Molecular sensors and modulators of thermoreception

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Introduction

Temperature affects nearly every aspect of function in organisms ranging from cell metabolism to animal behaviors. Animals have thus evolved various robust sensory mechanisms permitting them to select their preferred temperatures, while avoiding thermal extremes, an essential process for animals to maintain temperature homeostasis. The perception of temperature is initiated by the activation of thermoreceptors on peripheral nerve endings in mammals. How-ever, the molecular identity of thermoreceptors has been a mystery for a long time. A breakthrough was achieved when the first temperature-sensitive ion channel of thermoreceptors was identified. TRPV1, a heat-activated ion channel expressed on sensory neurons, is thus also dubbed as Thermo-TRP ion channel superfamily, they are thus also dubbed as Thermo-TRP ion channels (Fig. 1). Interestingly, equivalent temperature-sensitive ion channels and thermosensory mechanisms were also discovered in other organisms such as Drosophila. These thermo-sensitive ion channels, therefore, offer a molecular gateway for our understanding of thermal sensation and signaling.

Thermosensation and Thermoreceptors in Mammals

In mammals, temperature sensation is carried by specialized sensory neurons in the Dorsal Root Ganglia (DRG) and the trigeminal ganglia, which project their terminals to both peripheral tissues (e.g. skin) and the spinal cord in the central nervous system (CNS). These temperature-responding sensory neurons are thus the key to our understanding of a broad range of temperature sensation extending from heat, warm to cold.

Heat detection

An inward current triggered by noxious heat (> 42°C) was first observed from a subpopulation of DRG neurons. The molecule responsible for this heat-activated current was soon identified as TRPV1 using the expression cloning strategy. Indeed, when expressed in a heterologous cell system, TRPV1 was activated by heat with a similar thermal threshold of 42°C, and also by capsaicin, a known ingredient from hot chilli peppers causing a burning heat sensation. Moreover, mice deficient for TRPV1 exhibited impaired responses to noxious heat and showed reduced heat hyperalgesia caused by inflammation. These findings argue for TRPV1 as a heat sensor responsible for detecting heat temperature in mice. However, nerve fibers isolated from TRPV1-deficient mice responded to heat normally. Moreover, the heat avoidance behavior of mice was not impaired by deleting TRPV1 over the temperature range between 40°C and 50°C evaluated in a 2 temperature preference assay, but completely eliminated by ablating TRPV1-expressing (TRPV1+) neurons or by silencing TRPV1+ fibers. These results support the idea that there are other yet unknown molecular sensors within TRPV1+ neurons that mediate noxious heat detection.

What molecule functions as an additional heat sensor? In search for homologous genes to TRPV1, TRPV2 was identified as another heat-activated ion channel expressed on sensory neurons, albeit with a much higher heat activation threshold (> 52°C). However, the majority of TRPV2+ cutaneous nerve fibers did not respond to heat, and TRPV2-deficient mice exhibited no deficits in response to noxious heat over a broad heat range. Therefore, it is not likely that TRPV2 functions as a heat sensor.
TRPM3 and calcium activated chloride channel anoctamin 1 (ANO1) are another 2 recently identified ion channels that respond to noxious heat (Fig. 1). TRPM3 and ANO1 exhibit steep temperature dependence and can be directly activated by heat over 40°C and 44°C, respectively, when they were heterologously expressed in HEK293 cells.14,15 Of interest, both TRPM3 and ANO1 are mainly expressed in small diameter nociceptive neurons and the majority of them also co-express TRPV1, suggesting a role of these ion channels in heat nociception. Indeed, responses to noxious heat in mice was significantly reduced by either deleting TRPM3 or ANO1, similar to that observed in TRPV1-deficient mice.4,5 However, there remains a large proportion of heat responding neurons after deleting TRPM3 combined with pharmacologically blocking TRPV1.14 It remains to be determined whether blocking TRPV1 and TRPM3 together with ANO1 can further eliminate remaining fractions of heat-responding neurons. Collectively, these data suggest that sensory neurons employ multiple and redundant heat sensors within TRPV1+ neurons to transduce noxious heat, presumably robust thermosensory mechanisms are required for reliably detecting and avoiding damaging stimuli, such as extreme heat, which otherwise can cause irreversible tissue injury.

**Warm sensation**

The identification of heat transducers prompted the search for sensors responsible for detecting warm temperatures. The attempt led to the cloning of TRPV3 by several labs around similar time.16-18 TRPV3 was activated by innocuous warm temperatures (>33°C).16,18,19 However, TRPV3 was mainly expressed in skin and keratinocytes without significant expression in DRG.18,19 The unique TRPV3 expression profile led to the proposal that TRPV3 acts as a warm receptor in the skin responsible for detecting physiological range of temperatures. Indeed, in one report, mice lacking TRPV3 exhibited deficits in response to both innocuous and noxious heat.20 However, these deficits were not observed in another TRPV3-null mice line with a different gene background.21 TRPV3 may thus have only an assisting role in mediating warm and/or heat perception. In support for this idea, mice with TRPV3 overexpressed in keratinocytes did not display significant altered thermosensory behaviors until functions of the heat receptor TRPV1 were masked by a pharmacological inhibitor.22 A more recent study employing TRPV3 and TRPV1 double knockout mice provided more direct evidence supporting the notion that skin-derived-TRPV3 and sensory neuron-localized TRPV1 have a cooperative role in mediating warm and heat temperature sensation.23 TRPV4 was initially recognized as an osmolality sensor.24,25 It was soon found that TRPV4 can also be activated by warm temperatures over 27°C.26,27 Interestingly, similar to TRPV3, TRPV4 is highly expressed in skin epidermal keratinocytes, but not in DRG.28,29 As expected, both TRPV3 and TRPV4 contribute to different components of currents elicited by warm temperatures in primary skin keratinocytes,19,30 suggesting that keratinocytes may act in concert with sensory neurons to transduce thermal information. As predicted, TRPV4-deficient mice displayed deficits in detecting warmer temperatures.31,32 Puzzlingly, TRPV3/TRPV4 double knock-out mice did not exhibit significant deficits in either thermo-sensory behaviors or thermal nociception.33 These studies suggest that there are other as-yet-unknown significant warm sensing mechanisms that may compensate warm sensation.

In addition to acting on thermo-sensitive ion channels on the cell membrane, temperature rises can also cluster and activate STIM1, an ER Ca2+ sensor, leading to the activation of the store-operated ion channel Orai1 and Ca2+ influx, implying that STIM1 also acts as an intracellular heat sensor. However, it remains to be established whether this heat signaling mechanism contributes to warm and/or heat transduction in somatosensory neurons.

**Cold sensation**

Following the identification of the heat-activated TRPV1 channel, it was suggested that there exists a similar thermoreceptor for detecting cold temperatures, because a moderate cooling can directly elicit an inward ionic current from a subpopulation of sensory neurons.34 Indeed, molecule responsible for mediating the cold-induced current was later on identified as the TRPM8 ion channel.36,37 TRPM8 can be activated by a broad range of cold temperatures ranging from innocuous cooling (<26°C) to noxious cold (<1°C), and also by cooling compounds such as menthol. Consistently, mice lacking TRPM8 lost the ability to sense cold (up to 15°C) and exhibited pronounced deficits in cold-avoiding behaviors.38-40 Furthermore, pain induced by noxious cold was also prevented by either genetically deleting TRPM8 or by pharmacological blocking TRPM8,41,42 in line with TRPM8 activation by noxious cold. These studies conclusively demonstrated that TRPM8 is a *bona fide* principal cold

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**Figure 1.** A schematic diagram depicting the temperature sensitive ion channels. Ion channels are ordered according to their relative activation threshold to temperatures.
sensor in animals. However, the ability to detect noxious cold largely remains in TRPM8-deficient mice, suggesting that there are other significant unknown cold sensing mechanisms.

The attempt to seek another cold sensor for transducing noxious cold led to the identification of TRPA1 using a bioinformatic approach.43 TRPA1 is indeed can be activated by an average of 17.5°C, much lower than that of TRPM8.43 However, this proposal caused a continued debate surrounding the cold sensitivity of TRPA1, with some supporting, while others disproving.44 A recent study demonstrated that TRPA1 is sensitive to cold even when reconstituted into lipid bilayers, lending strong support to the idea that TRPA1 is cold-sensitive intrinsically.45 However, there is again no consensus on whether TRPA1 contributes to acute noxious cold sensation in animals, with some endorsing,46,47 and others not.7,42,48,49 Despite these differences, it is agreed that TRPA1 plays a significant role in pathological cold signaling, such as cold hypersensitivity associated with nerve injury and chemotherapy.48,50-52 In contrast to the controversial role of TRPA1 in cold transduction, TRPA1 was well documented as a polymodal nociceptor for integrating various environmental and endogenous damaging stimuli such as mustard oil and oxidative stress that elicit pain.55 Interestingly, in contrast to the cold-sensitive mammalian TRPA1, invertebrate TRPA1, such as rattlesnake and *Drosophila* TRPA1, is heat sensitive.54,55 The robust heat sensitivity of rattlesnake TRPA1 was proposed to enable rattlesnake to use infrared radiation to detect warm-blooded prey.54

TRPC5 is another TRP ion channel reported to respond to innocuous cold temperature (<37°C) (Fig. 1). However, there are no changes in temperature-sensing behaviors in TRPC5-null mice, thus TRPC5 may only act as a thermal modulator in cold transduction.

In summary, thermo-TRP ion channels function as thermosensors for detecting different spectrum of temperatures. But there are also other unknown mechanisms cooperative for sensing different ranges of temperatures.

**Modulation of Thermal Sensors**

Thermosensors have their inherent thermal activation threshold. The threshold for temperature activation, however, can be modulated by a variety of factors (e.g., inflammatory mediators), leading to abnormal thermo-sensation, such as heat hyperalgesia induced by inflammation. Therefore, understanding thermal modulation of thermosensors is crucial for elucidating abnormal thermo-sensation associated with diseases such as pain. Here I discuss the modulation of TRPV1 and TRPM8, 2 well-accepted thermo-sensitive ion channels, under both physiological and pathological conditions. TRPA1 modulation will also be discussed due to its significant role in pathological cold signaling. However, as TRPV2 and TRPC5 do not function as thermo-sensors, and either TRPV3 or TRPV4 alone does not contribute significantly to thermo-sensation, they are thus not the focus of this review.

**Modulation of TRPV1**

**Physiological modulation**

TRPV1 is believed to be intrinsically heat sensitive. However, different populations of TRPV1+ neurons exhibit differential heat sensitivities, and capsaicin-responding neurons are not always sensitive to heat.57,58 The varied heat sensitivities of TRPV1 in sensory DRG neurons under the basal condition suggest that there exist additional thermal modulators.

We have recently discovered that PKCBII is such a crucial modulator that causes varied heat-induced responses across different populations of TRPV1+ neurons.59 Here, PKCBII is co-expressed in only a subset of TRPV1+ neurons, and markedly enhances their responses by phosphorylating TRPV1 at T705. Interestingly, co-expressed PKCBII is constitutively active as a result of direct binding to TRPV1 and forming a local TRPV1-PKCBII complex (Fig. 2). Therefore, different basal phosphorylation at T705 may underlie varied heat sensitivities of TRPV1, and TRPV1-PKCBII complex-containing neurons may represent a subset of hypersensitive nociceptive neurons.

The membrane lipid PIP2 is another critical factor involved in regulating the heat sensitivity of TRPV1. However, there is a continuing controversy regarding the exact role of PIP2 in TRPV1 activation, with some supporting an inhibiting role,60,61 and some advocating an stimulating effect,62-69 whereas others favoring both activating and inhibiting roles depending on certain conditions.70,71 Different approaches used for manipulating the cellular PIP2 level may underlie the difference, with some including PIP2 into the whole-cell recording pipette65,67 and some applying PIP2 directly to an inside-out excised patch,63,68,69 whereas others reconstituting PIP2 and purified TRPV1 in an artificial liposome or into planar lipid bilayers.61,64 It should be noted that most of these studies are conducted in expression or reconstitution system, which may also contribute to variable conclusions. A missing study is to determine the role of PIP2 in native sensory neurons. In this respect, it will be interesting to know whether different levels of PIP2 are present in different populations of TRPV1+ neurons and thus influence their heat sensitivity.

The negatively charged head groups of PIP2 underlie most of its functional effect. PIP2 acts primarily by binding to positively charged residues on ion channels through the head groups. The identification of PIP2 effector regions or sites on TRPV1 is thus important for elucidating the acting mechanisms of PIP2. In this regard, both a distal C-terminal region (777~820) and a TRP domain in the proximal C-terminal region (682~725), rich in polycationic residues, were identified as the PIP2 binding region.63,72 A recent study further identified R575 and R579 in the S4-S5 linker, and K694 in the TRP domain, as specific PIP2 binding sites on TRPV1 structure.69 It is interesting to note that PIP2 was predicted to bind at the interface between the transmembrane domain and the cytoplasmic domains of TRPV1, lined with the identified basic residues, similar to that observed in the structure of Kir2.2.69 However, how PIP2 exactly binds to TRPV1 can only be answered after resolving the structure of TRPV1 in complex with PIP2.
tein-coupled receptors (GPCR) that couple to either Gs and/or Gq, leading to the activation of PKA and PKC respectively, anchoring both PKA and PKC to the C terminus of TRPV1, thus assembled into a macro-protein signaling complex.47,58,86 Correspondingly, the sensitization of TRPV1 induced by both PKA and PKC was blunted either by knocking down AKAP79/150 or by disrupting mutual interactions between TRPV1 and AKAP79/150.47,87,88 Importantly, inflammatory heat hyperalgesia was inhibited by interfering with the interaction between TRPV1 and AKAP79.89,90 These studies suggest a possible novel analgesic approach by antagonizing the TRPV1-AKAP79/150 interaction.

Intriguingly, another complex formed between TRPV1 and GABA B1 receptor was recently identified.91 Here, activated GABA B1 inhibits TRPV1 sensitization and inflammatory pain caused by inflammatory mediators by preventing TRPV1 phosphorylation. It will be interesting to know whether GABA B1 acts by interfering in the interaction between TRPV1 and AKAP79/150.

Pathological modulation

TRPV1 is activated by noxious heat. In disease conditions such as inflammation, the heat activation threshold of TRPV1 is markedly lowered down so that even pleasant warm temperatures can be felt to be very painful, a process known as heat hyperalgesia. It is caused by the sensitization of TRPV1 by a variety of inflammatory mediators released during tissue injury and inflammation, including bradykinin (BK),60,73 prostaglandin E2 (PGE2),74,75 nerve growth factor (NGF),60,76 ATP,77 substance P,78 cytokines (e.g. IL-6),79 chemokines (e.g., CCL3),80 endothelin-181 and proteases.82–84 Most of these agents bind to G protein-coupled receptors (GPCR) that couple to either Gs or Gq, leading to the activation of PKA and PKC, which then phosphorylates TRPV1 at S116 and S502/S801, respectively, leading to the sensitization of TRPV185 (Fig. 2). Mutating these PKA and/or PKC phosphorylation sites markedly impaired TRPV1 sensitization induced by these agents,85 suggesting that TRPV1 phosphorylation at these sites is critical for inflammatory heat hyperalgesia.

Interestingly, the same mutation of PKCε phosphorylation sites (S502/S801), however, did not affect the basal TRPV1 thermal sensitivity, which is determined by phosphorylation at T705 by PKCβII.85 On the other hand, mutating PKCβII phosphorylation site T705 had no effect on sensitizing TRPV1 induced by BK. Therefore, PKCβII and PKCε control basal thermal sensitivity and sensitization of TRPV1, respectively, by phosphorylating distinct PKC sites. Notably, TRPV1 phosphorylation by PKCε depends on the scaffolding protein AKAP79/150, which anchors both PKA and PKCε in close proximity to TRPV1 by binding to the C terminus of TRPV1, thus assembled into a macro-protein signaling complex.47,58,86 The responsiveness of TRPV1 to heat is not only influenced by the thermal gating of TRPV1, but also affected by the number of ion channels trafficking to the cell membrane. The dynamic trafficking of TRPV1 is a tightly-regulated process. Many protein kinases, such as PKC, PKA, Src kinase and cyclin-dependent kinase 5, were shown to promote the forward trafficking of TRPV1 to the cell membrane, contributing to thermal hyperalgesia.75,76,92 On the other hand, inhibition of TRPV1 internalization induced prolonged thermal hyperalgesia.93 Taken together, both enhanced gating and trafficking of TRPV1 are responsible for enhanced TRPV1 responses to heat, leading to inflammatory hyperalgesia.

Modulation of TRPM8

Physiological modulation

TRPM8 responds to both innocuous and noxious cold and exhibits different cold activation threshold across different populations of sensory neurons. Based on the different activation threshold, TRPM8- neurons were classified into 2 main categories, with one subpopulation activated by a low-threshold (LT) cold (>26°C) and another responding to a high-threshold (HT) cold (<24°C).94,95 However, the mechanisms that govern different cold threshold among TRPM8- neurons are not completely understood. In one study, different levels of TRPM8 expression was proposed to be one of the mechanisms, because LT TRPM8- neurons are often associated with higher TRPM8 responses and vice versa.95 The same study also implicated different expression of shaker-like Kv1 channels in setting the threshold...
of TRPM8^+ neurons, with LT neurons containing lower expression of outward K^+ currents and HT neurons associated with higher level of K^+ currents. A further analysis of TRPM8^+ neurons identified TASK3, a 2-pore-domain K^+ leak channel (K2P), to be highly enriched in TRPM8^+ neurons and critical for specifying the threshold of HT TRPM8^+ neurons. However, in other studies, A type K^+ currents and voltage-gated Na^+ currents were thought to be critical in specifying cold activation threshold of TRPM8^+ neurons. It is possible that a complex interplay and concerted action of different ion conductance shape the excitability of TRPM8^+ neurons. What remains little known is why TRPM8 per se exhibits different cold sensitivities in different subpopulation of neurons and what determine the varied cold sensitivity of TRPM8.

PIP_2 is a well-established factor critical for maintaining TRPM8 activity by binding to the TRP domain in the C terminus of TRPM8. Addition of synthesized PIP_2 activates TRPM8, whereas depletion of PIP_2 inhibits TRPM8 by inducing a 5-phosphatase. Interestingly, different basal temperatures can alter the interaction of PIP_2 with TRPM8, which was thought to be responsible for changes in temperature thresholds for TRPM8 activation induced by different pre-exposed ambient temperatures. Furthermore, the metabolic products of membrane lipids due to phospholipase A2 activation can alter TRPM8 thermal sensitivity. For example, lysophospholipids (LPLs) shifts TRPM8 cold activation threshold toward warm temperature, whereas another product, arachidonic acid, inhibits TRPM8 activation by cold. There is also evidence showing that TRPM8 thermal responses are inhibited by lipid rafts, a cholesterol-rich membrane micro-domain where TRPM8 tends to reside. It is thus tempting to wonder whether these different lipids are crucial in specifying different cold sensitivities of TRPM8 in sensory neurons.

Pathological modulation

It is known that a moderate cooling (innocuous cold) inhibits pain mediating an analgesia effect, but noxious cold causes pain. Paradoxically, TRPM8 can mediate both processes. During inflammatory condition, TRPM8 sensitivity is susceptible to alteration by inflammatory mediators, leading to inflammatory hyperalgesia and cold hypersensitivity. Of note, a brief application of BK rapidly inhibited TRPM8 in DRG neurons, an event presumably leading to the inhibition of TRPM8-mediated analgesia and thus contributing to inflammatory hyperalgesia. The effect is mainly mediated by the BK receptor B2R, a Gq-coupled GPCR. However, the underlying mechanisms for BK-induced TRPM8 inhibition had been unclear. It has been suggested to be caused by either depletion of PIP_2 due to activation of PLCβ or by activation of downstream PKC. However, we found that neither of these mechanisms is critical, instead activated Gq directly inhibits TRPM8 by binding to the channel forming a local protein complex independently of downstream GPCR signaling (Fig. 2). Notably, PIP_2 cannot activate TRPM8 anymore in the presence of activated Gq, suggesting that Gq is a potent regulator of TRPM8 activity. Interestingly, activated G_q and G_11 inhibit TRPM8 to a markedly different degree, despite they have similar capability of inducing PIP_2 hydrolysis. Further supporting the idea that direct inhibition of TRPM8 by G_q is separable from PIP_2 hydrolysis-mediated TRPM8 inhibition. However, it is not known whether these 2 mechanisms act concomitantly to inhibit TRPM8 during activation of a G_q-coupled receptor. In contrast to BK-induced TRPM8 inhibition, artemin, a glial cell-derived neurotrophic factor, sensitizes TRPM8-mediated cold responses in mice, leading to cold hypersensitivity. However, the sensitizing effect of artemin was not demonstrated at the cellular level and the underlying potential signaling mechanisms remain to be established. The opposing effects of BK and artemin may be caused by the colocalization of their respective acting receptors (i.e. B2R and GFRα3) in analgesia- and pain-mediating TRPM8^+ neurons, respectively, thereby contributing to inflammatory hyperalgesia and cold hypersensitivity, separately.

Modulation of TRPA1

As a key damage sensing ion channel, it is not surprising that TRPA1 is targeted by many inflammatory mediators (e.g. BK and PGE2), leading to pain hypersensitivity. Similar to TRPV1, TRPA1 can be potentiated by BK and PGE2, which activates Gq and Gs-coupled GPCR, respectively, resulting in the activation of phospholipase C (PLC) and PKA. Blocking PLC and PKA prevented the sensitization of TRPA1 induced by BK, and activation of PLC and PKA evoked TRPA1-mediated hyperalgesia. Mechanistically, activation of PLC/PKA pathways enhances trafficking of TRPA1 to the cell membrane, suggesting that the PLC and PKA pathways potentiates TRPA1 by promoting forward trafficking of the channel. Interestingly, several downstream signaling messengers of the Gq-PLC pathway such as Ca^{2+}, diacylglycerol (DAG) and arachidonic acid (AA) can directly activate TRPA1, and was suggested to be a mechanism underlying BK-elicited excitation of sensory neurons and pain. A similar direct action on TRPA1 was also observed with prostaglandins (PG). However, PG excites TRPA1 via 15d-PGJ2, a metabolite of PGD2, without the involvement of intracellular signaling. Puzzlingly, none of these studies investigated whether these modulation mechanisms can alter the cold sensitivity of TRPA1.

Thermo-Modulation by Other Ion Channels

Thermo-reception not only depends on the temperature sensitivity of thermos-sensors, but also relies on the membrane excitability and transducing capability of thermo-sensitive neurons, which is determined by several K^+ channels and voltage-gated sodium channels, respectively. Therefore, activities of these channels can significantly influence thermo-reception. Of note, the 2 pore domains background K^+ channels (K2P) TRENK-1, TRENK-2 and TRAAK are sensitive to temperature increases (Fig. 1). They are thus proposed to hyperpolarize both heat- and cold-sensitive neurons and antagonize the depolarizing effect evoked by thermo-sensors, leading to a shift of temperature threshold of thermo-sensitive neurons. Voltage-gated sodium channels
in inflammation and tissue injury, the thermo-sensitivity of ther- morceptors was subjected to be regulated by a variety of factors, leading to thermal hyperalgesia. Thereby, thermo-transduction is governed by both thermal sensors and modulators. Despite rapid progress in our understanding of thermoreception, many ques- tions remain. For example, what are molecular entities for detect- ing heat and noxious cold, independently of thermo-TRP ion channels? It is still not known whether LT TRPM8 neurons mediate cold analgesia and HT TRPM8 neurons cause cold pain. Understanding these fundamental questions will be critical for elucidating pathological thermo-sensations and open up novel targets for therapy of related diseases.

Concluding Remarks

Thermoreception is fundamental to animals. Many tempera- ture-sensitive ion channels and receptors have been identified and some of them act as molecular thermometers involved in thermo-sensation. Under pathological conditions such as

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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