Therapeutic Potential of TRPM8 Modulators

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Abstract: The perception of temperature is a key tenet of sensory physiology and is critical in not only acute responses to changes in our environment, but also fundamental in regulating homeostatic mechanisms like core body temperature [1]. The somatosensory system is able to detect subtle changes in ambient temperature due to the coordinated efforts of thermosensory neurons which express temperature-sensitive members of the TRP channel family [2]. Remarkably, the range of temperatures that these channels respond to covers the entire perceived temperature spectrum, from warm to painfully hot, from pleasingly cool to excruciatingly cold. Moreover, many of these channels are receptors for ligands that elicit distinct psychophysical sensations, such as the heat associated with capsaicin and the cold felt with menthol [2]. The latter of these was influential in the discovery of the first TRP channel shown to be responsive to temperatures in the cold range (<30°C), TRPM8, a member of the melastatin TRP channel subfamily [3, 4]. *In vitro*, the channel is a receptor for a number of compounds which evoke the psychophysical sensation of cold (such as menthol and icilin), and is activated by temperatures that range from innocuous cool (26-15°C) to noxious cold (<15°C). Recent genetic evidence shows that TRPM8 is the predominant mammalian cold sensor and is involved in most, if not all, aspects of cold thermal transduction [5-8]. These studies demonstrated that TRPM8 mediates transduction of innocuous cool and noxious cold, hypersensitivity to cold caused by inflammation or nerve injury, and provides the analgesic effect produced by cold or chemical cooling compounds. This review highlights these findings and suggests some potential uses of both TRPM8 antagonists and agonists in the treatment of pain.

Keywords: TRP channel, TRPM8, pain, cold, menthol.

COLD SENSING, MENTHOL, AND TRPM8

The perception of nonpainful, cool temperatures begins when the skin is cooled as little as 1°C from normal skin temperature of 32°C. However, once temperatures approach 15°C, the perception of cold pain is felt, with qualities described as burning, aching, and prickling. Indeed, when subjects are asked to provide verbal descriptors for the sensation of a noxious cold stimulus *vs* that of noxious heat, a wider range of words are used for cold than for heat [9]. Thus, the perception of cold generates a diverse, and perhaps more ambiguous, range of sensations compared to heat. At the level of the afferent nerve, a subset of both Aδ- and C-fibers from either the dorsal root (DRG) or trigeminal ganglia (TG) respond to cold temperatures with thermal thresholds for activation ranging from below 30°C to near freezing. Cold fibers account for approximately 15-20% of all afferents, demonstrating that there is significant diversity in the types of neurons that respond to cold, as well as an expansive range of cold activation thresholds.

Most cold-sensitive neurons are also sensitive to the cooling compound menthol, a cyclic terpene alcohol found in mint leaves [10]. It is well known that moderate concentrations of menthol induce a pleasant cool sensation, such as that felt when using menthol-containing products. However, when present at higher doses menthol can be noxious, causing burning, irritation, and pain [11]. In influential studies conducted by Hensel and Zotterman, it was shown that menthol elicits a sensation of "cool" by increasing the threshold temperature for activation of cold fibers. Indeed, they hypothesized that menthol exerted its actions on "an enzyme" that was involved in the activation of these nerves [12]. Consistent with this postulate, topical application of menthol to the skin of healthy subjects evokes cold hypersensitivity by sensitizing cold-sensitive C fibers in a manner that mimics cold allodynia observed in patients with neuropathies of various etiologies [13]. Indeed, in a patient with a small-fiber neuropathy in which the chief complaint was cold allodynia, responsiveness of C-fibers to menthol and cold were significantly enhanced [14].

At the cellular level, a small fraction of neurons from either the dorsal root (DRG) or trigeminal (TG) ganglia respond to cold temperatures, with thresholds for activation below 30°C. Such stimuli evoke a robust influx of calcium in these cells similar to that caused by menthol. Indeed there is a strong correlation between menthol and cold sensitivity *in vitro* [3, 5, 15]. Cooling generates an inward current when DRG neurons are held at negative membrane potentials, with an average temperature threshold near 29°C [16]. This threshold shifts to warmer temperatures when the recordings are conducted in the presence of menthol, as was predicted in Hensel and Zotterman's original hypothesis [12]. Similar responses are observed in cultured TG neurons and both menthol and cold evoke rapidly activating, nonselective

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cation conductances that are characterized by strong outward rectification [3]. Thus, menthol has been most useful in identifying and describing mechanisms of cold sensing in both acute and pathological settings. Moreover, menthol was critical in identifying the predominant cold sensor in mammals.

Two independent groups using different experimental approaches concurrently cloned TRPM8 from sensory afferents and identified it as a cold- and menthol-sensitive ion channel [3, 4]. The first group used menthol to expression clone a complementary DNA (cDNA; a synthesized copy of an RNA transcript) from rat TG neurons that could confer menthol sensitivity to cells [3]. The second group searched for TRP channel–like sequences in mouse DRG neurons and tested these channels for temperature sensitivity [4]. With these divergent approaches, both groups simultaneously identified TRPM8 (also referred to as trp-p8 or CMR1), a member of the melastatin channel subfamily [17]. TRPM8 has also been identified as a transcriptional marker of prostate epithelia [18].

Biophysically, cold and menthol-evoked TRPM8 currents have surprisingly similar properties to those recorded in both cultured DRG and TG using similar experimental paradigms. These include ion selectivity, menthol potency, and voltage dependence of membrane currents induced by either cold or menthol [3]. Like almost all TRP channels, TRPM8 is a nonselective cation channel that displays strong outward rectification and has relatively high selectivity for calcium and little selectivity among monovalent cations [3]. More remarkably, TRPM8 currents are also evoked by temperature decreases with an activation temperature threshold of ~26°C, with activity increasing in magnitude down to 8°C. Interestingly, this broad range spans what are considered both innocuous cool (~30–15°C) and noxious cold temperatures (<15°C). The relatively warm activation threshold for TRPM8 has been suggested to preclude the channel from mediating responses to noxious cold temperatures. However, care must be taken in such assumptions when the context of channel expression *in vivo* is considered. As with all neuronal environmental sensors, the channel's active site is predominantly the peripheral nerve terminal in cutaneous structures, such as the skin, and surface skin temperatures are considerably colder than those recorded subcutaneously. For instance, Morin and Bushnell correlated subcutaneous skin temperatures (recorded at a site 1 mm below the skin surface) and found that when a subfreezing temperature of -5°C was applied to the skin for 30 seconds, subcutaneous temperatures was reduced to only ~17°C [9]. Thus, the ranges of temperatures that activate TRPM8 *in vitro* are more reflective of a noxious cold sensor than one detecting innocuous cool.

In both TG and DRG, TRPM8 is expressed in <15% of small-diameter sensory neurons, consistent with the proportion of neurons shown to be cold- and menthol-sensitive in neuronal cultures [3, 4, 19-21]. TRPM8 transcripts are more abundant in trigeminal *vs* dorsal root ganglia, and those expressing this channel have a diverse neurochemical phenotype, suggesting that distinct subsets of TRPM8 neurons may mediate different aspects of cold sensing [3, 4, 19, 20]. A cohort of mouse TRPM8 neurons are both Aδ and C-fibers based on co-expression with neurofilament 200 (NF200) and peripherin, respectively, as well as in the location of their peripheral terminals in structures such as the tooth [20]. Thus, first and second cold pain sensations are likely dependent on TRPM8 activation [22]. Moreover, a fraction of TRPM8 neurons also express the nociceptive markers TRPV1 and calcitonin gene-related peptide (CGRP), further supporting a role for this channel in cold nociception [19, 20]. Thus, TRPM8 defines a small and discrete population of sensory afferents that innervate tissues known to be highly sensitive to cold and nociceptive stimuli.

PHYSIOLOGICAL ROLES OF TRPM8

As stated above, TRPM8 becomes active at innocuous temperatures below 26°C *in vitro*, and the steep temperature-dependence of TRPM8 currents extends its activity into the noxious range, reported to begin at temperatures lower than 15°C in psychophysical studies [9, 23]. Thus, it was not clear if TRPM8 mediates detection of innocuous cool, noxious cold, or both. Insights into these and other questions regarding the role of this channel *in vivo* came to light in 2007 when three independent groups reported on the phenotype of genetically modified mice lacking functional TRPM8 channels [5-8]. One of the characteristic difficulties in analyzing cold evoked behaviors using traditional methods, such as the cold plate, is that rodents seem little bothered by cold temperatures in comparison to the robust responses generated by noxious heat. In the standard paw withdrawal assay, where measurements such as number of paw lifts or latency to lifts are recorded, previous reports have found rodents to be poorly responsive. For example, in rats placed on a surface chilled to 5°C latencies of longer than 1 minute have been observed [24, 25]. Moreover, there is significant variability in these behaviors between research studies. In the analyses of control mice in the three TRPM8 knockout studies, the time to paw withdrawal at near freezing temperatures ranged from 5-50 seconds (5, 20, and 50 seconds) between the three studies [5, 6, 8]. These significant differences in animal behavior highlight the difficulty of these assays, and demonstrate the need for additional experimental paradigms.

One valuable assay to assess cold behaviors is the two-temperature preference test which was conducted on all three TRPM8-null lines [7]. In these assays, animals are presented with a choice between two surfaces that are independently temperature controlled with one held typically near 30°C, the preferred thermal clime of wildtype mice [26, 27]. When both surfaces are set the same temperature, mice will explore and spend equal time on both surfaces. However, once the temperature of one surface is shifted to colder temperatures (or hot), wildtype mice show a preference for the 30°C side. In the case of mice lacking functional TRPM8 channels, there are robust deficits in their ability to show preference for the warmer side, yet discrepancies emerged as to the exact temperature ranges affected [7]. Bautista *et al*., showed that TRPM8-nulls display no preference until temperatures near 10°C, where they do increase their time on the 30°C side, but not to the levels exhibited by wildtype mice [5]. These data suggested that other mechanisms may also detect noxious cold temperatures. Indeed another member of the TRP channel family TRPA1 has been proposed to detect cold temperatures below 17°C [28, 29]. However, a

definitive role for TRPA1 to be shown in cold evoked activity *in vitro* and *in vivo* has yet to be shown definitely [15, 30]. Thus it is not clear what role, if any, TRPA1 serves in cold perception [31]. Nonetheless, Colburn *et al*. found that their line of TRPM8-nulls were incapable of discriminating temperatures below 5°C [6]. Moreover, in cold plate assays, Colburn *et al*. found that TRPM8-nulls exhibit longer paw withdrawal latencies at 0°C than their wild-type littermates [6], whereas no differences in behavioral responses were observed in the two other mouse lines under similar experimental conditions [5, 8]. Nonetheless, these results establish the necessity of TRPM8 in thermosensation, and demonstrate that *in vivo*, the channel mediates the detection of innocuous cool and perhaps a component of noxious cold.

Injury greatly impacts thermosensation, resulting in heightened sensitivity to temperatures in the innocuous range (allodynia), and in the already painful range (hyperalgesia). Indeed, menthol is commonly used as a mechanism to mimic cold allodynia in human subjects [13, 32]. Thus, one outstanding question regarding TRPM8 is whether or not the channels serve a role in cold evoked hypersensitivity under different pathological conditions [23]. To address this, Colburn *et al*. used two different models to assess the involvement of TRPM8 in injury-evoked hypersensitivity to cold [6]. In a model of neuropathic pain where the sciatic nerve is chronically irritated, wild-type animals shake their hind legs in response to the evaporative cooling caused by acetone application. Injured TRPM8-null mice, however, display no such nocifensive behaviors, similar to uninjured control animals. They obtained similar results when using a model of inflammatory injury, paw injections of Complete Freund's adjuvant (CFA). Taken together, these results suggest that TRPM8 may mediate the majority of cold allodynia and hyperalgesia.

Menthol and cooling are commonly used as topical analgesic [11, 33]; thereby suggesting that activation of TRPM8 may lead to pain relief. The first indication that TRPM8 may serve this role arose when Proudfoot *et al*. demonstrated that topical application of cold or cooling compounds produces a temporary analgesic effect mediated by TRPM8-expressing afferents [34]. Using the same rodent model of neuropathic pain described above, it was observed that paw withdrawal latencies in response to mechanical or heat stimuli were significantly attenuated in animals first treated with cold or cooling compounds such as icilin. Similarly, pain behaviors were also reduced in models of inflammatory pain. The analgesia persisted for over 30 minutes after which the animals regained their hypersensitivity similar to before the cool stimuli were applied and at levels of those not pre-treated with cold or cooling-compounds. Only modest cooling and low doses of cooling compounds produced analgesia and when TRPM8 expression was reduced this form of analgesia was abolished. This role for TRPM8 in analgesia was further confirmed by analyses of TRPM8-null mice. Dhaka *et al*. using formalin (a compound that evokes acute pain followed by inflammation) injections into wildtype mouse hindpaws found that cooling to 17ºC and 24ºC produced a marked decreases in pain behaviors (licking and lifting hindpaws) during the acute pain phase [8]. However, mice lacking TRPM8 did not behave similarly in that they continued to show nocifensive responses even after exposed to the cool surface and were indistinguishable from wild-type animals that were not exposed to cool temperatures. Together these data indicate that TRPM8 is mediating the analgesia provided by cool temperatures and cooling compounds, suggesting that modest activation of TRPM8 afferent nerves can serve as an endogenous mechanism to promote pain relief.

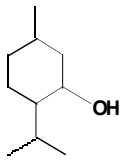
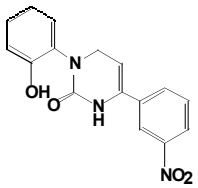
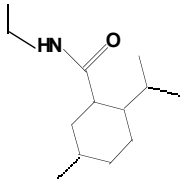
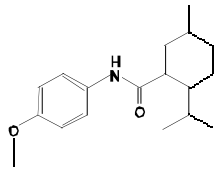
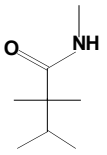
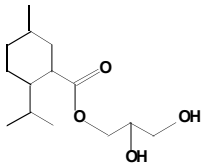
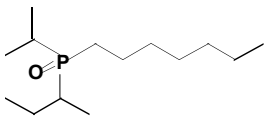
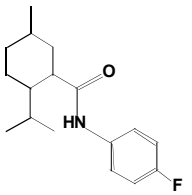
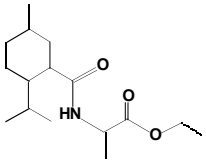
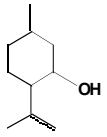
In addition to the roles of TRPM8 in somatosensation and nociception, the channel may be required for thermoregulation and homeostatic mechanisms. Intragastric administration of TRPM8 agonists has been found to increase thermogenesis [35]. Moreover, intravenous administration of a super cooling compound induces a robust thermogenic type behavior characterized as "wet dog shakes" [36]. A role for TRPM8 in thermoregulation would not be entirely unexpected, as other temperature sensitive ion channels, particularly TRPV1, have been shown to play a role in regulating body temperature as well [37-40].

TRPM8 PHARMACOLOGY

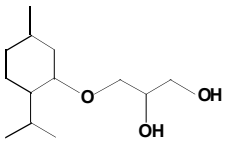
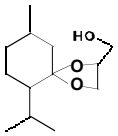
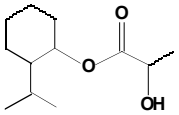
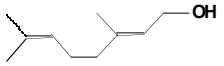
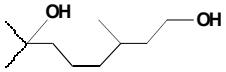
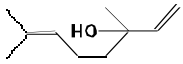
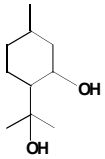
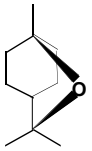
A growing list of cooling compounds has been shown to activate TRPM8 with various potencies and efficacies (see Table **1**). Indeed one of the poorest TRPM8 agonists is menthol with a potency in the mid micromolar range [3]. To date few of these compounds have been tested in animal models to ensure that their cooling effects are specific to TRPM8. The most potent TRPM8 agonist is WS-12 [41], which is one of many cooling compounds that came out of an extensive synthesis and evaluation program by Wilkinson Sword Ltd. to develop such compounds that did not have the minty sensations associated with menthol [10, 42]. In addition, Wei independently identified another cooling compound, icilin (also known as AG-3-5) in the early 1980s which bears little resemblance to menthol structurally but is more potent and effective in activating TRPM8 [3, 36]. Of note, when given intravenously, it will induce the characteristic shivering or "wet dog" shakes noted above. Interestingly, the mechanism whereby icilin activates TRPM8 is different than that of menthol or cold in that it requires a coincident rise in cytoplasmic calcium, either *via* permeation through the channel or by release from intracellular stores, in order to evoke TRPM8 currents [43]. This requirement of a calcium rise for TRPM8 activity is not needed for cold- or menthol-induced channel activity, suggesting the channel can be activated by multiple mechanisms. Additionally, a critical amino acid was identified, which when mutated renders icilin incapable of activating TRPM8. This residue was located between the second and third trans-membrane domains of the channel, a region known to be important for capsaicin sensitivity of TRPV1 [44], suggesting a conserved mechanism for ligand activation of these thermosensitive TRP channels.

While various compounds activate TRPM8, a more relevant class of molecules that may be of use clinically are those that antagonize or block the channel (Table **2**). Surprisingly, many of these compounds also antagonize the heat-gated channel TRPV1, suggesting a conserved mechanisms amongst thermosensitive channels [45]. Capsazepine, N-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl)piperazine-1-carboxamide (BCTC) and Thio-BCTC,

Table 1. TRPM8 Agonists

Compound	Structure	EC50	Assay	Reference
Menthol		66.7 μM	Cation currents	(McKemy, Neuhausser *et al*. 2002)
Ag-3-5 (icilin)		0.20-0.36 μM	Cation currents	(McKemy, Neuhausser *et al*. 2002; Behrendt, Germann *et al*. 2004)
WS-3		3.7 μM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
WS-12		193 nM	Ca^{2+} microfluorimetry	(Bodding, Wissenbach *et al*. 2007)
WS-23		44 μM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
WS-30		5.6 μM	Ca^{2+} microfluorimetry	(Bodding, Wissenbach *et al*. 2007)
WS-148		4.1 μM	Ca^{2+} microfluorimetry	(Bodding, Wissenbach *et al*. 2007)
CPS-113		1.2 μM	Ca^{2+} microfluorimetry	(Bodding, Wissenbach *et al*. 2007)
CPS-369		3.6 μM	Ca^{2+} microfluorimetry	(Bodding, Wissenbach *et al*. 2007)
Coolact P		66.0 μM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)

(Table 1) contd…..

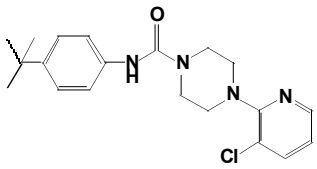
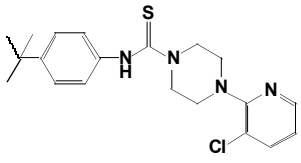
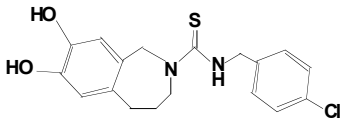
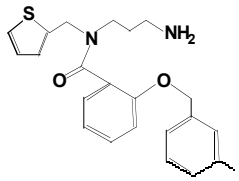
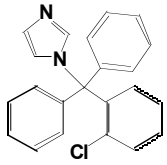
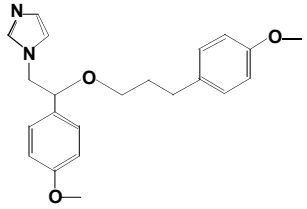
Compound	Structure	EC50	Assay	Reference
Cooling Agent 10		6.0 μM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
FrescolatMGA		4.8 μM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
FrescolatML		3.3 μM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
Geraniol		5.9 mM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
hydroxycitronellal		19.6 mM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
Linalool		6.7 mM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
PMD38		31 μM	Ca^{2+} microfluorimetry	(Behrendt, Germann *et al*. 2004)
eucalyptol		3.4-7.7 mM	Cation currents, Ca^{2+} microfluorimetry	(McKemy, Neuhausser *et al*. 2002; Behrendt, Germann *et al*. 2004)

known TRPV1 antagonists, were found to inhibit TRPM8 activity using a fluorimetric imaging plate reader (FLIPR) assay [46]. This is in addition to capsazepine's known roles as a TRPV1 antagonist with non-specific activity on voltage-gated calcium channels and nicotinic acetylcholine receptors [47-50]. BCTC has also been shown to be a TRPA1 agonist [51]. *N*-(3-aminopropyl)-2-{[(3-methylphenyl) methyl]oxy}-*N*-(2-thienylmethyl)benzamide hydrochloride salt (AMTB) was recently shown to be an TRPM8 blocker in cellular assays [52]. Moreover, intravenous application of AMTB was able to diminish the frequency of volume-induced bladder contractions, without any effects on contraction. These results suggest that TRPM8 afferents are good targets for treating overactive and painful bladder conditions. Finally, clotrimazole, an anti-fungal medication, was shown in a recent study to activate TRPV1 and TRPA1 (consistent with its commonly reported side effects of irritation and burning), while also serving as a potent TRPM8 antagonist [53]. Together, these antagonists present a range of pharmacological tools to regulate TRPM8 function. However, each of these compounds has off-target effects and, while of interest pharmacologically, these results complicate the search for selective agents for these channels that are such good targets for drug discovery.

REGULATION OF TRPM8 CURRENTS

Temperature sensation is a dynamic process and like other sensory systems, we can easily adapt to cold temperatures, a process observed in both psychophysical and cellular assays. Similarly, cold- or menthol-induced TRPM8 currents will adapt or desensitize in a calcium-dependent manner during prolonged stimulation [3, 54]. In addition to Ca^{2+} sensitivity, TRPM8 adaptation is also temperature dependent, meaning less adaptation is observed at colder temperatures, and that channel activity can be recovered, but only when the cell is returned to temperatures near that of skin (~32°C) [3, 54]. These observations suggest that a Ca^{2+} and temperature dependent process mediates adaptation at the cellular and channel level. TRPM8 activity is also highly sensitive to plasmalemmal levels of phosphatidylinositol 4,5-bisphosphate (PIP_2) [54-56]. These observations led to the hypothesis that adaptation occurs as a result of TRPM8-mediated Ca^{2+} entry leading to activation of phospholipase C (PLC), thereby promoting breakdown of PIP2. This hypothesis was recently confirmed when it was shown that Ca^{2+} and receptor independent activation PLC and PLC independent reductions in PIP_2 levels leads to TRPM8 adaptation [54]. Thus, adaptation occurs due to cellular changes in the levels of PIP_2, which modulates the ability of

Table 2. TRPM8 Antagonists

Compound	Structure	IC_{50}	Other Effects	Reference
BCTC		0.5-0.8 μM	TRPV1 antagonist; TRPA1 agonist	(Behrendt, Germann *et al*. 2004; Madrid, Donovan-Rodriguez *et al*. 2006)
Thio-BCTC		3.5 μM	TRPV1 antagonist	(Behrendt, Germann *et al*. 2004)
Capsazepine		18 μM	TRPV1, voltage-gated calcium channel, nicotinic acetylcholine receptor antagonist	(Behrendt, Germann *et al*. 2004)
AMTB		7 μM	NA	(Lashinger, Steiginga *et al*. 2008)
Clotrimazole		200 nM	TRPV1, TRPA1 agonist	(Meseguer, Karashima *et al*. 2008)
SKF96365		1.0 μM	Pore blocker of Ca^{2+} channels	(Malkia, Madrid *et al*. 2007)

TRPM8 to respond to cold or menthol. Of note, adaption, with reduced PIP_2 levels does not lead to shifts in either menthol or cold sensitivity of the channel, but does cause a shift in voltage-dependence of TRPM8 currents indicative of a change in channel gating [54]. At the molecular level, a number of amino acid residues have been identified in the carboxy-terminal domain of the channel, adjacent to the sixth transmembrane domain, that appear to be involved in PIP_2's effects on TRPM8 [56]. Interestingly, these residues are near the highly conserved TRP box of the channel and are found in other PIP_2-sensitive TRPM channels, including TRPM4 and TRPM5 [57].

In addition to direct effects of PIP_2 breakdown, increased kinase activity has also been suggested to lead to reduce TRPM8 activity [58]. A consequence of PIP_2 breakdown is the generation of diacylglycerol (DAG) and inositol-trisphosphate (IP_3), which are 2nd-messangers that promote protein kinase C (PKC) activity. Phosphorylation is a common mechanism whereby channel activity is modulated, and increased PKC activity causes decreased TRPM8 membrane currents. Interestingly, PKC activation does not lead to increased incorporation of phosphate on TRPM8, but rather a decrease in phosphorylation. Thus, the PKC-mediated effects are not due to direct phosphorylation of TRPM8, but that PKC plays a role upstream of channel phosphorylation.

Intracellular pH also regulates TRPM8. When pH is increased to above physiological levels, TRPM8 activity is inhibited. These effects of pH are thought to be mediated intracellularly, but there is disagreement on the effects of pH on cold-, menthol-, and icilin–evoked currents. Andersson *et al*. reported that menthol's ability to activate TRPM8 is unaffected by pH, but that cold and icilin responses are inhibited [59]. Behrendt *et al*. also found that icilin was less effective in activating TRPM8 at high pH, but in contrast, menthol-evoked responses were also suppressed [46]. Thus, it seems likely that either cell-to-cell variation in temperature thresholds for cold, or altered sensitivity due to experience and pathological state of the neuron, may be a result of TRPM8 regulation *via* cellular levels of PIP_2, protons, or

kinase activity. This level of channel modulation may also account for the complexity and variability in cold-evoked temperature responses observed both *in vivo* and *in vitro*.

CONCLUSIONS

The elucidation of TRP channels as molecular detectors of thermal stimuli addressed a fundamental issue in sensory transduction: how are thermal stimuli converted into neuronal activity? These proteins have now become interesting and potentially valuable drug targets for a range of conditions from pain, visceral organ function, and thermal homeostasis. While much has been made regarding the role of the companion channel TRPV1 in such studies, we are at the beginning in our understanding of how modulation of TRPM8 can be beneficial clinically. Both direct genetic evidence, as well as indirect data from the effects of menthol and cooling, clearly shows that the channel serves a fundamental role in sensory physiology. Indeed the breadth of the *in vivo* properties of TRPM8 are surprising, raising the fundamental question of how can a single sensor of environmental stimuli lead to such a diverse assortment of functions, ranging from acute cold detection, injury evoked hypersensitivity and analgesia. One likely hypothesis is that while TRPM8 enables neurons to respond to cold, the cellular context of channel expression is the final determinant of our percept. Indeed, there may be distinct and non-overlapping TRPM8-mediated neural pathways that are segregated into transducing temperature, pain and analgesia. The goal now is to identify these pathways to determine the molecular, cellular and anatomical substrates that engender TRPM8 with these functions. Nonetheless, the identification of TRPM8 established the first molecular detector of cold stimuli, and confirmed Hensel and Zotterman's half-century-old hypothesis for how menthol makes us cool.

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