Transient Receptor Potential Ion Channels as Participants in Thermosensation and Thermoregulation

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Abbreviations: cDNA, complementary DNA; GABA, gamma-amino butyric acid; LPS, lipopolysaccharide; mRNA, messenger RNA; NADA, N-arachidonoyl dopamine; PO/AH, preoptic/anterior hypothalamus; TRP, transient receptor potential; TRPV, transient receptor potential vanilloid subfamily; TRPA, transient receptor potential ankyrin subfamily; TRPM, transient receptor potential melastatin subfamily.
ABSTRACT
Living organisms must evaluate changes in environmental and internal temperatures in order to mount appropriate physiological and behavioral responses conducive to survival. Classical physiology has provided a wealth of information regarding the specialization of thermosensory functions among subclasses of peripheral sensory neurons and intrinsically thermosensitive neurons within the hypothalamus. However, until recently, the molecular mechanisms by which these cells carry out thermometry have remained poorly understood. The demonstration that certain ion channels of the Transient Receptor Potential (TRP) family can be activated by increases or decreases in ambient temperature, along with the recognition of their heterogeneous expression patterns and heterogeneous temperature sensitivities, has led investigators to evaluate these proteins as candidate endogenous thermosensors. Much of this work has involved one specific channel, TRPV1, which is both a receptor for capsaicin and related pungent vanilloid compounds and a “heat receptor”, capable of directly depolarizing neurons in response to temperatures > 42 degrees C. Evidence for a contribution of TRPV1 to peripheral thermosensation has come from pharmacological, physiological, and genetic approaches. In contrast, although capsaicin-sensitive mechanisms clearly influence core body temperature regulation, the specific contribution of TRPV1 to this process remains a matter of debate. Besides TRPV1, at least six additional thermally sensitive TRP channels have been identified in mammals, and many of these also appear to participate in thermosensation. Moreover, the identification of invertebrate TRP channels whose genetic ablation alters thermally-driven behaviors makes it clear that thermosensation represents an evolutionarily conserved role of this ion channel family.

KEYWORDS
Thermotransduction; Transient Receptor Potential; Capsaicin; Temperature; Vanilloid
A. Introduction
The mechanistic basis of temperature perception and thermoregulation has been a topic of interest to physiologists for well over a century. Despite substantive advances at the physiological level, however, our understanding of thermotransduction at a molecular level has lagged behind that of many other sensory systems. This review will examine recent evidence indicating that ion channels of the Transient Receptor Potential (TRP) family are evolutionarily conserved participants in the peripheral mechanisms by which we sense hot and cold temperatures and that these channels may also contribute, directly or indirectly, to thermoregulation.

B. Transient Receptor Potential (TRP) Channels Participate in Peripheral Thermosensation

1. TRPV1
   a. The capsaicin receptor, TRPV1: a sensor of pungent vanilloid chemicals
      Many of our current views regarding the molecular nature of temperature transduction in the peripheral nervous system can ultimately be traced to a somewhat different but long-recognized sensory phenomenon, namely that certain pepper fruits produce a sense of pain or pungency when they come in contact with broken skin or mucous membranes such as those in the oral cavity or eye. Additional consequences of exposure to these peppers include sweating (which results in a substantial hypothermia) and cardiovascular effects such as tachycardia (16, 50, 128, 133). For thousands of years, these phenomena have led to the incorporation of “hot” peppers into diets and medicinal treatments in various cultures around the world. The principal ingredient responsible for pepper pungency, capsaicin, was purified in the 19th century (139) and was later demonstrated to be an acyl amide derivative of homovanillic acid (8-methyl-N-vanillyl-6-noneamide) (96). The notion that the pungent extract of peppers could directly activate sensory afferents can be traced back over a century (48), but was refined much later with the demonstration that only a subset of primary afferent neurons, particularly those C- and A-delta fibers responsible for pain and warmth perception, could be activated by capsaicin (64, 80, 88, 107, 132). Besides acute pain, capsaicin also produces a transient state of enhanced sensitivity to both thermal and mechanical stimulation (15, 132). However, as illustrated in the classic studies of Jancso, Szolcșanyi and colleagues in the 1960s, these “gain of function” effects are followed by “loss of function”, in that topical or systemic treatment of mice, rats, or guinea pigs with capsaicin produce subsequent desensitization to the effects of this compound (63). What’s more, the effects of capsaicin extended beyond homologous desensitization. Reduced capsaicin excitability is often accompanied by reduced responsiveness to noxious thermal and/or mechanical stimuli, as well as a loss of local neurogenic inflammation evoked by application of mustard oil to the skin (20, 34, 44, 50, 61, 63). Under certain experimental conditions, this polymodal impairment can be explained by neuronal death. For example, high doses of capsaicin administered to neonatal rats were shown to produce a lifelong loss of a specific subset of small-diameter neurons from dorsal root ganglia (61). This neurotoxicity apparently stems from capsaicin-evoked calcium overload of neuron terminals and mitochondrial swelling (60, 62). Neuronal death is not an obligate consequence of capsaicin exposure, however. Topical application of capsaicin to the cornea can produce ultrastructural...
changes in the nerve terminals without obvious axonal degeneration (136). In human skin, intradermal capsaicin administration triggers a degeneration of nerve terminals within the epidermis that can be observed histologically within one day. Under these circumstances, however, limited epidermal reinnervation occurs over a period of weeks, along with partial return of cutaneous sensation (106, 120). Moreover, in adult rats, low doses of capsaicin have been shown to produce C-fiber nerve block that is entirely reversible (150). Thus, capsaicin dose and animal age are important contributing factors to the spectrum of capsaicin action (49). Despite such variability in the extent and timecourse of its effects, the ability of capsaicin to broadly impair the function of a susceptible neuron has historically served as the basis for dichotomization of many neurophysiological processes into “capsaicin-sensitive” versus “capsaicin-insensitive” categories.

Another key finding in this field was the realization by Blumberg and colleagues that resiniferatoxin, a compound derived from the latex of the succulent plant *Resiniferii euphorbia*, resembles capsaicin not only in its functional properties (pungency, ability to evoke hypothermia and to desensitize animals to capsaicin), but also in the fact that it possesses a vanilloid chemical moiety (31, 126). This observation, together with structure-activity studies performed earlier (134, 135), led to the proposal that capsaicin and resiniferatoxin may be acting on a receptor site selectively expressed by susceptible neurons. Proof of this notion came with the demonstration that [3H]resiniferatoxin could bind with high affinity to the membranes of small-diameter peripheral sensory neurons and that this binding could be displaced by capsaicin (127, 158). Further support for the existence of a “vanilloid receptor” and insight into its mechanism of action arose from the findings that small-diameter peripheral sensory neurons could be selectively depolarized by capsaicin or resiniferatoxin through the opening of a nonselective Ca2+-permeable cation channel (5, 38, 87, 100, 156, 157, 159) and that a structural analog of capsaicin, capsazepine (11), or the cationic dye, ruthenium red (4, 35, 105), could block neuronal effects evoked by vanilloid compounds.

The molecular identity of the vanilloid receptor emerged from an expression cloning study in which capsaicin-evoked calcium influx was monitored in an epithelial cell line that had been transfected with complex mixture of cDNAs derived from a sensory ganglion library. Without the introduction of foreign DNA, these cells showed no change in cytosolic calcium upon challenge with vanilloid compounds. However, of the thousands of cDNAs tested, one proved sufficient to render these epithelial cells capable of responding to either capsaicin or resiniferatoxin with a large influx of sodium and calcium ions. The protein encoded by this cDNA, TRP vanilloid 1 (TRPV1, also called VR1) turned out to be a novel ion channel subunit of the Transient Receptor Potential (TRP) family (19). Members of this family, of which there are at least 30 mammalian examples, contain six putative transmembrane domains, with large cytosolic amino and carboxyl termini, a pore-loop region interposed between transmembrane domains 5 and 6 and, in some cases, several ankyrin repeat domains in the amino terminus. Four TRP channel subunits are thought to coassemble to form a functional ion channel (25). TRPV1 was initially found to be expressed only in small-to-medium diameter neurons in dorsal root, trigeminal and nodose ganglia, consistent with the selectivity of vanilloid
action (19, 45, 142). Although subsequent studies employing more sensitive techniques have demonstrated TRPV1 to be expressed in many other neuronal and nonneuronal locations (91, 93, 116, 143), the expression level in small-diameter peripheral sensory neurons appears to be at least 30-fold higher than anywhere else in the body (115).

b. TRPV1 as a polymodal sensor of chemical and heat stimuli
In heterologous expression systems, TRPV1 can be activated not only by vanilloid compounds, but alternatively by a number of other chemical and physical stimuli. For example, extracellular protons can activate this channel, apparently by titrating acidic residues in the vicinity of the pore loop domain (19, 70, 142). In addition, a number of endogenously occurring lipid molecules have been shown capable of acting as TRPV1 agonists. These fall into two general classes: lipoxygenase products of arachidonic acid, such as 5HPETE (57) and amide derivatives of arachidonic acid such as arachidonyl ethanolamide (anandamide) (165) and N-arachidonyl-dopamine (NADA) (56). Most recently, monovalent and divalent cations have also been shown to directly activate TRPV1 (2).

Perhaps most relevant to the focus of this article, however, is that TRPV1 activity can be influenced by ambient temperature, an effect that is manifest in two ways. First, slight warming above room temperature (e.g., to 37°C) potentiates the responsiveness of TRPV1 to its chemical agonists. Second, and more strikingly, TRPV1 can be activated in the absence of exogenous chemical ligands by simply increasing ambient temperature to >42°C. In voltage-clamp experiments, this effect can be observed in either the whole cell configuration or in excised membrane patches. The latter finding suggests that thermal activation of TRPV1 is a direct or membrane delimited process, as opposed to one driven by soluble second messenger molecules (19, 142).

The apparent threshold of ~42°C for thermal activation of recombinant TRPV1 is noteworthy for several reasons. First, it is very close to the threshold for the psychophysical perception of pain in human skin (79, 108). Second, it is close to the threshold for activation of C-fiber nociceptors assayed in vivo or in excised skin-nerve preparations (10, 108). Third, it is close to the threshold for endogenous heat-evoked cationic currents recorded in a subset of small-diameter neurons in dissociated sensory ganglion cultures (21, 75, 95, 111). Indeed, in such cultures, there is a strong correlation between cellular responsiveness to capsaicin and responsiveness to heat (75, 95). Together, these findings suggest that TRPV1 might act physiologically as a transducer of noxious heat, as well as a detector of capsaicin, acid, and arachidonic acid metabolites.

c. TRPV1 null mice exhibit partial deficits in peripheral heat transduction
One approach that has been used to directly evaluate the postulated contribution of TRPV1 to peripheral temperature sensation is the generation and characterization of TRPV1 gene knockout mice (See Table 1 for summary). Mice lacking TRPV1 are developmentally normal and grossly indistinguishable from their littermates. However, as predicted from the properties of recombinant TRPV1, these mice and sensory neurons derived from them are completely devoid of behavioral or physiological responses to capsaicin or resiniferatoxin (17, 28). In contrast, analysis of thermosensation in these
animals has produced a much more complex picture. In dissociated cultures of dorsal root ganglia, TRPV1 null neurons exhibit a dramatic decrease in the prevalence of heat-evoked cationic currents. In fact, none of these neurons respond to temperatures <50°C, in contrast to the ~40% of wild-type neurons that do so (17, 28, 164). Likewise, in vivo recording from wide-dynamic range neurons in the deep spinal cord dorsal horn during heating of the hind paw has revealed a drastic reduction in heat-evoked signaling to these presumed second-order spinal projection neurons. The induction of expression of the immediate early gene, c-fos that is normally observed in the dorsal horn following hind paw heating is also significantly reduced in TRPV1 null mice. Consistent with these findings, TRPV1 null mice were found in one study to exhibit prolonged latencies for tail withdrawal in several different assays of acute thermal nociception (17). Of note, these deficits were confined to the highest temperatures tested in each assay, typically >50°C.

An even more dramatic phenotype in the TRPV1 null mice was observed following inflammation of the hind paw with mustard oil, complete Freund’s adjuvant or carrageenan. These insults typically produce decreased thresholds or latencies of withdrawal from mechanical or thermal stimuli. In mice lacking TRPV1, however, only mechanically-evoked responses were enhanced, whereas thermally-evoked behavioral withdrawal failed to sensitize (17, 28). The basis for this deficit most likely stems from the fact that heat- or capsaicin-evoked responses are dramatically enhanced following exposure of sensory neurons to inflammatory mediators such as bradykinin, nerve growth factor, and ATP. One consequence of this sensitization is that even normal body temperature may suffice to activate nociceptive neurons (83, 142). In some cases, these mediators have been shown to enhance TRPV1 expression levels on the cell surface or at nociceptor terminals (68, 163). In other cases, TRPV1 phosphorylation by protein kinases or the removal of inhibitory phospholipids have been proposed to render the channel more responsive to agonist stimulation (12).

The recent development of TRPV1-selective antagonists has provided additional tools for the dissection of TRPV1 thermosensory function. In general, these antagonists inhibit both capsaicin- and heat-evoked currents in cultured dorsal root ganglion neurons, consistent with the TRPV1 knockout data (37, 39, 110). In animal models, the TRPV1 antagonists assayed to date have not been shown to affect acute thermally evoked withdrawal behaviors. However, they have consistently reduced thermal hyperalgesia and, in some cases, mechanical hyperalgesia, resulting from peripheral inflammation (39, 51, 110). At present, it is difficult to reconcile the lack of acute thermosensory effects with the phenotype of TRPV1 null mice. However, these findings render indisputable the contribution of TRPV1 to thermotransduction in the context of inflammatory hyperalgesia. Assessment of the clinical utility of TRPV1 antagonists for the treatment of acute and chronic pain is under way (109).

Together, the findings outlined in the previous paragraphs support a role for TRPV1 in peripheral transduction of painful heat stimuli, particularly in the context of inflammation. However, it should be noted that TRPV1 null mice and tissues derived from them still exhibit significant thermal responsiveness. For example, in an isolated skin-nerve-dorsal root ganglion-spinal cord explant system, no differences were observed
between wild-type and TRPV1 null mice in the measurable thermal response characteristics of peripheral sensory neurons, as recorded at their cell bodies within the dorsal root ganglion (160). In slightly simpler isolated skin-nerve preparations, the reported effect of TRPV1 gene disruption on heat-evoked sensory neuron activation has been inconsistent (17, 164). Even in dissociated dorsal root ganglion cultures, currents in response to temperatures >53°C were observed in a rare population of medium diameter neurons (~10% of total) (17). Likewise, in the behavioral assays described above, significant residual responsiveness to heat was observed in TRPV1 null mice, particularly at lower stimulus temperatures (<50°C) (17, 28). Finally, thermal hyperalgesia produced by surgical injury of the sciatic nerve was found to be normal in mice lacking TRPV1 (17). Together, these observations demonstrate the existence of TRPV1-independent mechanisms for heat transduction in the peripheral nervous system. In addition, they emphasize the fact that the dominant thermotransduction mechanisms may differ in healthy versus diseased states.

2. A diverse family of thermally sensitive TRP channels
The residual responsiveness to heat stimuli in mice lacking TRPV1 or following TRPV1 antagonism argues that additional peripheral heat transduction mechanisms must exist. In addition, there is considerable neurophysiological evidence for the existence of distinct populations of peripheral sensory neurons that are selectively activated upon the exposure of skin to temperatures >53°C, nonpainful warmth, nonpainful cold, or painfully cold temperatures (<18°C) (102, 108). Given the thermosensitivity of TRPV1, a logical hypothesis was that temperature transduction over these other temperature ranges might also be mediated by TRP channels. This possibility has, indeed, been borne out. Many of these channels are closely related to TRPV1, and have therefore been placed into the TRP vanilloid (TRPV) subfamily, whereas others belong to distinct TRP subfamilies. All of the thermally responsive TRP channels identified thus far are nonselective cation channels. However, as described below, their thermal response properties and patterns of expression vary considerably, and may allow them to account for a number of thermosensory phenomena that have been described physiologically.

a. TRPV2
This protein was initially identified from mouse and rat as a channel subunit with 50% amino acid identity to TRPV1 (18, 72). Within sensory ganglia, TRPV2 (also called GRC or VRL-1) is highly expressed within a subpopulation of medium-to-large diameter neurons that give rise to Aδ and Aβ fibers. In contrast with TRPV1, however, TRPV2 is also highly expressed in a number of other neuronal and nonneuronal locations, including the spleen and brain. TRPV2 is insensitive to capsaicin, but does show responsiveness to elevated ambient temperatures. In recombinant systems, TRPV2 exhibits a threshold for activation of ~52°C, approximately 10°C higher than that of TRPV1. This temperature response pattern corresponds to that of a subset of medium-diameter Aδ peripheral neurons recorded in vivo (36, 90), as well as that of a relatively minor (~10%) subpopulation of dissociated sensory neurons from rats and mice (17, 95). Direct evidence for a contribution of TRPV2 to temperature sensation in vivo is still lacking.
However, it may be of relevance that this channel can reportedly heteromultimerize with TRPV1 (84, 114). This finding raises the possibility that TRPV2 may contribute to thermosensation within a temperature range lower than that required to activate recombinant TRPV2 homomultimers.

b. TRPV3 and TRPV4

Two additional members of the TRPV subfamily of ion channel proteins are TRPV3 (104, 121, 162) and TRPV4 (also called OTRPC4, VRL-2 or VR-OAC) (29, 85, 123), both of which share 40-50% homology with TRPV1. TRPV3 can be activated by warm temperatures, with a threshold between 34 and 39°C. Moreover, repetitive stimulation of TRPV3 with heat results in a current response of gradually increasing amplitude (104, 121, 162). In addition to heat, TRPV3 can alternatively be activated by camphor (92), irritant extracts from oregano and cloves (161), or synthetic boron compounds containing diphenyl groups, such as 2-aminoethoxydiphenyl borate (22, 23, 55). TRPV4 was first described as an ion channel that could be activated by hypoosmotic stimuli (85, 123). It can alternatively be activated by endogenous cytochrome P450-generated metabolites of arachidonic acid, including 5’.6’-epoxyeicosatrienoic acid (153) or by certain synthetic phorbol esters (152). In addition, however, like TRPV3, TRPV4 can be activated by warm temperatures. Depending on the study, the threshold for TRPV4 activation has ranged from ~25°C to ~34°C (42, 154). Electrophysiologically, TRPV4-mediated currents are distinguishable from TRPV3-mediated currents based upon a relatively pronounced desensitization upon repeated or prolonged thermal challenge. TRPV3 and TRPV4 share another feature key to this discussion. Namely, both are expressed at very low levels in sensory neurons, but are prominently expressed within keratinocytes, the stratified epithelial cells that are the major constituents of skin epidermis (42, 104, 161). Consistent with this expression pattern, electrophysiological studies have demonstrated that both sensitizing TRPV3-like and desensitizing TRPV4-like heat-evoked currents can be recorded in cultured keratinocytes (23, 24, 92). Indeed, each of the respective response types is lost in keratinocytes from the corresponding knockout mice (Table 1) (24, 92). TRPV1 expression has also been reported in human keratinocytes (30, 59). However, in the mouse, functional data regarding TRPV1 expression in these cells have been inconsistent(13, 24). This may reflect differences between keratinocytes in vivo versus in culture.

The thermal responsiveness of TRPV3 and TRPV4, together with their expression patterns, has led to the hypothesis that, in the skin, some aspects of heat transduction may not be confined to sensory neurons. Rather, activation of thermally-gated channels on skin keratinocytes may contribute to temperature sensitivity (42, 104). In this model, activated keratinocytes would signal, presumably via diffusible chemical substances, to sensory neurons whose terminals are deeply embedded in the epidermis. Keratinocytes can release many different paracrine factors, such as ATP, acetylcholine, opioid peptides, and cytokines, among others, that have the capacity to activate, sensitize or inhibit the sensory nerve endings (40, 74, 77, 161). However, to be validated as a thermosensory signal, a given candidate would need fulfill at least three criteria. First, it would need to be released in response to thermal activation of TRPV3, TRPV4, or other heat transducers. Second, it would need to have a measurable, rapid effect on the “warmth”
sensitive neuronal population in the skin. Third, this effect would need to reverse upon skin cooling. Such validation will undoubtedly require the convergence of biochemical and pharmacological analysis with direct physiological monitoring of neuronal activity or thermally-driven behaviors. Although the “keratinocyte signaling hypothesis” has not yet been directly tested in vivo, physiological evidence has accumulated for the contributions of both TRPV3 and TRPV4 to peripheral thermosensation (Table 1). First, both TRPV3 and TRPV4 knockouts exhibit prolonged latencies in behavioral withdrawal responses to acute peripheral heating. In the case of TRPV3, this is manifested as a prolonged latency in the hot plate and tail immersion assays at relatively high temperatures (> 48°C) (92). In the case of TRPV4, there is a more subtle defect in acute thermally-evoked withdrawal responses in the tail immersion assay, confined to the range between 45 and 46°C (81). In addition, these mice exhibit decreased thermal hyperalgesia following peripheral inflammation with carrageenan (140). Second, both knockout lines exhibit defects in thermotaxis paradigms, in which one monitors floor selection temperature either in a thermal gradient or in a chamber that consists of multiple discrete floor temperatures. Interestingly, the abnormal phenotypes of TRPV3 null and TRPV4 null mice are different. TRPV3 null mice exhibit delayed selection of a thermal preference zone on a gradient, compared with wild-type littermates, but eventually settle within the same temperature range. Also, unlike wild-type mice, TRPV3 nulls exhibit less preference for 35°C versus room temperature during a ten minute assay (92). In contrast, TRPV4 null mice select a preferred temperature range with normal kinetics, but settle in a slightly warmer range of the thermal gradient than do their wild-type controls. In the two-temperature selection scheme, the TRPV4 mice show an increased preference for 34 over 30°C compared with wild-type mice, but a normal degree of preference for 34 over 36°C (81). Taken together, these data suggest that TRPV3 may be contributing preferentially to the speed of thermal selection behavior, but not the final thermal preference, whereas TRPV4 may be more important in dictating the precise range of preferred temperatures, within the innocuous range. Interestingly, mice lacking TRPV1 exhibited no detectable defects in either thermal selection paradigm (92, 118). This observation argues that thermosensory channels with lower activation thresholds exhibit dominant effects over higher-temperature channels during thermal response behaviors evoked by temperatures in the innocuous range.

c. TRPM8
TRPM8 (also called CMR1) is a member of a the TRPM (melastatin) subfamily of TRP channels (89, 103). These channels differ from many other TRP family members in that they possess a much longer amino terminal cytosolic domain that is devoid of ankyrin repeats. Menthol has long been recognized as a “cooling-mimetic” chemical that produces the sensation of cooling without actually reducing skin temperature (46). In a remarkable parallel with the identification of TRPV1, homology-based and function-based cloning of a “menthol receptor” resulted in the identification of TRPM8 as an ion channel that could be activated by either menthol or cool temperatures. The apparent threshold for TRPM8 activation by cold is ~25°C, consistent with a role in innocuous cooling perception. Moreover, this threshold is shifted to warmer temperatures by the simultaneous presence of menthol, mirroring the characteristic psychophysical effects of this compound (89, 103). Additional support for the participation of TRPM8 in
physiological cold transduction comes from the observation that its expression is confined largely to a subpopulation of small-diameter peripheral sensory neurons that under normal conditions are distinct from those that express TRPV1 (89, 103, 122). There is, as of yet, no evidence for epidermal expression of TRPM8. However, an ortholog of this protein was initially identified in a human prostate cancer line (146), suggesting that it may, under some circumstances, be expressed in non-neuronal epithelial cells.

e. TRPA1
TRPA1 was initially identified as a theoretical protein (ANKTM1) expressed in cultured fibroblasts (67). However, like TRPM8 it was subsequently “rediscovered” as a TRP channel that exhibits strong expression within sensory neurons. TRPA1, like members of the TRPM family, possesses a relatively long cytosolic amino terminal domain. However, the large number (14) of ankyrin-repeat motifs located within the TRPA1 N-terminus prompted its classification in a new (TRP Ankyrin) subfamily. TRPA1 appears to be highly co-expressed with TRPV1 in a subset of small to medium-diameter peripheral sensory neurons (69, 122). Support for a functional role of TRPA1 in nociceptive neurons has come from the demonstration that pungent chemical ligands, including isothiocyanates such as those found in mustard oil, wasabi, and garlic, as well as other irritant chemicals such as acrolein, are capable of activating this channel (6, 9, 69). Indeed, there is a substantial loss of mustard oil responses in vivo and in vitro following TRPA1 gene disruption (8, 78). In addition, however, it has been suggested that TRPA1 may act as a sensor of painfully cold temperatures. Evidence in support of such a role comes from three sources. First, recombinant TRPA1 has been reported by some labs (122), but not others (69, 94), to be activated by temperatures < 18°C. Second, antisense “knockdown” of TRPA1 has been reported to reduce behavioral hyperresponsiveness to cold following inflammation of the rat paw or sciatic nerve injury (99). Third, mice lacking TRPA1 have been reported in one study to exhibit blunted behavioral responses to a cold metal surface or to acetone-mediated cooling of the hind paw skin (Table 1) (78). The latter finding is somewhat surprising, given that acetone is unlikely to cool the skin to the low temperatures required to activate recombinant TRPA1. Moreover, cold responses were not evaluated in dissociated sensory neurons in that study. In contrast with these findings, another group failed to observe TRPA1-dependent alterations in cold responsiveness in several different behavioral assays or in cultured sensory neurons (8). Possible reasons for these discrepancies include the incomplete elimination of the TRPA1 gene in both studies and consequent potential production of a mutated partial TRPA1 protein, as well as the inclusion of female mice in some behavioral studies. Regardless, the apparent involvement of this channel in temperature transduction certainly warrants further attention.

e. TRPM4, TRPM5, TRPM2
These members of the TRPM subfamily, like TRPV3 and TRPV4, can be activated by warming to temperatures >15°C for TRPM4 and TRPM5 (138), or >35°C for TRPM2 (141). Apparently, none of these channels is expressed in peripheral somatosensory neurons or in skin keratinocytes, suggesting that they are not involved in temperature sensation in skin. Interestingly, though, TRPM5 is expressed in epithelial taste cells in
the tongue and is required for the ability of warm temperatures to enhance sweet tastant-evoked firing of gustatory neurons in mice (138). This effect may be related to a fascinating psychophysical phenomenon in humans referred to as “thermal taste”, whereby warm temperatures applied to the tongue create a perception of taste, even in the absence of exogenous tastants (26).

3. Nonmammalian TRP channels are also important for thermosensation
Nonmammalian species also possess TRP channel isoforms that participate in sensory functions that include photosensation, chemosensation, and mechanosensation, as well as thermosensation (71, 148). The most compelling examples of nonmammalian channels with thermosensory function have come from the analysis of Drosophila TRP proteins (82, 113, 144). Interestingly, these have thus far all proven to be members of the TRPA subfamily. A more detailed description of invertebrate TRP channel function can be found elsewhere (149). However, one important theme to emerge is that the molecular basis of thermosensation exhibits a substantial degree of evolutionary conservation, including specialization of TRP channel function over different temperature ranges.

4. Frontiers in peripheral temperature sensation
As outlined in the previous sections, there is now a wealth of evidence to support the contribution of TRP channels to peripheral temperature sensation in both mammalian and nonmammalian species. Although many TRP channels are potential players in this process, supportive data are currently strongest in the cases of TRPV1, TRPV3, TRPV4, TRPM8 and invertebrate TRPA channels. A model summarizing the mammalian findings is presented in Figure 1. However, much remains to be learned regarding peripheral mechanisms of thermotransduction. For example, to what extent do different temperature-gated TRP channels carry out redundant versus complementary roles in cold and heat detection? How are signals mediated by these channels integrated and evaluated at the level of the spinal cord and brain to evoke behavioral or physiological responses? Do most of these channels function as homomultimers or do heteromultimeric channels increase the mammalian thermosensory repertoire? It also remains to be determined whether it is the keratinocyte-expressed pools of TRPV3 and TRPV4 that account for these channels’ contributions to heat perception. If it is, then via what signals might TRPV activation in keratinocytes alter the activity of adjacent “thermosensory” afferents? Finally, do other mechanisms, besides the activation of TRP channels, contribute to peripheral thermosensation?

C. TRP channels and mechanisms of body temperature regulation

1. TRPV1 and body temperature regulation

a. Thermoregulatory effects of exogenous vanilloid compounds
As described above, intraoral or subcutaneous capsaicin or resiniferatoxin administration produces in mammals not only a perception of pain, but also a robust thermoregulatory response. Within 30 minutes of treatment, there is a rapid and profound (up to 7°C) reduction in basal core temperature (65). This hypothermic response typically reverses within 2-3 hrs, but if the vanilloid dose is sufficiently high, it is often followed by a
period of relative hyperthermia that can last several days (101, 131). The precise efferent events triggered by vanilloid exposure are complex, in that they appear to include both heat-loss (e.g., tail skin vasodilation, increased level pressing for cool air) and heat-production (reflected by increased O2 consumption) processes (32, 65, 76, 101, 131). The latter apparently results from activation of catecholamine release from the adrenal medulla (76). However, during the first few hours, it is the heat loss mechanisms that dominate. Like nociceptive responses to vanilloid compounds, these thermoregulatory responses desensitize, such that repeat administration one to several days after an initial high-dose challenge fails to produce changes in core temperature. Moreover, capsaicin-desensitized rodents exhibit reduced vasoregulatory responses to whole-body warming, as well as reduced behavioral escape from warm environments (53, 65). Thus, both physiological and behavioral thermoregulatory mechanisms depend upon capsaicin-sensitive pathways.

b. Location of TRPV1 effects on thermoregulation

Two general hypotheses have emerged for the mechanisms by which vanilloid compounds exert their thermoregulatory effects. One is that they excite peripheral capsaicin-sensitive nerves (either somatosensory afferents in the skin or vagal afferents within the peritoneal cavity) that send projections via polysynaptic pathways to the preoptic/anterior hypothalamus (Figure 2, Mechanism 1). There, the synaptic activation of warmth sensitive neurons is proposed to trigger the heat-loss reflex via a reduction in sympathetic vasoconstrictor outflow to tail skin, as well as behavioral heat-loss mechanisms. In this model, peripheral capsaicin-sensitive neurons include “warmth receptors” that signal mild increases in skin temperature to the central nervous system. Consistent with this notion, capsaicin pretreatment of the human tongue results in a shifted threshold for psychophysical warmth discrimination towards higher temperatures (132). Further support for a peripheral site of action comes from the observation that intravenous resiniferatoxin can also produce cold-seeking behavior as well as hypothermia in rats (3). A direct action on vascular afferents might be occurring in this context, given the ability of capsaicin to promote the dilation of mesenteric arterioles through the neurogenic release of neuropeptides (49). However, the hydrophobicity of resiniferatoxin also makes it possible that its hypothermic effect results from the activation of extravascular afferents. Visceral warmth-sensitive afferents represent another potential peripheral site of capsaicin action, given the robust expression of TRPV1 in neurons that innervate visceral tissues (142) and the demonstrated ability of capsaicin to promote neurogenic peptide release in those neurons (49). In general, capsaicin-evoked signaling from the periphery most likely reaches the PO/AH via dorsal root ganglia, the spinal cord, and the parabrachial nucleus of the pons. However, a contribution from TRPV1-expressing vagal afferents that project to the nucleus of the solitary tract (142) cannot be excluded.

An alternative (but not mutually exclusive) idea is that circulating vanilloids can penetrate the brain and act directly on warm-sensitive neurons within the hypothalamus (Figure 2, Mechanism 4). For example, they might excite intrinsically warmth-sensitive hypothalamic neurons, resulting in an acute net heat loss response. This hypothesis has been far more controversial, but warrants consideration based on several experimental
observations. First, direct application of capsaicin to the preoptic/anterior hypothalamus has been shown to trigger hypothermia in rats (66). Second, following hypothalamic desensitization with capsaicin, thermoregulatory responses to direct hypothalamic warming are reduced (43, 66). Third, 3H resiniferatoxin binding has been reported in the preoptic/anterior hypothalamus in vitro (1). Fourth, systemic treatment with high doses of capsaicin have been shown by some investigators to produce neurotoxicity within the PO/AH (112, 137). Fifth, mRNA encoding TRPV1 has been detected in the hypothalamus (116), although immunoreactivity for TRPV1 is considerably lower there than in other brain subregions (91, 143). Sixth, capsaicin has been shown to modulate the firing of a subset of PO/AH neurons in anesthetized rats as well as the release of glutamate and GABA from presynaptic terminals in hypothalamic explants (54, 73, 116). Seventh, newly-developed TRPV1-selective antagonists have revealed that acute systemic inhibition of this channel results in a transient hyperthermia (7, 125). A compelling feature of this latter effect is that it is opposite in direction to that produced by TRPV1 agonism. At the least, this finding argues that body temperature is under tonic vanilloid receptor influence, though the site and nature of such tone is unclear. Eighth, although the apparent threshold for TRPV1 activation in recombinant systems is often described, for the sake of simplicity, as being ~42°C, the temperature dependence of this channel’s activity extends well below this value (28, 147). Furthermore, mechanisms such as posttranslational modification (97), alternative splicing (93, 117, 151), or heteromultimerization (84, 114) might substantially alter the TRPV1 temperature response profile.

Despite the findings outlined above, other observations call into question any significant role for intrahypothalamic capsaicin receptors in physiological body temperature regulation. First, there has been no consistent relationship demonstrated between warmth-sensitivity and either capsaicin responsiveness or TRPV1 immunoreactivity in PO/AH neurons in explants or dissociated cultures. Second, findings from electrophysiological studies of warmth-sensitive neurons in organotypic hypothalamic slices are generally consistent with models in which thermal responsiveness can be explained without invoking the selective expression of heat-gated nonselective cationic conductances. Rather, these models have included differential expression levels of “housekeeping” channels whose activation and deactivation kinetics are known to be sensitive to ambient temperature. These channels, which influence membrane resistance, as well as the prepotential and afterpotential components of the action potential, include IA-type voltage-gated K+ channels, hyperpolarization-activated cyclic nucleotide channels, and two-pore K+ “leak” channels (27, 41, 155). One notable exception to this view comes from a study by Kobayashi et al., in which a thermally-activated nonselective cationic channel with a threshold of ~37°C was reported from hypothalamic slices. Unfortunately, no assessment of capsaicin sensitivity was made in that study (52). Third, capsaicin-mediated impairment of thermoregulation can be achieved without measurable impairment of intrinsic PO/AH thermosensitivity (98). Fourth, unlike capsaicin-desensitized animals, mice devoid of TRPV1 exhibit apparently normal environmental temperature selection, normal basal core temperatures, and a normal ability to defend core temperature when placed in a warm or cold environment (See Table 1 for summary) (17, 58, 130). Capsaicin-evoked hypothermia is also completely absent in these mutant
animals, whereas ethanol-evoked hypothermia is intact, indicating that TRPV1 is specifically essential for the capsaicin effect (17, 58). One exception to these general findings is that in one study (130), but not the others, TRPV1 null mice exhibited a slightly increased circadian fluctuation in core body temperature, reflected in both a lower daytime nadir and a higher nighttime peak. Neither the mechanistic basis of this difference nor the reason for the difference between the studies is clear.

How might these apparently disparate results be reconciled? One possibility, albeit a highly unlikely one, is that TRPV1 does, in fact, participate directly in warmth transduction in hypothalamic neurons (Figure 2, Mechanism 4), but that its expression and/or function are artifactually lost during culture or organotypic slice preparation. In this case, one would also need to argue that TRPV1 null mice have compensated for the lack of this channel by upregulating other thermodtransduction mechanisms or relying entirely on extrahypothalamic input for warmth assessment. A diametrically opposed possibility is that TRPV1 plays no role whatsoever in central thermosensation, and that all effects of capsaicin on thermoregulation are mediated via input from capsaicin-sensitive extrahypothalamic mechanisms. In this model, TRPV1 could either be participating directly in thermodtransduction at extrahypothalamic sites or could be relevant only in the context of vanilloid-evoked excitation or desensitization of those sites. This view would appear to be disputed by the effects of intrahypothalamic capsaicin administration on thermoregulatory response to direct hypothalamic warming. However, one might postulate that these effects of capsaicin are mediated by non-TRPV1 targets. A repetition of these classical pharmacological experiments in mice lacking TRPV1 could address any such off-target effects. A third, “intermediate”, possibility is that TRPV1 confers capsaicin sensitivity upon a population of neurons (with cell bodies intrinsic or extrinsic to the hypothalamus) that synthetically stimulate warm-sensitive PO/AH neurons and/or inhibit cold-sensitive PO/AH neurons (Figure 2, Mechanisms 2 and 3). In these two models, TRPV1 need not be acting as a thermal transducer. Rather, it could be regulated by other stimuli such as endocannabinoid lipids, which potently activate this channel in the range of normal core body temperature. Here, again, one would need to invoke some sort of compensation for the absence of this channel in TRPV1 null mice. The availability of TRPV1-selective antagonists and the site-specific application of these antagonists during direct assessment of warmth-receptive hypothalamic neuronal firing may help to distinguish among these possibilities.

One final note is that considerable evidence has emerged for a contribution of TRPV1 and/or TRPV1-expressing cells to fever produced by systemic administration of the bacterial endotoxin, lipopolysaccharide. The first indication of such a contribution came from the observation by Szekely and colleagues that capsaicin-desensitized rats exhibit an exaggerated fever response to LPS (129). Later studies involving the relatively nonselective TRPV1 antagonist, capsazepine, or TRPV1 knockout mice have yielded a somewhat differing picture. Capsazepine administration to rats reduces the amplitude of the first phase of a triphasic fever response to intravenously administered LPS (33). In contrast, mice lacking TRPV1 were reported to exhibit a depressed amplitude of the second and third phases of intraperitoneal LPS-induced fever. In the latter study, the first febrile phase was masked by stress-induced hyperthermia, and could not be evaluated.
However, LPS-induced c-FOS induction in a number of hypothalamic loci related to fever, including the PO/AH did not differ between wild-type and TRPV1 null mice (58). The simplest interpretation of these findings is to invoke the participation of TRPV1 and TRPV1-expressing neurons in extrahypothalamic afferent pathways resulting in the fever response or in efferent heat gain/retention mechanisms, as opposed to a direct role for TRPV1 in hypothalamic thermoregulatory centers. Again, the availability of TRPV1-selective pharmacological tools should help to resolve these possibilities.

2. Other TRP channels and body temperature regulation

It also remains to be fully explored whether other TRP channels, besides TRPV1, participate in thermoregulatory responses. TRPV4, for example, is expressed in the medial preoptic area and the median preoptic nucleus and exhibits a thermal response profile similar to that of warmth-sensitive hypothalamic neurons (42, 85, 86). However, several findings argue against a significant role for TRPV4 in central mechanisms underlying thermoregulation. First, TRPV4 immunoreactivity in the hypothalamus appears to be confined to terminals, as opposed to cell somata, suggesting an extra-PO/AH origin (155). Second, the TRPV4 agonist 4PDD failed to modulate MPA neuronal activity in slices (73). Third, neither basal core temperature nor core temperature responses to environmental warming were altered in TRPV4 null mice (81, 86). However, it is well recognized that osmolarity and temperature can interact at hypothalamic neurons (119, 145). Thus, similar to TRPV1, a modulatory role for TRPV4 in central thermosensation, thermoregulation, or the integration of osmoregulation and thermosensation cannot be excluded. TRPV3 expression in the hypothalamus has not been examined in any detail, though the mRNA for this molecule can be detected there (H. Lee and M. Caterina, unpublished observation). Mice lacking TRPV3 exhibit apparently normal core body temperature (92). However, a detailed analysis of thermoregulation in these animals has yet to emerge. Similarly, the potential contributions of TRPM8 and TRPA1 to intrinsic cold sensitivity within the hypothalamus have yet to be evaluated. Table 1 provides a summary of data related to thermoregulation that has been obtained from TRP channel knockout mice.

D. Conclusions

Over the past decade, evidence in favor of a role for TRP channels in temperature sensation has emerged from a number of mammalian and nonmammalian systems. These data can be most readily explained by the existence of multiple thermally-sensitive TRP channels, each contributing to temperature sensation over a different range and in different anatomical locations. However, as described above, much remains to be learned regarding the extent to which these channels underlie the myriad thermosensory functions that have been documented in the peripheral and central nervous systems. The creative use of genetically modified model organisms, TRP subtype-specific antagonists and the innovative approaches that have permeated classical thermal physiology will be required to explore these issues fully.
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Competing Interests
The author does not declare any competing interests

FIGURE LEGENDS
Figure 1. Models for thermosensation over a range of skin temperatures. **Top)** The sensation of burning pain can be evoked by activation TRPV1 in nociceptive neurons in the skin. Many TRPV1-expressing neurons also express TRPA1, which has been proposed to participate in the transduction of painfully cold temperatures. A distinct class of nociceptive heat-activated neurons expresses TRPV2, though the participation of this channel in temperature transduction *in vivo* has not yet been demonstrated. Although the nerve endings shown are epidermal, the additional presence of dermal heat-sensitive endings cannot be excluded. **Middle)** Warm temperatures apparently activate TRPV3 and TRPV4, which are expressed prominently in epidermal keratinocytes. One way this information might reach the nervous system is through the release of chemical substances (X) that act on “warm-sensitive” nerve terminals within the epidermis. At higher temperatures, activation of TRPV3 and TRPV4 also contributes to the perception of painful heat, perhaps via keratinocyte-mediated activation of nociceptive neurons. **Bottom)** Modest skin cooling most likely activates TRPM8, which is expressed in a distinct subset of sensory neurons.

Figure 2. Possible mechanisms for TRPV1-mediated hypothermia in response to heat or capsaicin. In all four cases, increased output from warm-sensitive neurons of the preoptic/anterior hypothalamus (PO/AH) (black filled oval) and/or decreased output from cold-sensitive neurons (gray filled oval) triggers an early net heat-loss response via autonomic effector control centers in the brain. Not represented is an apparently concomitant but weaker activation of heat-gain mechanisms that becomes obvious only after several hours (see text). **Mechanism 1.** TRPV1 activation at the peripheral terminals of cutaneous or visceral warm fibers, whose cell bodies are in dorsal root ganglia, leads to stimulation of neurons within the most superficial layer of the spinal cord dorsal horn. These neurons decussate, then ascend within the spinothalamic tract, and send collateral branches to the parabrachial nucleus in the pons. Pontine projections to the PO/AH then activate warm-sensitive neurons and/or inhibit cold-sensitive neurons. **Mechanisms 2 and 3.** Activation of TRPV1 on the presynaptic terminals (from the pons or elsewhere) impinging on the PO/AH results in increased excitatory neurotransmitter release onto warm-sensitive neurons (2) or increased inhibitory neurotransmitter release onto cold-sensitive neurons (3). **Mechanism 4.** Activation of TRPV1 intrinsic to warm-sensitive neurons results in their direct activation. Solid lines or filled arrowheads indicate excitatory actions. Dashed lines indicate inhibitory actions. Hypothalamic circuitry is simplified from reference (14).
REFERENCE


110. **Rami HK, Thompson M, Stemp G, Fell S, Jerman JC, Stevens AJ, Smart D, Sargent B, Sanderson D, Randall AD, Gunthorpe MJ, and Davis JB.** Discovery of


Table 1. Summary of thermosensory and thermoregulatory findings in knockout mice lacking temperature-gated TRP channels. References are indicated at right.

**TRPV1**

**In vitro thermosensation**
- Loss of moderate threshold heat-evoked currents from cultured sensory neurons  
  16, 27, 163
- Reduced amplitude of heat-evoked firing in C-fibers in skin-nerve prep  
  16
- Reduced proportion of heat-sensitive C-fibers in skin-nerve prep  
  16
- No change in heat-sensitive C-fibers in skin-nerve prep  
  163
- No change in heat-evoked CGRP release from skin  
  163
- No change in heat-evoked neuronal firing in skin-DRG-spinal cord preparation  
  159

**In vivo thermosensation**
- Reduced heat-evoked spinal cord c-FOS induction  
  16
- Reduced heat-evoked activation of spinal cord dorsal horn neurons  
  16
- Reduced behavioral withdrawal from acute heat (hot plate, radiant heat, tail immersion)  
  16
- No change in behavioral withdrawal from acute heat  
  27
- Reduced thermal hyperalgesia following inflammatory insult  
  16, 27
- No change in thermal selection on temperature gradient  
  91, 117

**In vivo thermoregulation**
- No alterations in core body temperature or thermoregulation in heat or cold  
  16, 57
- Accentuated circadian fluctuation of body temperature  
  129

**TRPV3**

**In vitro thermosensation**
- Reduced sensitizing currents evoked by heat in keratinocytes  
  91
- No change in sensory neuron responses to heat?  
  91

**In vivo thermosensation**
- Reduced behavioral withdrawal from acute heat  
  91
- Delayed thermal selection on gradient  
  91

**In vivo thermoregulation**
- No alterations in core body temperature  
  91

**TRPV4**

**In vitro thermosensation**
- Reduced desensitizing currents evoked by heat in keratinocytes  
  23

**In vivo thermosensation**
- No change in acute thermal nociception (radiant heat, hot plate)  
  85, 123
- Reduced thermal hyperalgesia following capsaicin administration  
  139
- Longer latency in hot-plate assay at 45-46°C  
  80
- Thermal selection shifted towards warmer temperatures on gradient  
  80

**In vivo thermoregulation**
- No alterations in core body temperature or thermoregulation in heat or cold  
  80, 85

**TRPA1**

**In vitro thermosensation**
- No evidence of changes in cold sensitivity of cultured DRG neurons  
  8

**In vivo thermosensation**
- No change in acute cold-evoked behavioral withdrawal (acetone, cold plate)  
  8
- No change in cold-related thermal preference  
  8
- Reduced acute cold-evoked behavioral withdrawal (acetone, cold plate) in females  
  77
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