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Sensing hot and cold with TRP channels

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Abstract

The past decade has witnessed the cloning of a new family of ion channels that are responsive to temperature. Six of these transient receptor potential (TRP) channels are proposed to be involved in thermosensation and are located in sensory nerves and skin. The TRPV1, TRPV2, TRPV3, and TRPV4 channels have incompletely overlapping functions over a broad thermal range from warm to hot. Deletion of the individual TRPV1, TRPV3, and TRPV4 channels in mice has established their physiological role in thermosensation. In all cases thermosensation is not completely abolished – suggesting some functional redundancy among the channels. Notably, the TRPV2 channel is responsive to hot temperatures in heterologous systems, but its physiological relevance in vivo has not been established. Cool and cold temperatures are sensed by TRPM8 and TRPA1 family members. Currently, the pharmaceutical industry is developing agonists and antagonists for the various TRP channels. For instance, TRPV1 receptor agonists produce hypothermia, while antagonists induce hyperthermia. Recent investigations have found that different regions of the TRPV1 receptor are responsive to temperature, nociceptive stimuli, and various chemical agents. With this information, it has been possible to develop a TRPV1 compound that blocks responses to capsaicin and acid while leaving temperature sensitivity intact. These channels have important implications for hyperthermia research and may help to identify previously unexplored mechanisms in different tissues that are responsive to thermal stress.

Keywords: *Thermo-TRP channels, temperature regulation, behavior, mutant mice, cell culture, electrophysiology*

Introduction

Maintenance of body temperature is a basic physiological process for many different organisms. Mammals defend their body temperatures through a variety of physiological and behavioral responses which help them to maintain temperature within a narrow range that is optimal for ongoing biochemical processes. Innate acute nocifensive behaviors permit escape from environmental conditions of intense cold or heat so that tissue damage, morbidity, or mortality do not ensue. Responses to temperature change require the organism to possess sensors that can detect thermal changes in the environment. Very recent work has identified a number of receptors in the skin that respond to temperature changes in the environment. Six different thermo-sensitive receptors have been newly identified to play important roles in thermoregulation and these receptors are members of the transient receptor potential (TRP) ion channel family and have been termed thermo-TRP channels (Table I).

TRP channels that sense heat

TRP channels were first identified in a phototransduction mutant in *Drosophila* that showed a transient instead of a sustained response to light [1]. Since that time, the first mammalian TRP channel was found to reside in primary sensory neurons and it was termed TRPV1 (TRP vanilloid receptor type 1) [2–4] (Table I). Four identical TRPV1 subunits, each containing six transmembrane domains with a pore loop between domains 5 and 6, assemble to form the channel [5]. In heterologous systems this channel is weakly Ca^{2+}-selective, and when stimulated by capsaicin, resiniferatoxin, acid, endocannabinoids, certain eicosanoids, and heat (>43°C), it produces

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Table I. Characteristics of thermo-TRP channels.

Channel	Temperature sensitivity	Tissue distribution
TRPV1	≥43°C	Central and peripheral nervous systems, tongue, bladder, skin
TRPV2[+]	≥52°C	Central and peripheral nervous systems, widely expressed
TRPV3	≥33°C	Central nervous system, keratinocytes
TRPV4	28–42°C	Central and peripheral nervous systems, kidney, inner ear, endothelial tissues, skin
TRPM8	<15–<28°C	Peripheral nervous system
TRPA1	<18°C	Peripheral nervous system, hair cells

[+]The role of this channel in thermal sensation has not been established in vivo.

outwardly rectifying cation currents [2–4, 6]. As an aside, in avian species TRPV1 responds to heat but not capsaicin [7]. Since in whole-cell recordings, excised patches with TRPV1 can be activated by increased temperature, it is likely that this receptor is stimulated directly by the proton mode of the receptor [6, 8]. TRPV1 is expressed primarily in small- to medium-diameter peptidergic and non-peptidergic sensory neurons within the dorsal root, trigeminal, and nodose sensory ganglia that are characteristic of nociceptive C- and Aδ-fibers [2, 6]. It is also found within brain [9, 10]. In the periphery, termini of capsaicin-sensitive neurons release the neuropeptides, substance P and calcitonin gene-related peptide that can lead to neurogenic inflammation [11]. Recently, two splice variants of TRPV1 have been identified [12]. When murine TRPV1β is expressed with TRPV1α channels, it exerts dominant negative actions; the human variant is stimulated by acid and capsaicin [13]. Additional TRPV1 mRNA species have been visualized in kidney and brain [14, 15]. TRPV1-knockout mice have been generated and electrophysiological responses to capsaicin, acid, and heat (43°C) were reduced or absent in these mutants, while responses to >50–55°C were conserved [16, 17]. In tail immersion assay, withdrawal latencies at temperatures >48°C were protracted for TRPV1-knockout animals, but they had normal latencies at lower temperatures [16]. A similar relationship was seen with the hot-plate assay [16]. Despite this result, another group was unable to detect any genotype differences in the hot-plate or radiant heat assays [17]. Nonetheless, inflammation-induced heat hyperalgesia was virtually abolished in TRPV1-knockout animals [16]. When capsaicin was injected into the plantar skin of the hind-paw, robust licking and paw shaking were observed in wild type mice [16]. By comparison, little or no response was evident and edema was less apparent in the knockouts. In a skin-nerve preparation ~50% of small dorsal root ganglion cells from wild type mice were responsive to heat stimuli up to 47°C, only 16% of cells from knockout mice were activated by heat [16]. Nevertheless, no genotype differences were observed in thermal thresholds for units that were stimulated by heat stimuli; however, the mean evoked discharge was decreased by ~45% in units from mutant mice. Interestingly, peripheral injection of capsaicin was sufficient to lower body temperature by ~6°C in wild type animals and it took up to 2 hours for recovery; TRPV1-knockout mice were completely unresponsive. Additional experiments with TRPV1-knockout mice have revealed that they are deficient in mounting a fever response to lipopolysaccharide [18]. Although these collective findings strongly support a role for TRPV1 receptors in thermal responses, they cannot account for all responses to heat.

Effects of TRPV1 on temperature regulation have been investigated in more detail. Capsaicin and resiniferatoxin are known to produce hypothermia [19, 20]. As described above, body temperatures of TRPV1-knockout mice were not affected by capsaicin administration [16]. Subsequent studies with mutants have shown that the circadian rhythm for temperature, tolerance to decreased (4°C) or increased (35°C) environmental temperatures, and responses to ethanol-induced hyperthermia were normal [18]. However, fever production by lipopolysaccharide was attenuated in TRPV1-knockout mice. In a separate investigation [21], the amplitude of daily body temperatures were higher for TRPV1-knockout animals than for wild type controls. As a control, wild type mice were treated with capsaicin for 2 consecutive days to down-regulate the receptor [21]. Interestingly, the amplitude of the daily body temperatures was even higher than that in the knockout mice. When the TRPV1 mice were placed into a 37°C environment for 30 min, body temperatures were increased to the same extent in wild type and mutant animals; the capsaicin-desensitized animals had to be removed from the conditions after 15 min because of development of imminent heat-stroke (i.e., their body temperatures reached 42°C). These results indicate that TRPV1-knockout animals can defend their body temperatures against acute overheating. Notably, these experiments also reveal that down-regulation of the channel more severely effects temperature regulation than deletion of the gene – possibly due to off-target effects in the intact animal and compensatory effects

in the mutant mouse. Importantly, this difference in response has important implications for the development of compounds to be used for pharmacotherapy.

Additional temperature regulation experiments have been conducted with TRPV1 agonists and antagonists. TRPV1 receptor agonists cause hypothermia and shivering at least through skin vasodilation and reduced metabolism [22]. Recent work with a variety of distinct TRPV1 receptor agonists and antagonists have found them to produce hypothermia and hyperthermia, respectively in rats, mice, dogs, monkeys, and humans, but not in TRPV1-knockout mice [23]. The hyperthermia appears to be transient and tolerance to repeated drug administration develops, suggesting that other mechanisms are involved in maintaining homeostasis. Pharmacological antagonism of the TRPV1 channel also produces hyperthermia in humans [24]. Together, these data indicate that TRPV1 is tonically active and that it plays a role in the regulation of body temperature through a variety of thermoeffector mechanisms in the periphery and central nervous systems [c.f., 10, 25].

TRPV2 has been found to mediate high-threshold heat sensations (>52°C) in vitro. Since it is expressed in medium- to large-diameter sensory neurons, it is thought to be associated with Aδ fibers [3] (Table I). TRPV2 is also found within the brain [10, 25]. In contrast to TRPV1 channels, TRPV2 is not responsive to capsaicin and its distribution across tissues is wider [26]. Sensory C-fiber neurons from TRPV1-knockout mice that do not contain putative TRPV2 immunoreactivity respond normally to noxious heat [27]. However, only one such neuron was identified in this study. Investigations by other researchers have localized TRPV2 primarily to myelinated fibers that were mechanically sensitive; no evidence for expression in C-fibers was observed [28]. The few TRPV2-containing cells that did respond to heat also were active to cold stimuli. While TRPV-2 channels appear to be activated by heat in vitro, evidence that the channels mediate sensation to heat in vivo is lacking.

In heterologous systems TRPV3 is activated by camphor and increases in thermal temperatures (>33°C) [29–32] (Table I). As compared to other TRP channels, TRPV3 shows a biphasic activation profile [33, 34]. The initial sensitizing phase develops gradually and is followed abruptly by a secondary phase with a current of larger amplitude and the loss of the outer rectifying current. TRPV3 is expressed in keratinocytes, but not in sensory neurons [30]. TRPV3 is also found within the brain [20, 25]. There is some evidence that TRPV3 may interact with TRPV1 and TRPV2 channels [31, 35]; however, the relationship between TRPV1 and TRPV3 channels is controversial [35]. Disruption of TRPV3 exerts no effects on body weight, body temperature, exploratory activity in the open field, or anxiety-like responses [29]. In a temperature preference test with a choice between two temperature zones, 92% of the wild type animals preferred to be on the 35°C plate, whereas only 64% of the mutants selected this plate over the room temperature one [29]. Another thermotaxis assay run with both plates at room temperature or one at room temperature and the other at 15°C failed to distinguish responses between the genotypes [29]. In a linear temperature gradient assay (15–55°C), wild type mice developed a preference for zones between 30–38°C within the first 25 minutes [29]. Knockouts took 60 min to develop this preference. In a tail-immersion test, tail-flick was delayed in the TRPV3-knockout animals at 48, 50, and 52°C; a similar protracted response was seen with the hot-plate assay at 45, 50, and 55°C [29]. Electrophysiological recordings with cultured keratinocytes showed that most wild type cells responded with gradually increasing current responses to 37°C pulses, whereas cells from knockout animals were unresponsive to this stimulus or displayed TRPV4-like desensitization responses [29]. Hence, disruption of *Trpv3* in mice serves to impair responses to noxious thermal stimuli, but it does not abolish them.

TRPV4 channels are also sensitive to heat stimuli at 25–34°C in heterologous systems [36] (Table I). TRPV4-knockout mice have been developed and these mutants show impairments in osmotic regulation or alterations in vasopressin secretion [37, 38]. TRPV4 is expressed in skin, endothelium, kidney, dorsal root ganglion, and brain [37, 38–40]. Importantly, TRPV4-knockout mice were deficient in responses to tail pressure and acid nociception; however, responses to radiant heating or to a hot plate (35–50°C) were not different from those of the wild type controls [37, 41, 42]. Electrophysiological recordings revealed that ~80% of the hind-paw femoral nerves of wild type mice were responsive to 40°C, while only ~15% of nerves in TRPV4-knockout samples were activated [42]. When temperature was increased from 25 to 50°C, nerves from wild type mice began responding at 33°C, whereas those from knockout animals did not respond until 40°C was reached [42]. Notably, the numbers of heat-sensitive neurons and the magnitudes of evoked activities of the 40°C-sensitive neurons were similar for wild type and TRPV4-knockout mice. Additionally, no genotype differences were noted in responses at 50°C. Since inflammation or swelling can increase sensitivity to temperature [40], animals were tested for hot-plate (40°C) responses before or 20 minutes after injection of carrageenan into the hind-paws [42]. Despite there being no genotype differences in swelling to carrageenan, wild type

latencies to escape to 40°C were decreased from baseline following injection while those for mutants were not distinguished between baseline and carrageenan conditions. Following carrageenan administration latencies to escape from 37.5 and 42.5°C were also prolonged for TRPV4-knockout mice, while both genotypes responded similarly to 50°C. Electrophysiology studies indicated that while carrageenan-induced discharges were different from the magnitude of those induced by temperature, the threshold for warmth-sensitive neural activity was reduced. Parenthetically, it should be emphasized that hypotonicity, such as swelling, can lead to production of 5′,6′-epoxyeicosatrienoic acid which can directly activate TRPV4 channels [44, 45]. These findings indicate that TRPV4 is responsive to thermal hyperalgesia at warm temperatures and that this channel is responsible for determining the sensitivity of the response. It should be emphasized, however, that heat may activate TRP4 through indirect mechanisms since in whole cell configurations in excised membrane patches TRPV4 is not activated by heat [46].

In a separate set of experiments, Lee and co-workers [5, 47] evaluated thermal selection responses in TRPV4 mice. In thermal gradient and thermal selection assays, TRPV4-knockout mice preferred higher temperatures than wild type controls. In tail immersion tests, withdrawal responses were prolonged at 45 and 46°C, but were not different from wild type animals between 47 and 50°C. Inconsistencies between tail immersion [5, 47] and hot plate results [37, 41, 42] may be attributed to differences in sensitivities of the tail and paws, how the tests were scored, or in genetic background. In the hyperalgesia assay with complete Freund's adjuvant, no genotype differences were observed in the thermal selection assay [5, 47]. These findings are in contrast to those from Todaka and colleagues [42] and bring into question the role of TRPV4 in hyperalgesia. In a separate experiment, body temperatures were not different between genotypes in terms of circadian rhythmicity at 25°C or following an abrupt increase in ambient temperature from 25 to 35°C [5, 47]. These findings support the idea that TRPV4 is not required for thermoregulation, but is necessary for detection of thermal changes in the periphery.

Aside from nervous tissues, TRPV4 channels have been found in kidney, endothelial cells, and keratinocytes. In kidney, TRPV4 is postulated to play a role in systemic osmoregulation [39]. In endothelium deletion of the TRPV4 gene has been found to alter membrane permeability and Ca^{2+}-influx [40, 48–52]. Keratinocytes have been found to contain several different types of TRP channels, in particular they possess TRPV3 and TRPV4 channels [30, 32, 36]. Although TRPV1 channels have been found in human keratinocytes [53, 54], they have not been reliably identified in mouse keratinocytes [33]. However, whether all three channels exist within the same cells and, if co-expressed, how they may function within the same cell is unknown. Chung and colleagues [33] conducted an electrophysiology experiment with primary keratinocytes to address this question for TPRV3 and TRPV4 channels. They found these cells detect warm temperatures by two distinct mechanisms. The TRPV3 responses were characterized by a slow increase in current amplitude and a protracted sensitization to repetitive warm thermal stimulation. The TRPV4 responses appeared to involve rapidly activating, weakly rectifying, desensitizing currents. The latter responses were absent in TRPV4-knockout cells and they reappeared when the null cells were transfected with murine TRPV4 cDNA. Hence, keratinocytes possess distinct responses mediated by TRPV3 and TRPV4 channels to signify changes in mild temperatures. Additional research has shown that TRPV3 and TRPV4 form homodimers [35]. Presently, it is unclear whether they can form heterodimers with hybrid functional characteristics.

Keratinocytes are found primarily in the outer layer of the skin where they form tight junctions with nerves of the skin, and can modulate lymphocyte and Langerhans cell function [55]. Keratinocytes are involved in making keratin that serves to protect the skin and underlying tissue from environmental damage (e.g., UV radiation, heat, and water loss). These cells arise from proliferative cells located in the basal layer of the epidermis and migrate to the surface. Additional epithelial cells include melanocytes, Langerhans cells, Merkel cells, and sensory nerve terminals. The inner skin layer or dermis contains connective tissue, hair follicles, sebaceous and sweat glands, nerves and sympathetic fibers, blood and lymphatic vessels, and specialized cells such as Pacinian corpuscles. Results from the TRPV3 and some TRPV4 experiments indicate that keratinocytes participate in thermal sensation. What is not clear is how keratinocytes can communicate with sensory neurons. It has been proposed that the non-neuronal keratinocytes may signal to sensory neurons through the release of some soluble factor (perhaps ATP) [5, 56]. This type of mechanism has already been reported for the gustatory system for tastant detection by epithelial taste buds and gustatory sensory afferent terminals [57]. Since non-peptidergic and peptidergic nociceptive innervations of epithelia are segregated spatially within the skin and spinal cord [58], it may be the case that similar parallel distinct neural pathways exist for sending temperature information from keratinocytes to sensory neurons.

TRP channels that sense cold

Although TRPV1, TRPV3, and TRPV4 play roles in heat detection, TRPM8 (TRP channel, subfamily M, member 8) and TRPA1 (TRP channel, subfamily A, member 1) or ANKTM1 (ankyrin-repeat, transmembrane, member 1) are responsive to cool or cold temperatures. Typically, innocuous cool includes temperatures from 30 to 15°C with noxious cold involving temperatures below 15°C [59–61]. TRPM8 channels respond to ''cooling'' compounds such as menthol and to reductions in temperature [62–64]. It should be emphasized that TRPM8 demonstrates steep temperature-dependence that extends from cool (<28°C) to noxious cold (<15°C) [65] (Table I). Interestingly, reductions in intracellular pH inhibit the cold-induced activation of TRPM8 [66]. Since in whole-cell recordings of excised patches TRPM8 can be activated by changes in temperature, it is likely that this receptor is stimulated directly by cool temperatures and that it does not require some soluble factor for activation [67]. TRPM8 is expressed in 5–10% of the small diameter tyrosine kinase receptor (TrkA) positive cells in dorsal root ganglion – suggestive of C-fiber and Aδ-fiber localizations; however, these cells do not co-express calcitonin gene-related peptide or substance P or other markers of nociception [63]. In a more recent study, investigators targeted green fluorescent protein to TRPM8-expressing cells using bacterial artificial chromosome technology and demonstrates that this channel is expressed in C-fibers and Aδ-fibers of the trigeminal or dorsal root ganglion [68]. These neurons terminate as peripheral nerve endings in skin in areas that mediate responses to innocuous cool, noxious cold, and nociception.

The gene for *Trpm8* has been disrupted in mice by three independent groups [69–71]. Bautista and colleagues [69] observed trigeminal neurons from TRPM8-knockout mice to be unresponsive to menthol or icilin, while Colburn and coworkers [70] found menthol sensitivity to be reduced in their mutants. Menthol responses were reduced also in mutant dorsal root ganglia neurons [71]. When cold-sensitive trigeminal neurons were subjected to a cooling gradient (30 to 8°C over 30 seconds) or to a 18°C stimulus, responses were distributed according to menthol-sensitive and –insensitive neurons [69]. The former neurons had an average threshold of 22°C, but when cooled from a holding temperature of 22°C the activation threshold shifted to 16°C – suggesting plasticity of response and adaptation. The latter neurons had a slower cold activation rate and had an average response threshold of 12°C regardless of starting temperature. Importantly, they were not mustard oil sensitive suggesting that TRPM8 does not participate in this response [69]. Deletion of TRPM8 only affected the menthol-sensitive group where the numbers of cells and magnitude of responses were diminished [69]. Despite this fact, TRPM8-deficient neurons displayed normal responses to heat. Similar results were seen with skin-nerve preparations where responses in C-fibers and Aδ-fibers were reduced in TRPM8-knockout samples, while mechanical responses were intact [69]. In behavioral tests mutant mice were deficient in the acetone-evoked evaporative cooling test [69, 71]. Mutants also could not discriminate warmer from cooler surfaces, but could detect cold – however, the thresholds for cold detection were different among the groups [69–71]. No genotype differences were observed for detection of heat or pressure. It should be noted that the differences in cold detection thresholds were not unexpected because of voltage-dependent gating characteristics of TRPM8 (as well as TRPV1) render these channels sensitive to temperature-induced shifts in their voltage-dependent activation curves [56, 64, 72]. When cold-plate responses were examined at 0°C, wild type mice responded within ~8 seconds, whereas TRPM8-knockouts took approximately twice as long but their individual latencies were still much shorter than the maximal 90 seconds allotted for the test [70]. However, Dhaka and colleagues [71] failed to observe a genotype difference at −1°C – perhaps because the temperature-dependence of the cold plate response is so steep. Although responses to mechanical allodynia were intact, neuropathic cold responses were deficient in mutant mice [70]. TRPM8-knockout mice were evaluated also whether cold could be analgesic to formalin injection [71]. On a 17°C cold plate, formalin-treated wild type mice displayed reduced responses during the acute (0–10 minutes after injection) and inflammatory (10–30 minutes after injection) phases of the test – demonstrating that cold is analgesic to formalin administration. TRPM8-knockout mice did not display the cold-induced analgesic response during the first 10 minutes of the test. Collectively, these data suggest that TRPM8 is important for thermal sensitivity and that it contributes to responses in the cold range; however, detection of noxious cold appears to involve additional mechanisms that may be due to other receptors, changes in vascular tone, alterations in nociception, or indirect effects from tissue damage or inhibition of warm-sensing fibers. The temperature and nociceptive results suggest that TRPM8 may be expressed at least in two different sets of neurons. One responds to cool temperatures to affect the perception of cool stimuli to control behavioral thermoregulation and analgesia, and a second population

transmits nociceptive information to influence nocifensive behaviors [8].

ANKTM1 or TRPA1, another member of the TRP family, has been cloned [73]. TRPA1 responds to cold temperature (<18°C), cannabinoids, mustard oil and other isothiocyanates, as well as mechanical stimuli (hair cells in inner ear), but not menthol [56, 74] (Table I). TRPA1 is expressed in a small population of TRPV1-containing, but not TRPM8-containing, sensory neurons, as well as with the nociceptive markers calcitonin gene-related peptide and substance P neurons [56, 75]. TRPA1 is not expressed with TRPM8 – indicating these channels do not coordinate at the level of individual sensory cells to integrate thermal information. Additionally, since TRPA1 is not expressed in heavily myelinated neurons, it has been suggested that it is localized to non-myelinated C- or lightly myelinated Aδ-fibers. Different splice forms of TRPA1 have been reported [56]. The short form does not posses transmembrane domains and does not constitute a functional channel. It serves to block transport of the long form of the receptor from the cytoplasm to the membrane. Experiments in heterologous cells have shown that TRPA1 is stimulated by formalin, whereas other members of the TRP family are not responsive [76]. Neurons from wild type trigeminal and dorsal root ganglia were activated also by formalin and the responses were blocked by a TRPA1 inhibitor (HC-030031), whereas neurons from TRPA1-knockout mice were unresponsive to formalin. Similarly, HC-030031 also blocked formalin-induced paw lifting, licking and flinching (Phase I), as well as, sensitization responses (Phase II) in wild type mice, but all formalin-induced behaviors were reduced in the knockout animals. Hence, TRPA1 appears to mediate nociceptive responses to formalin.

The temperature sensitivity of TRPA1 has been questioned by some investigators. For instance, when evaluating neurons that responded to cold (5°C), 95% were activated by menthol and the remainder were insensitive to mustard oil and menthol [75]. Only ~4% of the trigeminal neurons that responded to mustard oil were also activated by cold stimulus (5°C), but all of the cold-sensitive neurons were stimulated by menthol – suggesting that they had TRPM8 receptors [75]. The gene for *Trpa1* has been disrupted by two independent groups [77, 78]. Neurons from TRPA1-knockout mice were insensitive to allicin and mustard oil, but were fully activated by capsaicin, menthol, and Ca^{2+} [77]. When mustard oil was applied to the hind-paw, licking and flinching were abrogated in the knockout animals and inflammation was attenuated. Additionally, TRPA1-knockout mice showed no evidence of hypersensitivity to radiant heat or mechanical stimulation. When sensitivity of trigeminal neurons to cold stimuli (bath was reduced from ~23° to 6°C) was examined, 78% of the wild type cold-sensitive neurons responded to menthol while the remaining neurons were insensitive to it or mustard oil. A similar distribution was observed in TRPA1-knockout samples and these results were replicated in dorsal root ganglia neurons. In behavior no genotype differences were observed for acute nocifensive responses of shivering or paw withdrawal to cold surfaces (20, 10, 5, 0, −5, or −10°C), preference of cold surfaces over those at room temperature, acetone-evoked evaporative cooling, or for vestibular or auditory responses. Bradykinin has long been known to exert two actions on nociceptors: acute depolarization and protracted hypersensitivity to mechanical or thermal stimuli [79, 80]. When examined for phospholipase C-mediated bradykinin responses, trigeminal neurons from TRPA1-knockout mice were activated less by bradykinin than those from wild type animals [77]. When bradykinin was injected into the hind-paws of knockout mice and they were exposed to radiant heat, no evidence for hypersensitivity was observed. Nevertheless, both wild type and mutant mice developed thermal hypersensitivity to Freund's complete adjuvant which stimulates multiple inflammatory pathways. These findings suggest that TRPA1-knockout mice are responsive to a subset of inflammatory agents that activate phospholipase C signaling pathways. Results from these studies suggest that mustard oil and allicin stimulate TRPA1 receptors on primary afferent neurons to produce inflammatory pain, but do not mediate responses to cold temperatures or vestibular or auditory stimuli.

By comparison, some investigators find support for Story and colleagues' [73] contention that TRPA1 channels are activated by cold. Kwan and co-workers [78] have also disrupted *Trpa1* in mice. In concert with Bautista and collaborators [77], their mutants were not deficient in vestibular or auditory function and electrophysiological responses to mustard oil in neurons for mutant mice were almost completely lost. In a behavioral test, mustard oil was added to the drinking water and at the highest concentration wild type and heterozygous animals ceased drinking; homozygous mutants consumed a third less than normal. TRPA1-knockout mice were also less responsive than wild type animals to mustard oil injection in their hind-paw. Additionally, mutants were also less sensitive than wild type controls to bradykinin-induced mechanical hyperalgesia and to bradykinin injection into the hind-paw. Although both genotypes showed similar responses to a hot-plate (50, 52, 55°C), paw withdrawal responses to the cold plate (0°C) were reduced in mutants [78].

TRPA1-knockout mice were also less sensitive to acetone-induced evaporative cooling than wild type animals. Interestingly, the genotype difference was larger for females than males on the cold plate and acetone tests. Additionally, knockout mice showed reduced mechanical sensitivity to von Frey filaments and to blunt pressure in the Randall-Selitto test. In a model of peripheral neuropathic pain (ligation of the tibial and common peroneal nerves), no genotype differences in mechanical hypersensitivity were noted. Since this response is thought to be mediated by low-threshold, large, myelinated non-peptidergic Aβ-fibers [80, 81] and because TRPA1 is not expressed in these fibers [82, 83], the lack of a genotype distinction is consistent with TRPA1 expression results. Additional convincing evidence for TRPA1 playing some role in thermal sensitivity comes from work by Obata and co-workers [84]. Here, pharmacological blockade of TRPA1 in primary sensory neurons was sufficient to reverse the cold hyperalgesia produced by nerve injury and peripheral inflammation. Moreover, both conditions increased expression of TRPA1, but not TRPM8, in TrkA-containing dorsal root neurons. Intrathecal injection of TRPA1 antisense oligonucleotides depressed the induction of TRPA1 and cold hyperalgesia. More recent experiments using cultured vagal sensory neurons from nodose ganglion have identified a large fraction of neurons that respond to cold with a threshold $\sim$24°C [85]. The responses of these neurons were evaluated with TRPM8 and TRPA1 agonists and antagonists and these experiments suggested that TRPA1 channels were the primary mediators of the cold responses. These results were replicated in mouse nodose ganglion and neurons from TRPA1-knockout mice [note, mice from 78] showed a large reduction in the percentage of cold-responsive neurons. Similarly, Karashima and colleagues [86] found TRPA1 in heterologous cells to be responsive to the cold (26 to 10°C) and for trigeminal mustard oil responsive neurons from TRPA1-knockout mice [mice from 78] to be deficient in cold sensitivity. In wild type mice, TRPA1 trigeminal neurons had a lower threshold for activation to cool and cold temperatures, showed different time-courses, and were differentially sensitive to capsaicin than TRPM8 neurons. On the cold plate (0°C) and cold tail-flick ($-$10°C) tests, TRPA1-knockout mice were deficient compared to their wild type controls [86]. Hence, the bulk of the evidence supports a role for TRPA1 in mediating effects of cold temperatures. There may be several reasons for disagreement in the investigation of TRPA1-knockout mice. First, a fragment of TRPA1 was still expressed in Bautista and colleagues' [77] knockout animals. While this fragment did not appear to function as a dominant negative for capsaicin, menthol sensitivity, or Ca^{2+} handling, it may have exerted some unrealized effect on cold responses. Second, the presumed TRPA1-mediated behavioral responses to isothiocyanates could have been modulated by other receptors. Third, because various chemical agonists and thermal stimuli may gate the TRPA1 channel and activate other nociceptors, the final psychophysical responses may represent a summation or some combination of these inputs. In an analogous fashion responses to noxious cold may require additional inputs besides TRPA1 from converging neurons containing other receptors sensing cool (e.g., TRPM8). Finally, there is evidence that some cold-responsive neurons in dorsal root and superior cervical ganglia do not respond to menthol or mustard oil [87]. The threshold for cold temperatures was higher in dorsal root ganglion where only TRPM8 was expressed; TRPA1 was expressed at 10-fold higher levels in superior cervical ganglion than dorsal root ganglion. Hence, there may be additional receptors besides TRPM8 and TRPA1 that are sensitive also to cold. It this respect, Kv1 potassium channels can modulate the threshold for cold stimuli in TRPM8-expressing cells [88]. Additional non-TRP candidates for temperature regulation include background or TREK-1 potassium channels [67, 89, 90], Na^+/K^+ ATPase [91], the DEG/ENaC sodium channels [92], the voltage-gated sodium channel $Na_V1.8$ [93, 94], and the ATP-gated P2X3 purinergic receptor [91].

Heteroreceptor regulation of TRP channels

Activities of many TRP family members can be modulated by G protein coupled and Trk receptors that are coupled to phospholipase C and production of diacylglycerol, inositol phosphates, and/or changes in extracellular or intracellular Ca^{2+} concentrations [4, 91]. Parenthetically, there is evidence that some TRP channels can be modulated also by eicosanoids, endocannabinoids, and various other signaling molecules [4, 56, 91, 95, 96]. TRPV1 channels can be heterologously sensitized through actions of G protein coupled or tyrosine kinase receptors so that the channel opens at normal body temperature [97–99]. Many other TRP family members also show similar responses; however, activation of the TRPA1 channel can occur in the absence of Ca^{2+} and with Ca^{2+} availability augmenting the response [85]. Additionally, the TRPA1 channel has been proposed to be activated by bradykinin in two ways: through activation of the bradykinin receptor and stimulation of phospholipase C-mediated increases in intracellular Ca^{2+} and by Ca^{2+} influx through TRPV1 channels [77]. Since TRPA1 can be activated by thermal, mechanical, and various chemical stimuli, it may be an important integrator of information.

Since other TRP channels can also be polymodal transducers, their responses to thermal, nociceptive, chemical, and other changes may show plasticity where the threshold for activation and dose-response curve are shifted.

Promiscuous TRP channels

Although TRPV1, TRPV3, TRPV4, TRPM8, and TRPA1 channels respond to temperature, many also respond to pain. For instance, TRPV1 channels respond to inflammatory pain [16] and this condition results in peripheral sensitization where the threshold for activation is decreased. Under these circumstances, the threshold for heat pain is reduced from ~42°C to a level that is near body temperature [56]. In a model of peripheral nerve injury, TRPV1 levels are reduced in injured neurons but they increase in neighboring neurons [100]; thereby, potentially altering sensitivity to thermal stimuli. TRPV1 channels can be rendered more sensitive to stimuli through phosphorylation of the channel [101–103]; they can also be desensitized by dephosphorylation [104–105]. It should be noted, that local application of capsaicin has been used therapeutically for many years to produce analgesia for treatment of postherpetic neuralgia and diabetic neuropathy [106]. This therapy also reduces the sensitivity of the skin to thermal agents [107].

Development of compounds that target TRP channels

Although TRPV1 is the most studied and validated thermally-sensitive TRP channel, development of agonists and antagonists for this and other TRP family members are currently undergoing intense development [11, 22, 108]. Three different strategies have been applied: development of receptor agonists and receptor antagonists, as well as, developing compounds that can enter the pore of the TRP channels and exert actions. For instance, TRPV1 channels show time- and agonist-dependent increases in permeability that is due to phosphorylation of the channel by protein kinase C [109]. QX-314, a lidocane derivative, cannot block Na^+ channels when it is administered extracellularly because it cannot gain access to the inner face of the Na^+ channel [110]. When administered with capsaicin, QX-314 can enter TRPV1-containing cells through the TRPV1 channel and exert effects that last more than 10-times longer than lidocane alone. Notably, the threshold for pain and radiant heat sensitivities are reduced by this agent. In comparison to this mechanism, chronic administration of TRPV1 agonists can be used to desensitize the receptor, such that its actions are similar to that of antagonists that block actions at the receptor.

As described above, the TRPV1 channel and some other TRP family members may be activated by many stimuli. In the case of TRPV1, different domains of the receptor have been found to be responsive to different stimuli. For instance, the C-terminal region confers the channel with heat sensitivity [111]. Some investigators have been able to develop TRPV1 compounds (i.e., AMG-8562) that can antagonize responses to capsaicin and acid, but leave the heat-evoked responses intact [112]. This indicates that it is possible to target different functional domains of the receptor for selective activities. However, it should be emphasized that the pharmacological effects of antagonists do not always mimic results reported in the TRPV1-knockout mice [11, 21]. These differences may be attributed to off-target effects of the compounds, alterations in sensitization and/or down-regulation of the channels in different tissues with subchronic or chronic dosing, and compensation of some functions in the knockout mice. Additionally, long-term effects of administration have not been investigated. The future challenge in developing compounds that target TRP channels will be to stimulate, attenuate, or block certain physiological responses while leaving others intact.

TRP channels and studies of hyperthermia

The cloning and identification of receptors that can sense thermal changes present a number of new avenues for investigation for hyperthermia research. Aside from localization to sensory neurons and skin, TRP channels have been identified in other tissues. Currently, their physiological roles in these other tissues are unknown. It is also unclear the extent to which normal physiological responses to hyperthermia in brain and other tissues are mediated by these receptors. For instance, increases in body temperature are well-known to affect cognitive function. Agonist stimulation of TRPV1 channels has been reported to block long-term potentiation in hippocampus [10]. In this regard, it will be important in the future to determine whether hyperthermic conditions activate TRP channels in brain and other tissues and lead to changes in local tissue functions and behavior. Finally, one may anticipate that over time, polymorphisms in various TRP channel genes will be identified in humans and other organisms. In this context, it will be important to ascertain which mutations may render the individual more or less sensitive to hyperthermic stimuli or whether the physiological changes ascribed to hyperthermia are mediated by other mechanisms.

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