Lessons from peppers and peppermint: the molecular logic of thermosensation

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Sensory neurons report a wide range of temperatures, from noxious heat to noxious cold. Natural products that elicit psychophysical sensations of hot or cold, such as capsaicin or menthol, were instrumental in the discovery of thermal detectors belonging to the transient receptor potential (TRP) family of cation channels. Studies are now beginning to reveal how these channels contribute to thermosensation and how chemical signaling pathways, such as those activated by tissue injury, alter thermal sensitivity through TRP channel modulation. Analysis of TRP channel expression among sensory neurons is also providing insight into how thermal stimuli are encoded by the peripheral nervous system.

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Abbreviations
DRG dorsal root ganglia
PKC protein kinase C
PLC phospholipase C
TG trigeminal ganglia
TRP transient receptor potential
TRPV1 capsaicin receptor

Introduction

At any given moment we can experience a wide range of temperatures, from the warmth of a fire to the chill of a brisk wind. The sensation of temperature is initiated when a thermal stimulus excites primary afferent sensory neurons of the dorsal root (DRG) or trigeminal ganglia (TG). Once activated, these cells relay signals, through action potentials, from peripheral tissues to the spinal cord and brain, where they are integrated and interpreted to trigger appropriate reflexive and cognitive responses [1]. Psychophysically, we perceive heat to be uncomfortable or pain-producing (noxious) when temperatures exceed around $43°C$, whereas the threshold for noxious cold is in the vicinity of $15°C$. Indeed, our somatosensory system is capable of detecting thermal stimuli over a broad temperature spectrum, while enabling us to dis-criminate hot from cold, or noxious from innocuous stimuli. As subpopulations of primary afferent neurons show specific thermal activation thresholds [2,3•], it seems likely that our psychophysical perception is determined by a combinatorial code, in which specific cohorts of sensory neurons are activated at any given temperature. The identification of ion channels that detect heat or cold is now providing insight into the molecular logic of thermosensation, helping us to understand how cellular and psychophysical thresholds are determined at the biophysical and biochemical level.

The capsaicin receptor — a heat-activated ion channel in the pain pathway

Fundamental insights into the molecular mechanisms of thermosensation have come from the analysis of natural products that mimic sensations of heat or cold. Capsaicin is the pungent ingredient of chili peppers that elicits the familiar tingling and burning sensation associated with 'hot' spicy foods [4]. Capsaicin sensitivity has long been regarded as a functional hallmark of nociceptive (pain-detecting) sensory neurons and activates a non-selective cation channel permeable to both sodium and calcium ions [5]. Interestingly, most, if not all, capsaicin-sensitive nerve fibers (subpopulations of both unmyelinated C and thinly myelinated Aδ fibers) are also activated by noxious heat, with a 'moderate' thermal threshold of around $43°C$ [2]. The cloning and functional analysis of the capsaicin receptor, TRPV1 (also known as vanilloid receptor 1; VR1), provided an explanation for this correlation, and revealed for the first time how sensory neurons detect temperature at the molecular level [6]. When expressed in heterologous systems, TRPV1 can be activated not only by capsaicin but also by heat with a threshold temperature of $43°C$ and a high temperature dependence coefficient (Q_{10} value of greater than 20) [7,8]. A role for this channel in heat detection is further supported by gene knockout studies, which showed that DRG neurons from TRPV1-deficient mice have markedly reduced 'moderate' threshold heat responses [9,10].

The capsaicin receptor in thermal hyperalgesia: a polymodal detector of noxious stimuli

Another important phenotype of TRPV1-deficient mice is their failure to show hypersensitivity (hyperalgesia) to thermal stimuli following peripheral tissue injury [9,10]. Thermal hyperalgesia can be produced when inflammatory mediators sensitize peripheral nerve endings to physical or chemical stimuli [1,11]. *In vitro* studies suggest that some inflammatory mediators can sensitize TRPV1 by

acting on the channel directly, whereas others activate signaling pathways that converge on this channel. For example, injury promotes tissue acidosis and extracellular protons that are released serve as allosteric modulators of TRPV1 [7,8]. Sustained acid-evoked currents in sensory neurons are clearly diminished in TRPV1-deficient mice [9,10] and structure–function analyses have shown that a glutamate residue near the presumptive pore domain of the channel is an important structural determinant of acid sensitivity [12]. In addition to protons, injury or inflammation produces a large variety of lipid-derived second-messengers, such as anandamide or 12-HPETE that share structural similarity with capsaicin and may contribute to hyperalgesia by directly activating TRPV1 [13,14]. Recent structure–function studies suggest that capsaicin and these putative endogenous ligands (endovanilloids) bind to TRPV1 at the cytosol-membrane interface by interacting with residues located in a region spanning hydrophobic domains two through four [15•].

Tissue insult also produces a number of bioactive peptides that induce thermal hyperalgesia [1]. Among the most potent are bradykinin and nerve growth factor (NGF). Both of these agents activate phospholipase C (PLC) signaling systems, thereby promoting hydrolysis of the plasma membrane phospholipid phosphatidylino-sitol-4,5-biphosphate (PtdIns[4,5]P_2), which leads to release of calcium from intracellular stores and activation of downstream effectors such as protein kinase C (PKC). Several studies have implicated direct phosphorylation of TRPV1 by PKC as a mechanism of sensitization by bradykinin [16,17]. Alternatively, recent studies indicate that PLC-dependent hydrolysis of PtdIns(4,5)P_2 sensitizes TRPV1 through a mechanism independent of PKC activation [18•]. Specifically, this latter model suggests that PtdIns(4,5)P_2 exerts a direct inhibitory effect on TRPV1 such that PLC-mediated hydrolysis of PtdIns-(4,5)P_2 results in disinhibition of the channel. This results in the increased sensitivity of TRPV1 to both chemical and thermal stimuli, including a profound shift in thermal activation thresholds to lower temperatures, consistent with the hallmarks of thermal hypersensitivity. Recent structure–function studies have identified a putative PtdIns(4,5)P_2 binding site within the cytoplasmic carboxy-terminal tail of TRPV1, supporting the concept of a direct and functionally relevant phospholipid–channel interaction [19•].

TRPV1 is a distant relative of *Drosophila* transient receptor potential (TRP) ion channels, which mediate depolarization of photoreceptor cells in the fly eye following activation of PLC-coupled rhodopsin. A large number of mammalian TRP channels have now been identified in both vertebrate and invertebrate organisms [20], many of which appear to be activated or regulated by PLC-coupled 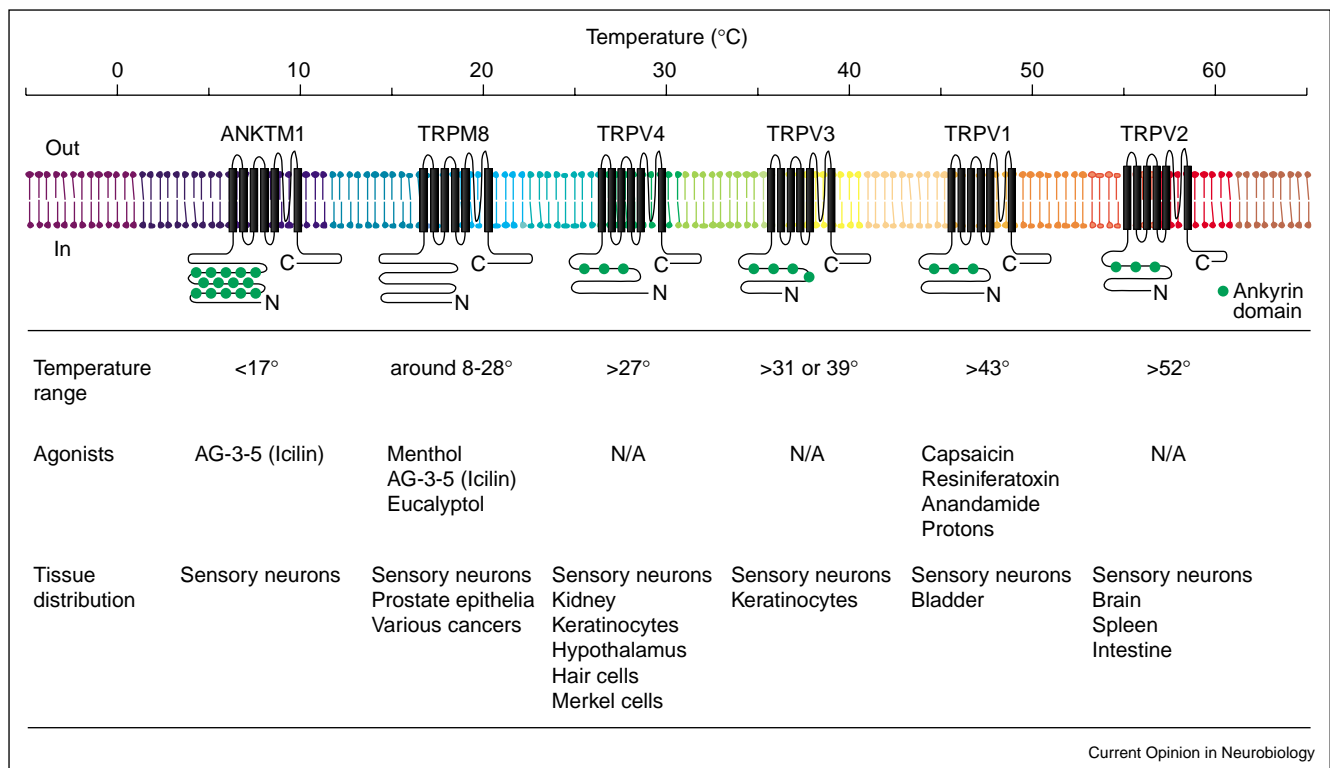receptors. Indeed, recent genetic and electrophysiological data now suggest that many TRP channels are regulated, both positively and negatively, by direct interaction with PtdIns(4,5)P_2 [21,22]. Thus, defining how bradykinin or NGF sensitize TRPV1 is crucial not only for delineating mechanisms of thermal hyperalgesia but also for understanding how this large family of TRP channels is regulated by a wide variety of physiological stimuli.

A growing family of heat sensitive ion channels

TRPV1-deficient mice are not completely heat insensitive [9,10], and heterologously expressed TRPV1 is activated only over a portion of the temperature spectrum to which our somatosensory system is responsive [7]. Thus, additional temperature sensitive ion channels were proposed to exist, and close homologs of TRPV1 were first in line as candidate thermosensors. Indeed, several related channels (now denoted as TRPV2, TRPV3, and TRPV4 in the current nomenclature [23]) have been cloned and shown to be activated by thermal stimuli in the warm to hot range when expressed heterologously (Figure 1; [24,25•–28•]).

The first identified TRPV1 homologue, TRPV2 (VRL1), is a candidate transducer of a high threshold heat response [24]. Medium diameter type I Aδ fibers that are lightly myelinated are characterized as mediators of high threshold noxious heat stimuli, because they respond to temperatures >52°C [1,2]. Evidence suggests that TRPV2 mediates these responses because of its distinct activation threshold of 52°C *in vitro*. Moreover, the cellular localization of TRPV2 is consistent with expression in type I Aδ fibers [24,29]. It is also expressed in tissues other than sensory neurons (brain, spleen), which suggests that it responds to physiological stimuli other than heat. TRPV4 was initially identified as an osmosensitive channel expressed in the kidney, the brain and the inner ear [30–32]. Recently it was shown that TRPV4 is activated at temperatures >25°C. Furthermore, its expression in hypothalamic neurons suggests that it may contribute to the central regulation of core body temperature [28•]. TRPV4 is also expressed in aortic endothelial cells in which non-selective currents with a comparable temperature profile have been recorded [33]. TRPV3 is the product of a gene in close proximity to TRPV1 [34], and heterologous expression studies suggest it is also heat sensitive with reported activation thresholds between 31 and 39°C [25•–27•]. Whether TRPV3 or TRPV4 are expressed in primary afferent neurons is still a matter of debate. Both channels are expressed in keratinocytes [27•,28•], in which they might function as warmth sensors in the skin by modulating calcium levels or activating release of paracrine signaling factors, such as ATP, that could excite nearby sensory nerve terminals. Further anatomical and genetic analysis of these channels should ultimately define their roles in thermosensation.

Figure 1

Temperature range	<17°	around 8-28°	>27°	>31 or 39°	>43°	>52°
Agonists	AG-3-5 (Icilin)	Menthol AG-3-5 (Icilin) Eucalyptol	N/A	N/A	Capsaicin Resiniferatoxin Anandamide Protons	N/A
Tissue distribution	Sensory neurons	Sensory neurons Prostate epithelia Various cancers	Sensory neurons Kidney Keratinocytes Hypothalamus Hair cells Merkel cells	Sensory neurons Keratinocytes	Sensory neurons Bladder	Sensory neurons Brain Spleen Intestine

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The current line up of thermosensitive TRP channels. Thermosensitive TRP channels respond to a wide range of ambient temperatures. This diagram (adapted from [35•]) depicts the known mammalian thermosensitive TRP channels, arranged according to their temperature response profiles when examined in heterologous expression systems. Listed below each channel are their reported thermal thresholds and the range of temperatures to which they respond. Together these channels can, theoretically, account for the detection of all commonly encountered thermal stimuli, from noxious cold to noxious heat. Known agonists and modulators are also listed below, some of which produce a psychophysical sensation of hot or cold, or sensitize primary afferent nerve terminals to thermal stimuli. Each channel is reported to be expressed in DRG or trigeminal neurons (at least at the transcriptional level) but several are also expressed outside of the sensory nervous system, suggesting multiple or alternative physiological roles for these excitatory ion channels.

Transient receptor potential channels turn cold

Further evidence for the essential role of TRP channels in thermosensation has come from the recent cloning of two ion channels proposed to mediate detection of cold temperatures [35•–37•]. Cool to noxious cold temperatures will depolarize a small subset (around 10%) of primary afferent fibers [38]. Historically, the mechanism of cold transduction was thought to involve inhibition of cellular processes required for maintenance of membrane polarization. Nerve fiber recordings had suggested that cold inhibits Na^+/K^+-ATPases, leading to membrane depolarization through leak currents [39]. However, Reid and Flonta [40] demonstrated that, at the cellular level, block of the Na^+/K^+-ATPase could not recapitulate the cell's response to cold, and suggested that inhibition of 'background' K^+ conductances also contributed to cold signaling. Viana *et al*. [41] expanded upon this idea by suggesting that cold-sensitive TG neurons lack a K^+-channel 'brake' current (IKD) that prevents the cold-

initiated depolarization. Cold-insensitive cells could then be made cold sensitive by inhibiting IKD with the blocker 4-aminopyridine. However, the molecular identities of putative K^+-channels that are involved in cold sensation have not yet been determined.

Alternatively, cold could depolarize neurons by directly activating an excitatory channel. Evidence to support such a mechanism has come from recent studies demonstrating that cooling of sensory neurons (below 27°C) activates non-selective cation conductances, leading to membrane depolarization and generation of action potentials [3•,35•]. As in the case of TRPV1, important insights into the mechanism of cold detection have come from the use of natural products, such as menthol, which elicit a psychophysical sensation of cold. Fifty years ago, Hensel and Zotterman showed that menthol shifts activation thresholds of cold-responsive TG fibers to warmer temperatures, leading them to suggest with great prescience that menthol is likely to "... exert its action upon an

enzyme, which is concerned in the thermally conditioned regulation of the discharge of the cold receptors." [42]. Validation of this idea came with the recent cloning of a cold- and menthol-sensitive TRP channel, TRPM8 (CMR1, trp-p8) [35[•],36[•]]. Previously identified as a transcriptional marker of transformed prostate epithelia [43], TRPM8 is now also known to be expressed in around 10% of all DRG or TG neurons, primarily within small diameter (around 18–20 μm) cells. TRPM8 does not appear to be co-expressed with many of the classical markers of nociceptive neurons, and may define a functionally distinct subpopulation [36[•]]. Menthol and other cooling compounds elicit cationic conductances in heterologous cells expressing TRPM8, with biophysical properties indistinguishable from those observed in cold- and menthol-sensitive neurons under similar conditions [35[•]]. Cold temperatures below 28°C evoke robust membrane currents through TRPM8, which saturate near 8°C. As in the case of capsaicin and TRPV1, these studies provide a molecular explanation for why menthol evokes a psychophysical sensation of cold. They also support the concept that TRP channels serve as principle detectors of thermal stimuli in the mammalian peripheral system (Figure 1).

In addition to TRPM8, Story *et al.* [37[•]] have recently identified another TRP-like channel, ANKTM1, that is activated near 17°C. ANKTM1 is insensitive to menthol and transcripts are expressed in fewer than 4% of sensory neurons. Unlike TRPM8, ANKTM1 is found exclusively in cells that also express TRPV1 and peptide markers of nociceptors, such as calcitonin gene-related peptide (CGRP). ANKTM1 has little sequence similarity to known mammalian TRP channels, and is most closely related to invertebrate TRP-like channels of the TRPN subfamily, named after the Drosophila channel NOMPC [44,45].

Thermal coding — a combinatorial affair?

Now that candidate thermal detectors have been identified, and their expression patterns and temperature sensitivity ranges characterized, we can begin to think about the cellular and molecular logic of thermal coding, at least with regard to peripheral mechanisms. According to this new information, distinct populations of primary afferent neurons (expressing one or more TRP channel) should be capable of detecting high (TRPV2) or moderate (TRPV1) noxious heat, warmth to noxious heat (TRPV3 and TRPV4), cool to noxious cold (TRPM8), or noxious heat and noxious cold (ANKTM1 and TRPV1). At the same time, a thermal stimulus of a given temperature may activate multiple sensory neuron subtypes. For example, an innocuous cold stimulus should activate TRPM8-expressing neurons to elicit a soothing cool sensation. However, as the stimulus intensity increases to the noxious cold range (below 15°C), then neurons expressing ANKTM1 (and TRPV1) should be recruited into action. Similarly, a noxious heat response will activate an even more complex cohort of cells and their receptors (i.e. cells

expressing TRPV1, TRPV2, TRPV3, or TRPV4). Thus, although there may not be a simple 'labeled line' for each thermal modality (i.e. warmth, noxious heat, cool, or noxious cold), it seems likely that a combinatorial code could account for each of these psychophysically distinct stimuli, at least when viewed from the level of the primary afferent fiber. Of course, such models will have to take into account both channel activation thresholds and the extent to which any given channel remains active at temperatures beyond its thermal threshold. Finally, electrophysiological recordings from second-order neurons in lamina I of the spinal cord suggest that distinct classes of cells exist that are responsive to innocuous cooling, nociceptive stimuli only, or multimodal thermal and mechanical stimuli [46,47]. Future studies that take advantage of newly identified thermal receptors should help to define neuronal connections between peripheral and central neurons and elucidate their roles in somatosensation.

Conclusions

The identification of hot- and cold-sensitive TRP channels has provided a framework for understanding the molecular mechanisms of temperature detection and thermosensation. By serendipity of evolution, the plant world has produced the TRPV1 and TRPM8 agonists capsaicin and menthol, thereby providing a compelling psychophysical link between these ion channels and our thermosensory experience. Gene knockout studies in mice have validated this connection for TRPV1, and future genetic analyses shall undoubtedly test somatosensory and nociceptive roles for the more recently identified TRP channels, addressing their putative involvement in the detection of thermal, mechanical or chemical stimuli. Although it now seems likely that TRP channels are the principle detectors of heat and cold in the peripheral nervous system, it is worth noting that other thermally sensitive proteins, such as K^+ channels, Na^+/K^+-ATPases and DEG/ENaC channels [48], may contribute to the overall temperature response profile of primary afferent neurons by modulating the extent, duration, or rate of heat- or cold-evoked action potential firing.

The identification of thermosensitive TRP channels and their genes now opens the way to addressing a variety of fascinating questions at a multitude of biological levels. These range from understanding the biophysical basis of TRP channel gating by physical stimuli to the analysis of neural circuitry and synaptic connections in animals whose sensory neurons are genetically marked using specific promoters (such as TRP channels or other functionally relevant genes). Some of these mechanisms may take time to unravel, but it promises to be an extremely interesting TRiP.

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