.*, 2005; Abeele *et al.*, 2006) raising the possibility that it can function as a component of SOC channel in VSMC. Importantly, this pathway involves $iPLA_2$ activation and LPLs generation (Smani *et al.*, 2004; Abeele *et al.*, 2006) making TRPM8 a likely factor in the development of atherosclerosis, but these roles are awaiting further investigation.

Although TRPM3 shows mechanosensitive properties, it was the TRPM4 channel that received much attention as the channel involved in myogenic constriction that can offer new insights in the molecular nature of myogenic tone control by Ca^{2+} and PKC (Earley *et al.*, 2004; Earley *et al.*, 2007; Brayden *et al.*, 2008). The authors used antisense technology to suppress TRPM4 expression in cerebral arteries [this was necessary due to the lack of selective TRPM4 blockers (Table 2)] and found reduction of pressure- and protein kinase C (PKC)-induced VSMC depolarization as well as pressure-induced vasoconstriction. Thus, both TRPM4 and the earlier studied TRPC6 have now been implicated in generation of the myogenic response due to their direct or indirect mechanosensitivity. Functional properties of TRPM4/5 channels also make them excellent candidates for various poorly understood CAN channels in VSMC, but any insight here is still missing and TRPM5 does not seem to be expressed in VSMC.

TRPM6 and TRPM7 channels regulate Mg^{2+} homeostasis that is reflected in their major roles in vascular Mg^{2+} transport and implicates them in hypertension (He *et al.*, 2005; Hamaguchi *et al.*, 2008; Touyz, 2008; Paravicini *et al.*, 2009). Furthermore, TRPM7 may be a novel mechanosensor in VSMC, the function of which can be altered in hypertension as reviewed by Touyz (2008). In VSMC, but not in endothelial cells, fluid flow increases TRPM7 current as the channel is translocated to the plasma membrane suggesting a TRPM7 role in cellular response to vessel injury (Oancea *et al.*, 2006). In addition, silencing TRPM7 by siRNA or its inhibition by 2-APB or Gd^{3+} promoted growth and proliferation of vascular endothelial cells as well as production of nitric oxide, the critically important endogenous vasodilator (Inoue and Xiong, 2009).

Challenges of studying TRPMs and future perspectives

Multifunctional non-selective TRPM cation channels are the important players regulating vascular function and potential new targets for treating vascular disease. Numerous pharmacological tools are available for the study of TRPM subfamily members, but there are important concerns considering that: (i) most ligands have limited selectivity and/or potency; (ii) most studies have been performed in artificial cell systems, with notable examples of differences in the pharmacological properties of recombinant TRPMs depending on the expression system; and (iii) heteromultimerization of TRPM isoforms and their interaction with non-TRP proteins in native vascular cells can also alter their pharmacological properties. Thus, any robust identification of functional roles of TRPM subtypes in native cells requires a combination of various approaches, the use of several ligands to characterize the pharmacological profile of the channel in question as well as its biophysical 'signature'.

Molecular biology approaches such as antisense or siRNA technologies have been widely used in TRP vascular research attempting to overcome the problems of limited selectivity of current pharmacological tools. Antibodies targeting extracellular loops near the channel pore region are also being introduced (Naylor *et al.*, 2008) and will undoubtedly aid this research. Genetic studies of hereditary disorders (exemplified by TRPM6 defect in HSH) and knockout mouse models are revealing specific roles of TRPMs in diverse biological processes, such as magnesium homeostasis, mast cell migration, inflammatory responses and sensing of cold and taste. These models will be indispensible in decoding specific roles of TRPM channels in the vasculature in future studies, with caution regarding possible compensatory up- or down-regulation of other ion channels or altered expression of transcription and growth factors.

With all this effort, there have been several exciting developments in the area of vascular roles of TRPM channels in recent years. These roles often conformed to the expectations based on the knowledge of the activation mechanisms and functional properties of heterologously expressed TRPM channels, but we have also seen several novel and unexpected developments, including the identification of mechanosensory roles of vascular TRPM4 and TRPM7 channels. Numerous other vascular functions of TRPMs can be envisaged based on their known properties as indicated by the question marks in Figure 1. This area of research presents many challenging tasks, and likely holds many new surprises. TRPM pharmacological tools discussed here will remain an invaluable resource in this continuing research.

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Conflict of interest

The author has no conflict of interests.

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