Published in final edited form as:

Mol Cell Endocrinol. 2012 April 28; 353(1-2): 68–74. doi:10.1016/j.mce.2011.10.023.

Regulation of TRPM8 channel activity

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Abstract

Transient Receptor Potential Melastatin 8 (TRPM8) is a Ca^{2+} permeable non-selective cation channel directly activated by cold temperatures and chemical agonists such as menthol. It is a well established sensor of environmental cold temperatures, found in peripheral sensory neurons, where its activation evokes depolarization and action potentials. The activity of TRPM8 is regulated by a number of cellular signaling pathways, most notably by phosphoinositides and the activation of phospholipase C. This review will summarize current knowledge on the physiological and pathophysiological roles of TRPM8 and its regulation by various intracellular messenger molecules and signaling pathways.

1. Introduction

Transient Receptor Potential Melastatin 8 (TRPM8) is a member of the TRP ion channel family; its major physiological activator is cold, and it is also activated by “cooling agents” such as menthol. TRPM8 is a well established physiological sensor of environmental cold temperatures. Its activators, cold and menthol have been used for many years as local pain relieving agents. This review will briefly summarize current knowledge on the physiology and pathophysiology of TRPM8, as well as its regulation by cellular signaling pathways.

2. TRPM8 channel discovery, general properties and structure

The first report with a short characterization of a cold- and menthol-sensitive current in peripheral sensory neurons was published in 2001 (Reid and Flonta, 2001). Shortly after this report two independent research groups cloned a cold- and menthol-sensitive ion channel from sensory neurons (McKemy et al., 2002; Peier et al., 2002). One year before these neuronal studies TRPM8 was also cloned from prostate epithelial cells, and was identified as a transcriptional marker there (Tsavalier et al., 2001).

TRPM8 is an outwardly rectifying, Ca^{2+} permeable, nonselective cation channel activated by temperatures below 26 °C. Many chemical compounds also activate TRPM8, including menthol, the supercooling reagent icilin (Peier et al., 2002; McKemy et al., 2002), and various other substances, such as the recently found selective agonists WS-12 and CPS-369 (Behrendt et al., 2004; Bodding et al., 2007; Sherkheli et al., 2010). These compounds shift the activation threshold of the channel to higher temperatures. Activation of TRPM8 is also voltage-dependent and agonists shift its voltage dependence to more negative membrane

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potentials (Brauchi et al., 2004; Voets et al., 2004). Several antagonists of TRPM8 have also been identified, including BCTC, thio-BCTC, capsaizepine, SKF96365 and AMTB (Behrendt et al., 2004; Malkia et al., 2007; Lashinger et al., 2008).

TRPM8 has 6 transmembrane (TM) domains with cytoplasmic C and N-termini, and the functional channel is formed by the homotetrameric assembly of 4 subunits (Ramsey et al., 2006). A unique TRP domain is located at the proximal C-terminal region (Clapham, 2003; Montell, 2001) followed by a coiled-coil region. TRPM8 unlike TRPC and TRPV channels does not contain any ankyrin-repeat domains in the N-terminal cytoplasmic domain. Residues located in the TM2 region and the TRP domain are involved in sensitivity to icilin and menthol, but not in sensitivity to cold (Bandell et al., 2006). For sensitivity to icilin the role of specific additional structural elements in the TM2-TM3 linker were shown that were not required for menthol activation (Chuang et al., 2004).

3. Physiological expression pattern

The cell bodies of temperature sensitive neurons are located in either trigeminal ganglia (TG) or dorsal root ganglia (DRG); their peripheral processes belong mostly to C and A δ fibers. TRPM8 protein and RNA was found in about 5-10 % of DRG and 10-15 % of TG neurons (McKemy et al., 2002; Peier et al., 2002; Okazawa et al., 2004; Abe et al., 2005; Sarria and Gu, 2010). The very low percentage of TRPM8 expressing neurons had been a significant hurdle in characterizing these channels in their native environment. To overcome this, two transgenic mouse lines were developed in which TRPM8 positive neurons express EGFP *in vivo* (Dhaka et al., 2008; Takashima et al., 2007). These models allow identification of TRPM8 expressing neurons *in vitro* and *in vivo* without prior exposure to the agonist. Detailed analysis of these EGFP expressing DRG neurons and their peripheral nerve endings demonstrate that they project to multiple termination zones localized in the superficial layer of glabrous and epidermis/dermal boundary of hairy skin, and TG nerve terminals innervate the oral cavity epithelium, distinct regions in the tooth pulp and dentin, and localized in the outermost tongue epithelium. Centrally they project primarily to lamina I and outer region of lamina II of the dorsal horn of the spinal cord, where they converge and relay sensory information to interneurons. DRG neurons expressing TRPM8 also innervate visceral organs such as the colon (Harrington et al., 2011).

Breathing cold air may induce airway constriction and asthma which is mediated by vagal afferent nerves (Jammes et al., 1983; Giesbrecht and Younes, 1995; Giesbrecht et al., 1993). TRPM8 expression in the vagal neurons innervating bronchopulmonary tissue and its cold activation has recently been reported (Xing et al., 2008). Airway constriction could also be evoked by inhalation of menthol or peppermint oil vapor (Kawane, 1996; dos Santos et al., 2001). On the other hand, menthol is a well known anti-cough agent, and accordingly, it was shown to attenuate respiratory irritation responses to cigarette smoke irritants (Willis et al., 2011). Therefore TRPM8 in the vagal neurons is a possible target for various respiratory disorders.

TRPM8 has also been shown to be expressed in a relatively distinct pattern in skeletal and smooth muscle, epithelia of the prostate, lungs, bladder and urogenital tract, where its function still remains mostly unclear (Stein et al., 2004; Mukerji et al., 2006). Functional expression of TRPM8 as well as co-expression with different TRPV channels was demonstrated in rat intralobar pulmonary arteries and aortic smooth muscle, where they play a role of the Ca²⁺ entry pathway in the arterial myocytes (Yang et al., 2006). TRPM8 mRNA and protein was also detected in rat tail, mesenteric, femoral arteries and the aorta (Johnson et al., 2009). Activation of TRPM8 with chemical agonists produced a dual effect.

On relaxed vessels it lead to small contraction and induced relaxation on precontracted ones. The first phenomenon could be explained by Ca^{2+} entry through the channel which confirmed previous similar observation of menthol effect on smooth muscle (Ito et al., 2008;Wright et al., 1997) and for the second effect the involvement of Ca^{2+} independent PLA_2 pathway was proposed (Johnson et al., 2009).

4. Behavioral experiments, knock-out animals

To investigate the *in-vivo* role of TRPM8 in detecting cold temperatures, three different groups reported successful generation of TRPM8 deficient mice. These animals had no difference from wild- type mice in overall appearance, general behavior, viability, core body temperature and anatomical structure of the sensory ganglia (Bautista et al., 2007;Colburn et al., 2007;Dhaka et al., 2007). Functionally TRPM8 knock-out mice demonstrated significantly reduced number of cold sensitive, and almost complete elimination of menthol sensitive DRG and TG neurons(Bautista et al., 2007;Colburn et al., 2007;Dhaka et al., 2007). A severe deficit was also observed in the cold sensitivity and responsiveness of C and A δ afferent nerve fibers (Bautista et al., 2007). Most importantly, behavioral experiments such as various temperature preference tests, confirmed the role of TRPM8 in detecting mildly cold temperatures (Bautista et al., 2007;Colburn et al., 2007;Dhaka et al., 2007).

TRPM8^{-/-} animals did not completely lose temperature sensitivity, however, especially to noxious cold stimuli (around 0°C), and a residual number of neurons responded with a intracellular Ca^{2+} increase to the cold activation (Bautista et al., 2007;Colburn et al., 2007;Dhaka et al., 2007;Story et al., 2003). This is consistent with the detection of a subpopulation of menthol-insensitive cold- sensitive neurons, and suggests TRPM8 independent mechanisms that are activated at noxious cold temperatures (Babes et al., 2006;Story et al., 2003;Smith et al., 2004;Munns et al., 2007). A possible candidate is the TRPA1 channel, which has originally been proposed to be a noxious cold-sensor (Story et al., 2003). This channel is clearly activated by a number of noxious compounds, including mustard oil and formaldehyde (Jordt et al., 2004), but its activation by cold temperatures is controversial (Knowlton et al., 2010). Other possible molecular components of cold sensitivity could be members of the two-pore (2P) domain potassium channel family. Some of these “leak” K^+ channels are more active at warm temperatures, thus their closure at cold temperatures depolarize the neuron. Indeed for the 2P channels TREK1 and TRAAK a regulatory role in nociceptor activation by cold was demonstrated, especially in the population that is not activated by menthol (Noel et al., 2009).

Data obtained in TRPM8 knockout studies also indicated that TRPM8 is required for cold allodynia (pain evoked by innocuous cold) in rodent models of neuropathic pain. Assessment of TRPM8 in the neuropathic cold hypersensitivity was performed on the chronic constriction injury (CCI) surgery model on the sciatic nerve for both wild and TRPM8 knock-out mice (Colburn et al., 2007). TRPM8^{-/-} mice exhibited no significant increase in the cold sensitivity similar to sham operated animals after CCI, while wild-type mice developed increased sensitivity to cold. Similar results on the cold- or menthol-induced nocifensive behavior after CCI were obtained by (Caspani et al., 2009). The involvement of TRPM8 also was demonstrated in the cold hypersensitivity after inflammation induced by Complete Freund’s Adjuvant (CFA) injection (Colburn et al., 2007). Furthermore, it was shown recently that TRPM8 antagonists are effective in reversing established pain in visceral pain models (Lashinger et al., 2008).

Topical application of menthol has been known for many years to induce a cooling sensation and act as an analgesic in certain conditions (Galeotti et al., 2002;Klein et al., 2010;Proudfoot et al., 2006;Wasner et al., 2008). Topical cold is also commonly used to

alleviate pain. The role of TRPM8 in cold- and menthol-induced analgesia was confirmed by experiments with TRPM8 knock-out mice (Dhaka et al., 2007). After injection of 2% formalin in the paw, mild cooling (17°C cold plate experiment) decreased both acute and inflammatory phase nociceptive response for the wild-type animals, while in TRPM8 deficient mice analgesia was observed only for the second, inflammatory phase. Using a rodent CCI model of neuropathic pain and several models of inflammatory pain (Proudfoot et al., 2006) demonstrated that low level of cooling or topical application of low concentrations of menthol or icilin produced temporary analgesia mediated by TRPM8, as demonstrated by antisense down-regulation of TRPM8. Involvement of the centrally mediated processes in the TRPM8 agonists-induced analgesia was confirmed by reversal of CCI-induced sensitization of behavioral response in thermal and mechanical tests after the intrathecal injection of icilin or menthol (Proudfoot et al., 2006).

Overall, TRPM8 is a major pathway responsible for the perception of noxious and innocuous ambient cooling temperatures, mediates cold-induced analgesia and plays a role in cold hypersensitivity in pathophysiological conditions.

5. TRPM8 and cancer

It is noteworthy that TRPM8 protein and mRNA expression was demonstrated in different types of cancer including prostate, breast, lung and colon, whereas it was only minimally expressed in the corresponding non-malignant tissue. The presence of TRPM8 in these tissues may be linked to the control of cell cycle progression, cell division and cell migration. In the prostate it was reported that TRPM8 is mostly expressed in the apical region of prostate secretory epithelial cells, with much lower expression in the basal regions (Bidaux et al., 2005;Bidaux et al., 2007). In the prostate cancer epithelial androgen-responsive LNCaP cell line, TRPM8 has been reported to be expressed both in the plasma membrane (PM) and the endoplasmic reticulum (ER). There is a relationship between its subcellular localization, functional Ca^{2+} entry through the PM and store depletion from the ER by channel activation and the androgen dependence of the cells (Zhang and Barritt, 2004;Thebault et al., 2005;Bidaux et al., 2007). Moreover, expression of TRPM8 is up-regulated in the differentiated carcinoma of prostate epithelia, but its expression is mostly abolished when the cells become de- differentiated, lost their luminal secretory phenotype and cancer developed to the androgen-independent stage (Tsavaler et al., 2001;Bidaux et al., 2005). Henshall and colleagues also observed that anti- androgen therapy lead to a great reduction of TRPM8 expression in prostate cells (Henshall et al., 2003). Dedifferentiation of the cells abolished TRPM8 activity in the PM, while TRPM8 in the ER remained functional regardless of the differentiation status of the cells (Bidaux et al., 2007).

Strong immunoreactivity for TRPM8 was found both in human pancreatic adenocarcinoma tissue and cell lines, compared to the trace level of TRPM8 in the normal tissue (Yee et al., 2010). Directed knock down of TRPM8 with siRNA lead to the arrest of cellular proliferation with increased proportion of the cells in G_0/G_1 phase and also an increase in non-apoptotic and apoptotic cell death (Yee et al., 2010;Zhang and Barritt, 2004). Expression of TRPM8 and its role in the cell survival was also shown for the human melanoma G-361 cell line (Yamamura et al., 2008).

Though the exact mechanism responsible for the up-regulated expression of TRPM8 in the majority of the cancer cells is unknown, in prostate cancer cells the presence of a putative androgen response element was demonstrated in the promoter region of TRPM8 and eleven androgen-response elements were identified in introns of the TRPM8 gene (Zhang and Barritt, 2004;Bidaux et al., 2005). It is possible that androgen hormones also change the level of TRPM8 expression in pancreatic adenocarcinoma through the same mechanism

because androgen receptors are variably expressed in human pancreatic adenocarcinoma cell lines (Konduri et al., 2007). For breast carcinoma and the breast cancer cell line MCF-7 the regulation of TRPM8 mRNA expression by estrogens was shown through ER2 receptors (Chodon et al., 2010).

The fact that in tissue specimens of patients with prostate cancer TRPM8 expression was up-regulated more than any other prostate specific gene compared to non malignant tissues (Fuessel et al., 2003) could mean that TRPM8 plays an important role in the pathophysiology of epithelial cells and suggests that it could be a future diagnostic/prognostic marker and potential therapeutic target.

6. Intracellular signaling pathways regulating TRPM8 activity

TRPM8 retains cold and menthol sensitivity even when removed from the cellular environment, in excised patches (Rohacs et al., 2005; Liu and Qin, 2005) and in planar lipid bilayers (Zakharian et al., 2010), indicating that cold and chemical activators act on the channel directly. Channel activity of the purified protein in planar lipid bilayers required the presence of its cofactors, phosphoinositides and inorganic polyphosphate which is tightly associated with the channel protein (Zakharian et al., 2009). Direct activation by its agonists, however does not mean, that signaling pathways do not regulate TRPM8 activity in a cellular context. Indeed the reported differences in cold-sensitivity of native neuronal and recombinant TRPM8 as well as differences in between individual cold sensing neurons (Reid, 2005) argue for the important role of the cellular environment in fine tuning TRPM8 activity.

6.1 Regulation by phosphoinositides

Phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P₂], commonly referred to as PIP₂ is a well established intracellular regulator of TRPM8. PtdIns(4,5)P₂ is a minor component of the inner leaflet of the plasma membrane, constituting approximately 1 % of the lipids there. Phospholipase C (PLC) enzymes hydrolyze PtdIns(4,5)P₂ to form the two classical second messengers inositol 1,4,5 trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ binds to its receptor in the endoplasmic reticulum and releases Ca²⁺, whereas DAG is an activator of protein kinase C (PKC) enzymes. PtdIns(4,5)P₂ regulates the activity many different ion channels, including members of the TRP family (Hilgemann et al., 2001; Suh and Hille, 2008; Nilius et al., 2008; Rohacs, 2009). The original evidence for the PtdIns(4,5)P₂ dependence of TRPM8 was obtained in excised inside-out patch measurements where phospholipids can be directly applied to the cytoplasmic surface of the patch membrane. Immediately after establishing the excised inside-out patch configuration channel activity showed significant decrease or rundown (Rohacs et al., 2005; Liu and Qin, 2005), which is a common characteristic for PtdIns(4,5)P₂ dependent channels and thought to be mediated by depletion of PtdIns(4,5)P₂ by lipid phosphatases (Huang et al., 1998; Zhang et al., 1999). The PtdIns(4,5)P₂ scavenger polylysine accelerated this rundown in excised inside-out patches (Rohacs et al., 2005) and inhibited channel activity when it was dialyzed through the patch pipette in whole-cell patch clamp experiments (Liu and Qin, 2005). Application of various PtdIns(4,5)P₂ analogues to the intracellular surface of the patch membrane restored channel activity (Liu and Qin, 2005; Rohacs et al., 2005). The effect of PtdIns(4,5)P₂ had a high level of specificity, since its precursor PtdIns(4)P and other phospholipids, such as PtdIns(3,4)P₂ and PtdIns(3,4,5)P₃ had much smaller effects (Rohacs et al., 2005). Direct activation of TRPM8 by PtdIns(4,5)P₂ was confirmed on the purified reconstituted TRPM8 protein in planar lipid bilayers (Zakharian et al., 2009; Zakharian et al., 2010), where activation of TRPM8 by both cold and menthol depended on the presence of PtdIns(4,5)P₂. Phosphoinositides in planar lipid bilayers activated TRPM8 with a very similar specificity profile to that observed in excised patches (Rohacs et al., 2005; Zakharian et al., 2010).

Both cold and menthol increased the apparent affinity of TRPM8 for PtdIns(4,5)P₂, i.e. resulted a shift in dose-response activation curve to the left both in excised patches (Rohacs et al., 2005) and in planar lipid bilayers (Zakharian et al., 2010). Voltage dependence of TRPM8 was also modulated in a similar manner by both cold and menthol (Voets et al., 2004) and by PtdIns(4,5)P₂ (Rohacs et al., 2005) all of which shifted I-V relationship to more negative voltages. Conversely, the apparent affinity of TRPM8 for PtdIns(4,5)P₂ is higher at positive than at negative voltages (Rohacs et al., 2005). Thus several different factors - chemical agonists, temperature, voltage and PtdIns(4,5)P₂ - regulate TRPM8 channel activity in a complex manner, each affecting the sensitivity to the others. Full description of this complex behavior would require an allosteric model that took into account interaction between all these factors.

6.2. Desensitization and adaptation of TRPM8

Most sensory modalities undergo adaptation, or desensitization, i.e. decreased activity despite the continuous presence of the stimulus. This is also true for TRPM8; both native and heterologously expressed channels show decreasing activity during cold or menthol activation in the presence of extracellular Ca²⁺ (McKemy et al., 2002; Okazawa et al., 2002; Daniels et al., 2008). It was proposed that Ca²⁺ influx through TRPM8 activates a Ca²⁺-sensitive PLC isoform, which in turn hydrolyses PtdIns(4,5)P₂ and the depletion of the lipid limits TRPM8 channel activity (Rohacs et al., 2005; Daniels et al., 2008; Yudin et al., 2011) (Figure 1A). This mechanism was subsequently demonstrated for several other TRP channels, including TRPV1 (Lukacs et al., 2007; Yao and Qin, 2009) TRPV6 (Thyagarajan et al., 2001; Thyagarajan et al., 2009) and TRPV2 (Mercado et al., 2010). Besides the well documented dependence of channel activity on PtdIns(4,5)P₂, the following data support this model for TRPM8. PLC activation in response to menthol, icilin and cold was shown by demonstrating the translocation of the PLC δ pleckstrin homology (PH) domains, that bind PtdIns(4,5)P₂, from the plasma membrane to the cytoplasm in TRPM8 expressing cells (Rohacs et al., 2005; Daniels et al., 2008; Yudin et al., 2011). Co-expression of the type I phosphatidylinositol-4-phosphate 5-kinase (PIP5K), which catalyzes formation of PtdIns(4,5)P₂ from its precursor PtdIns(4)P, significantly attenuated current desensitization, while co-expression of the Ca²⁺ sensitive PLC δ 1 isoform accelerated it (Rohacs et al., 2005). Dialysis of PtdIns(4,5)P₂ through the whole-cell patch pipette inhibited desensitization of menthol-induced currents both in expression systems and in DRG neurons (Yudin et al., 2011), see also Figure 1B. Direct activation of PLC with the benzenesulfonamide compound m-3M3FBS produced significant decrease in the cold and menthol-induced current in TG and in heterologous expressing systems (Daniels et al., 2008).

PLC activation however results in not only a reduction in PtdIns(4,5)P₂ levels, but also a number of other downstream signaling events. TRPM8 was also shown to be inhibited by PLC-independent reductions in PtdIns(4,5)P₂ levels in intact cells, using a number of different approaches. Inhibition of Phosphatidylinositol 4-kinases that are involved in synthesis of PtdIns(4)P, the precursor of PtdIns(4,5)P₂ by wortmannin and PAO caused a progressive decay in the menthol- and cold-induced TRPM8 currents (Liu and Qin, 2005). A membrane-bound PtdIns(4,5)P₂-specific 5'-phosphatase selectively reducing plasmalemmal PtdIns(4,5)P₂ also significantly decreased both menthol and cold- evoked currents and Ca²⁺ signal (Daniels et al., 2008). Depletion of PtdIns(4,5)P₂ using a rapamycin- inducible dimerization system inducing the translocation of a 5'-phosphatase to the membrane also inhibited menthol- or cold-induced currents (Varnai et al., 2006; Daniels et al., 2008). Similar results have been obtained with the voltage sensitive phosphatase cVSP that dephosphorylates PtdIns(4,5)P₂ in response to depolarization (Yudin et al., 2011). These data demonstrate that PtdIns(4,5)P₂ depletion is sufficient for channel inhibition.

Activation of PLC is expected to lead not only to reduction of PtdIns(4,5)P₂ levels, but also to the activation of PKC both via the formation of DAG and directly by Ca²⁺ influx through the channel. Even though phorbol esters, chemical activator of PKC were shown to inhibit TRPM8 currents (Premkumar et al., 2005; Abe et al., 2006), the synthetic DAG analogue OAG did not inhibit TRPM8 channel activity, raising doubts about the involvement of PKC in desensitization (Rohacs et al., 2005; Liu and Qin, 2005). PKC inhibitors were ineffective in one study to inhibit desensitization of menthol-induced currents (Yudin et al., 2011), and inhibited it in another (Sarria et al., 2011). IP₃, the downstream product of PLC activation, is unlikely to mediate current inhibition, since IP₃ injection into *Xenopus* oocytes, or IP₃ dialysis into TRPM8 expressing mammalian cells did not inhibit TRPM8 currents (Rohacs et al., 2005; Liu and Qin, 2005).

A recent study raised the possibility that Ca²⁺ - calmodulin (CaM) is also involved in acute desensitization of TRPM8 currents by showing that Ca²⁺-CaM inhibited TRPM8 in excised patches and that ophiobolin A, a CaM inhibitor inhibited acute desensitization (Sarria et al., 2011). The same study confirmed the role of PtdIns(4,5)P₂ depletion in tachyphylaxis, the decreased current amplitude upon repeated applications of menthol.

6.3 Regulation by cell surface receptors and protein kinases

TRPM8 channels are expressed in sensory neurons, which have a plethora of cell surface receptors, regulating the activity of these cells. Upon inflammation, for example, a “cocktail” of extracellular signaling molecules is released both from white blood cells and the nerve terminals themselves that increase the sensitivity of the sensory neurons to painful and non-painful stimuli. Most of these inflammatory mediators, such as bradykinin, extracellular ATP and various prostaglandins activate G- protein coupled receptors (GPCRs) that couple to either PLC or to adenylate cyclase (AC). In addition, various growth factors, such as Nerve Growth Factor (NGF) acting on receptor tyrosine kinases are also released, and may contribute to acute sensitization of the nerve terminals. There is ample literature on the effects of these inflammatory mediators on the capsaicin- and heat-activated TRPV1 channels (Rohacs et al., 2008). On the other hand, the effects of the activation of these cell surface receptors on TRPM8 just started to emerge recently. In contrast to TRPV1, which is generally rendered more sensitive to heat by these cell surface receptors, TRPM8 tends to be inhibited acutely by these inflammatory mediators (Linte et al., 2007). Two articles showed that bradykinin inhibited TRPM8 channel activity in DRG neurons, and this inhibition was alleviated by inhibitors of PKC (Premkumar et al., 2005; Linte et al., 2007). Pharmacological activation of PKC by phorbol esters reduced menthol responses of both recombinant and native TRPM8 channels (Premkumar et al., 2005; Abe et al., 2006). The mechanism by which PKC activation leads to TRPM8 inhibition is not yet clear. Individual mutations of none of the putative PKC phosphorylation sites eliminated the effect of phorbol esters on TRPM8 channel activity (Abe et al., 2006) and phorbol ester treatment resulted in reduced phosphorylation of the TRPM8 protein (Premkumar et al., 2005). Bradykinin receptor activation leads to PtdIns(4,5)P₂ hydrolysis, however the involvement of the decrease in the level of this phospholipid in the inhibitory effect of bradykinin was not yet tested. In an expression system, activation of muscarinic receptors by carbachol was proposed to inhibit TRPM8 activity via depletion of PtdIns(4,5)P₂ (Liu and Qin, 2005). Activation of NGF or PDGF receptors that activate PLC γ , was reported to inhibit TRPM8 via PtdIns(4,5)P₂ depletion (Liu and Qin, 2005; Rohacs et al., 2005) in recombinant systems, but this effect was not yet confirmed in native sensory neurons.

GPCRs that act through G_s protein activate AC enzymes, leading to the production of cAMP and the activation of protein kinase A (PKA). Chemical activators of this pathway, 8-Br-cAMP and forskolin were reported to inhibit TRPM8 channels (De Petrocellis et al., 2007). Another article reported inhibition of TRPM8 currents in DRG neurons by prostaglandin E₂,

which was concluded to be mediated by PKA (Linte et al., 2007). On the other hand, activation of α_2 -adrenergic receptors with clonidine was also reported to inhibit TRPM8 activity (Bavencoffe et al., 2010). These receptors act through Gi proteins thus inhibit AC activity. In this study, the effect of clonidine was alleviated by pretreatment with forskolin, a chemical activator of PKA, as well as activating β -adrenergic receptors that increase cAMP levels. Forskolin itself and 8-Br-cAMP had no effect on TRPM8 activity (Bavencoffe et al., 2010), in contrast to the previous reports (De Petrocellis et al., 2007; Linte et al., 2007). Another study has also not found a significant effect of either forskolin or 8-Br-cAMP on menthol induced Ca^{2+} responses in DRG neurons (Sarria and Gu, 2010). In conclusion, based on the limited number of publications so far, activation of PLC coupled cell surface receptors inhibit TRPM8 activity through depletion of $\text{PtdIns}(4,5)\text{P}_2$ and/or PKC, whereas the effect of the activation of the cAMP / PKA pathway is currently controversial.

6.4. Regulation by Phospholipase A₂ (PLA₂)

PLA₂ enzymes remove the acyl chain in the SN2 position of phospholipids, leading to the generation of polyunsaturated fatty acids, usually arachidonic acid, and lysophospholipids (Akiba and Sato, 2004). Ca^{2+} -independent phospholipase A₂ (iPLA₂) activation, and production of arachidonic acid and lysophospholipids were proposed to regulate TRPM8 activity (Vanden Abeele et al., 2006; Andersson et al., 2007). Lysophospholipids, products of PLA₂ activity, changed temperature threshold for TRPM8 activation towards body temperature stimulating activity of the channel above 30 C°. Furthermore, reduction of iPLA₂ expression with antisense knockdown lead to inhibition of TRPM8 activity, and PLA₂ inhibitors suppressed TRPM8 activation by icilin and cold, but menthol responses were less sensitive (Andersson et al., 2007). Extra- and intracellular application of lysophospholipids activated TRPM8 expressed in HEK293 cells (Vanden Abeele et al., 2006). Accordingly, PLA₂ inhibitors affected icilin- but not menthol-induced cold hypersensitivity *in vivo* (Gentry et al., 2010). Arachidonic acid, the other product of PLA₂ on the other hand was reported to inhibit TRPM8 activity (Andersson et al., 2007). Given the overall stimulating effect of PLA₂ on TRPM8, it is likely that positive effect of lysophospholipids dominate over the inhibitory effect of arachidonic acid. While products of PLA₂ modulate TRPM8 activity, reconstituted TRPM8 in the planar lipid bilayers retained its cold, menthol and icilin sensitivity (Zakharian et al., 2010) demonstrating that PLA₂ is not an absolute requirement for activation of TRPM8 either by cold or its chemical agonists.

7. Conclusions and future directions

Overall, TRPM8 has been convincingly demonstrated to be a major player in mediating physiological responses to moderately cold temperatures. The channel is also likely to mediate the analgesic effects of menthol, and cooling, and it is also involved injury-induced in cold hypersensitivity. On the cellular level, channel activity clearly depends on the presence of $\text{PtdIns}(4,5)\text{P}_2$, and the depletion of the lipid is involved in desensitization/adaptation of channel activity, with potential contributions of PKC and CaM. Clarification of the effects of inflammatory mediators and other extracellular signals probably require additional studies. Figure 2 shows a compilation of all signaling pathways that have been implicated in the regulation of TRPM8; to clarify the relative contributions of these pathways to regulation of TRPM8 in various conditions will require more work. Further questions include the molecular identity of additional cold sensors, and the apparent paradox that activation of cold fibers could lead both to analgesic effects and painful sensation. Also, essentially all experiments addressing regulation of TRPM8 by cellular signaling pathways were either performed on recombinantly expressed TRPM8, or in DRG/TG neurons. Even though the latter are well established models to study sensory ion channels, they are the isolated cell bodies of the sensory neurons, and relatively little is known about what happens

in the peripheral sensory nerve terminals, where cold sensation is initiated under physiological conditions.

Acknowledgments

This work was supported by National Institutes of Health Grants NS055159 and GM093290 and by a grant from the UMDNJ Foundation to T. R.

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Highlights Yudin et al

- TRPM8 is a cold- and menthol-activated Ca^{2+} permeable non selective cation channels, expressed in sensory neurons
- TRPM8 is a well established physiological cold sensor
- The activity of TRPM8 depends on the presence of phosphatidylinositol 4,5-bisphosphate (PIP_2)
- Calcium induced activation of phospholipase C and the ensuing depletion of PIP_2 mediates desensitization of TRPM8
- Various other cellular signaling pathways modulate TRPM8 activity

Figure 1.

Regulation of TRPM8 activity. A. Mechanism of desensitization of TRPM8: Ca^{2+} influx through the channel activates a Ca^{2+} sensitive PLC enzyme, presumably a PLC δ isoform, and the resulting depletion of PtdIns(4,5)P₂ limits channel activity, see text (6.2) for details. B. Representative traces from (Yudin et al., 2011) showing desensitization of menthol-induced current in mouse DRG neurons, left panel shows a control cell, right panel shows a cell which was dialyzed with 100 μM diC₈ PtdIns(4,5)P₂.

Figure 2.

Cartoon for the regulation of TRPM8 activity by various intracellular signaling pathways that has been implicated in the regulation of these channels. Extracellular signaling molecules activate G- protein coupled receptors (GPCR). Receptors coupling to Gq proteins activate PLC β -s leading to the formation of IP₃ and DAG, and a reduction in PtdIns(4,5)P₂ (PIP₂) levels. Both IP₃ and Ca²⁺ influx through TRPM8 increases cytoplasmic Ca²⁺, which together with DAG activates PKC, which may inhibit TRPM8 activity. Increased cytoplasmic Ca²⁺ will activate PLC δ isoforms which deplete PIP₂, and Ca²⁺ may also inhibit channel activity through CaM. Activation of Gs coupled receptors will activate adenylate cyclase (AC), which in turn activates protein kinase A (PKA) which may lead to channel inhibition, see text (6.3) for details. iPLA2 enzymes will hydrolyze phospholipids (PL) leading to the formation of lysophospholipids (LPL) and arachydonic acid (AA), see text (6.4) for details.