Review

Cell PRESS

Neuronal TRP channels: thermometers, pathfinders and life-savers

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Cation channels of the TRP superfamily are widely expressed in the nervous system, and important progress has been made in elucidating the gating properties and physiological roles of neuronal TRPs. Recent studies have firmly established the role of temperature-sensitive TRPs (thermoTRPs) as the principal molecular thermometers in the peripheral sensory system, and provided the first molecular insight into the mechanisms underlying the exquisite thermo- and chemosensitivity of these channels. Moreover, accumulating evidence implicates TRP channels in the development of the central nervous system. In particular, Ca^{2+} influx via TRPC channels appears to be a critical component of the signalling cascade that mediates the guidance of growth cones and survival of neurons in response to chemical cues such as neurotrophins or Netrin-1.

Introduction to the TRP superfamily

The TRP superfamily consists of proteins with six transmembrane domains (6TM) related to the product of the *Drosophila trp* (*t*ransient *r*eceptor *p*otential) gene. TRP proteins assemble as homo- or heterotetramers to form cation-permeable ion channels [1]. Based on sequence homology, the 28 mammalian TRPs are classified into six subfamilies: TRPC, TRPV, TRPM, TRPA, TRPP and TRPML [1,2]. A seventh subfamily, TRPN, has members in lower vertebrates and invertebrates only.

Members of the TRP superfamily are expressed in probably all mammalian organs and cell types, and in recent years great progress has been made in elucidating their involvement in health and disease [3]. Here we review current knowledge of the mode of action and physiological role of TRP channels in the nervous system, particularly focussing on their function in peripheral and central thermo- and chemosensation.

TRPs as peripheral thermosensors

Thermosensing can be considered as the most elementary sense, as it is absolutely crucial for our survival [4,5]. A prompt reaction to contact with noxiously cold or hot objects is crucial to prevent acute, potentially fatal, injury. Moreover, to maintain the core body temperature around 37°C, heat production and heat loss must equal in the steady state. This requires the permanent monitoring and integration of thermal information from skin (through peripheral thermoreceptors) and deep body structures (through central thermoreceptors), and the ensuing initiation of reflexes that promote heat production or heat loss [5].

Sensory nerve fibres that convey thermal information from the head and body arise from cell bodies in trigeminal (TG) and dorsal root ganglia (DRG) [6]. These peripheral sensory nerves are subdivided into three main categories. C-type fibres are characterised by small cell bodies, the lack of myelin sheets and slow conduction velocities. Both C-type and medium-sized, myelinated and more rapidly conducting Aδ-type fibres are responsible for conveying painful signals, including noxious cold or heat. The largest cell bodies of TG and DRG give rise to Aβ-type fibres. These are myelinated, rapidly conducting primary sensory fibres that convey nonnoxious signals, including warm or cool temperatures [6]. A subset of the TRP superfamily, dubbed thermoTRPs, is highly sensitive to temperature, and several thermoTRPs essentially serve as molecular thermometers in different cell populations of the peripheral sensory system [1] (Figure 1a,b).

TRPs in heat sensation

Excitation of heat-sensitive TG and DRG neurons results from the activation of nonselective cation channels (Figure 1c). Knowledge of the molecular bases of thermosensation has experienced an explosive growth since the cloning of the capsaicin receptor TRPV1, now a decade ago [5,7]. Under basal conditions and at the resting potential of a sensory neuron, TRPV1 starts conducting significant inward cation current when temperatures rise above ~43°C [5]. In line herewith, TRPV1-deficient mice specifically lack the subset of TG and DRG neurons that is excited by moderate heat (average threshold ~43°C) [8,9]. Although there is some discussion as to whether TRPV1 is involved in the acute response to painful heat stimuli [8–10] (see Table 1 for a summary of thermosensory behaviour in thermoTRP knockout mice), it is well established that sensitisation of TRPV1 by inflammatory mediators such as bradykinin, nerve growth factor (NGF) and prostaglandins underlies the heat hyperalgesia that one experiences in injured and/or inflamed tissue [8,9]. Moreover, TRPV1 is activated by exogenous chemicals that evoke a burning sensation, including vanilloid compounds, acid, ethanol, antifungal drugs such as clotrimazole and certain spider toxins [7,11,12].

The capsaicin-insensitive homologue TRPV2 is considered an attractive molecular candidate to explain the activation of large capsaicin-insensitive neurons at

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0166-2236/$ – see front matter doi:10.1016/j.tins.2008.03.002 Available online xxxxx

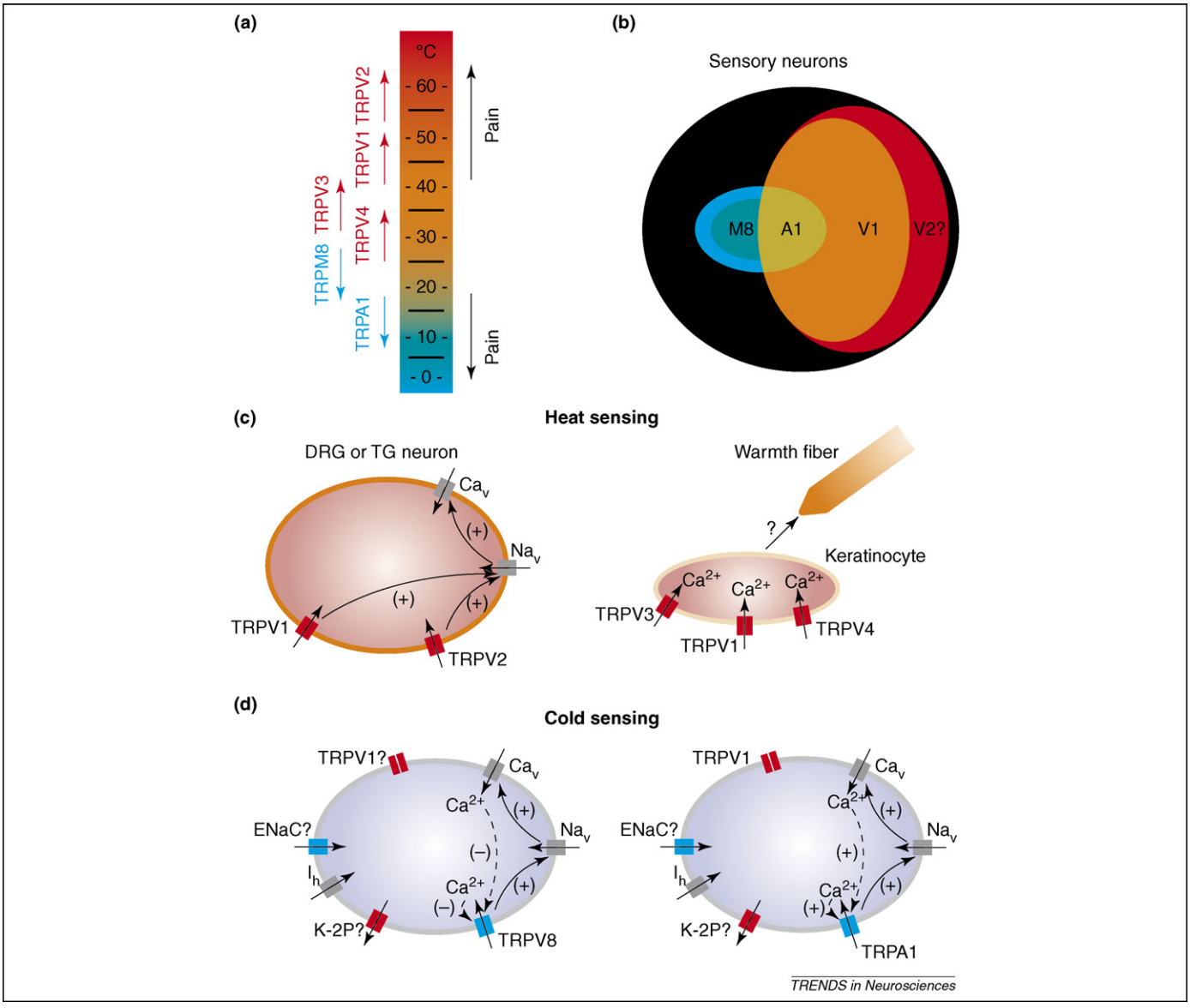

Figure 1. TRP channels in hot and cold sensation. **(a)** Diagram showing the temperature range of activation of the TRP channels involved in thermosensation. **(b)** Composition of the population of sensory neurons (DRG or TG) according to their thermal and agonist sensitivities. The ensemble of warm- and hot-sensitive neurons (red) contains the capsaicin-sensitive fraction expressing TRPV1 (orange). The cold-sensitive population (blue) contains the menthol-responsive cells expressing TRPM8 or TRPA1 (green). TRPA1-positive neurons are also activated by heat and capsaicin and can convey burning cold sensation. **(c)** Strong heat is detected by TRPV1 and/or TRPV2 in nociceptive nerve fibres. Activation of these channels causes cell depolarisation that triggers the opening of voltage-gated Na^+ and Ca^{2+} channels. Warmth is detected through the activation of TRPV3 and TRPV4 expressed in epithelial skin cells (keratinocytes), which excite sensory nerves via unknown paracrine signals. **(d)** Cool temperatures are primarily sensed by activation of TRPM8 and the consequent activation of voltage-gated Na^+ and Ca^{2+} channels. Ca^{2+} influx leads to desensitisation of TRPM8 channels through depletion of cellular PtdIns(4,5)P_2 [72]. Noxious cold can activate TRPA1 channels in a subset of nociceptive, TRPV1-expressing fibres. The activity-induced rise in intracellular Ca^{2+} further promotes channel opening [34,73]. The tetrodotoxin-insensitive voltage-gated Na^+ channel $Na_V1.8$, whose inactivation properties are resistant to cooling, is required for excitability at noxiously cold temperatures [74].

temperatures above ~50°C, as well as the residual nocifensive response to noxious heat stimuli in TRPV1-deficient mice [5]. However, some caution might be appropriate because heat-induced activation of TRPV2 is not fully understood and TRPV2-deficient mice have not yet appeared in the literature.

Two more related heat-activated channels, TRPV3 and TRPV4, exhibit already significant activity at body

Table 1. Thermosensation and thermoregulation in thermoTRP knockout mice

Response		TRPV1$^{-/-}$	TRPV3$^{-/-}$	TRPV4$^{-/-}$	TRPM8$^{-/-}$	TRPA1$^{-/-}$
Hot	Acute heat response	(+) (−)	(+)	(+)	(−)	(−)
	Heat hyperalgesia	(+)	(−)	(+)	ND	ND
Cold	Acute cold response	ND	ND	ND	(+)	(−) (+)
	Cold hyperalgesia	ND	ND	ND	(−) (+)	ND
	Chemical cooling	ND	ND	ND	(+)	(+)
Other	Temperature selection	(−)	(+)	(+)	(−)	ND
	Core temperature	(−)	(−)	(−)	(−)	ND
Selected references		[8,9]	[14]	[13,75]	[23–25]	[36,37]

(+) = significant difference from WT; (−) = no significant difference from WT; ND = not determined.

temperature, and are further activated by increases in temperature in the warm range [5]. Knockout mice lacking either TRPV3 or TRPV4 show mild but distinct deficiencies in thermosensation in the warm range, leading to altered thermal selection behaviour [13,14]. In particular, TRPV3-deficient mice have a lower preference for innocuous warmth, whereas TRPV4-deficient mice appear to select warmer temperatures than wild-type mice [13,14]. Intriguingly, the main sites of TRPV3 and TRPV4 expression in skin tissue are not the sensory nerve endings but the keratinocytes, which also express TRPV1 [15]. Thus, keratinocytes might play an active role in thermosensation, by signalling thermal information to the sensory nerves [15]. For example, ATP (acting on purinergic receptors) or NGF (sensitising TRPV1) can be released from keratinocytes and have been put forward as potential paracrine warmth signals (Figure 1c).

Heat-activated TRPM2, TRPM4 and TRPM5 have not been detected in sensory fibres or keratinocytes, and are therefore not likely to be involved in peripheral thermosensing. However, these channels might confer steep temperature dependence to processes such as insulin release [16], histamine secretion [17] and taste perception [18].

TRPs in cold sensing

A fraction (7–20%) of the whole population of TG and DRG neurons is excited upon lowering temperature. Before identification of cold-activated thermoTRP channels, cooling-induced depolarisation of these cold-sensitive neurons had been attributed to reduction of background K^+ currents, inhibition of the Na^+-K^+-ATPase, potentiation of the amiloride-sensitive ENaC Na^+ channel or activation of a nonselective cold-induced current [19]. A model was put forward in which cold-sensitive neurons were not associated with a specific cold-sensing molecule, but rather with a specific combination and dose of ionic channels and transporters [20].

This view had to be revised significantly following the characterisation of TRPM8 [21,22]. TRPM8 is expressed in a subset of TG and DRG sensory neurons, and can be functionally expressed as a nonselective cation channel that is strongly activated by cooling below ~28 °C [21,22]. Moreover, chemicals that evoke a cool sensation and excite cold-sensitive neurons, such as menthol, eucalyptol or icilin, were found to directly activate and sensitise TRPM8 [21,22]. Thus, TRPM8 fulfilled the criteria of a *bona fide* cold sensor that provokes depolarisation upon cooling (Figure 1d).

Three recent knockout studies have firmly established the central importance of TRPM8 in cold sensing, and provided important insight into the effect of cold sensing on mouse behaviour [23–25]. In TRPM8-deficient mice, the fraction of sensory neurons that respond to cooling or menthol was dramatically reduced (by at least 50%), indicating that TRPM8 is a principal but not the sole cold and menthol receptor in these cells. In behavioural experiments, mice exhibit strong deficits in the ability to discriminate warm from cool temperatures [23–25]. For example, whereas wild-type mice show a clear preference for the warm temperature range (30–35 °C) compared to lower temperatures, TRPM8-deficient mice show no clear thermal preference in the range between 15 and 35 °C [23–25]. Thus, TRPM8 is essential for mice to figure out where the environmental temperature is most 'comfortable,' that is, where core body temperature can be sustained at minimal energy expenditure. Moreover, these mice no longer exhibit the typical 'wet-dog shake' response to injections of the potent TRPM8 agonist icilin, and have a reduced nocifensive behaviour in response to painful acetone-induced evaporative cooling and longer response latencies when placed on a noxiously cold plate, indicating that TRPM8 is also involved in cold nociception [23–25]. Interestingly, TRPM8 is not only involved in cold-induced pain but also mediates the well-known analgesic effect of moderate cooling [23,26].

The residual sensitivity to cold in TRPM8-deficient mice, particularly to noxious cold (<15 °C), implies the existence of additional cold-sensing mechanisms [23–25]. Previous studies had also shown that around half of cold-activated TG and DRG neurons, mainly those activated in the noxious cold range, are also responsive to capsaicin [19,20]. TRPA1, which was initially cloned as a channel activated by cooling below ~17 °C and expressed in a subset of TRPV1-positive, nociceptive neurons [27], appeared a straightforward and attractive candidate to act as thermosensor in the noxious cold range (Figure 1d). Activation of TRPA1 in TRPV1-positive heat-sensitive neurons could easily explain why intense cold can evoke a burning sensation. Moreover, chemical agonists of TRPA1 such as cinnamaldehyde and mustard oil induce cold hyperalgesia in humans [28], whereas knockdown of TRPA1 expression suppressed injury- and inflammation-induced cold hyperalgesia [29].

A disturbing number of conflicting results show that the role of TRPA1 in cold sensation remains highly debated, however. First, some groups have consistently reported cold activation of heterologously expressed TRPA1 [27,30,31], whereas others were unable to reproduce these findings [32,33]. In a recent study, cold-induced activation of heterologously expressed TRPA1 was reported to be an artefact, representing Ca^{2+}-induced activation of the channel following cold-induced release of Ca^{2+} from intracellular stores [34]. However, yet another recent study shows that TRPA1 can be activated by cold independently of changes in intracellular Ca^{2+} [30]. Second, the correlation between TRPA1 expression and cold sensitivity in TG and DRG neurons is unclear. For example, a large proportion of DRG or TG neurons that respond to strong TRPA1 agonists such as mustard oil lack a clear cold response [33]. By contrast, the large majority of menthol-sensitive neurons, which were until recently catalogued as TRPM8-expressing cells, respond to cold stimuli [33]. However, the interpretation of these results is complex in light of the recent finding that menthol has a bimodal effect on TRPA1, activating it at low micromolar concentrations (<100 μM) and blocking it in the high micromolar to millimolar concentration range [35]. Unfortunately, analysis of cold sensitivity in TRPA1-deficient mice failed to settle the dispute. Whereas one study reported significant deficits in assays for noxious cold sensation (ice-cold plate withdrawal latency, acetone cooling) [36], a second study found no significant difference between TRPA1-deficient and wild-type mice in all tested aspects of cold sensation [37]. A

potential factor that might complicate the analysis of cold sensitivity of TRPA1-deficient mice is the dominant role of TRPM8 in both innocuous and noxious cold sensation (see above). Therefore, comparison of the thermal response between TRPM8 knockout and combined TRPM8/TRPA1 double knockout mice might give a more decisive answer regarding the involvement of TRPA1 in cold sensation.

ThermoTRPs in the brain?

Apart from the sensory system, the thermo- and chemosensory functions of thermoTRPs might also be relevant in central neurons. For example, expression of TRPV1 and/or functional effects of TRPV1 agonists has been observed in different cell types in all major regions of the brain. For example, TRPV1-deficient mice exhibit impaired hippocampal long-term potentiation, resulting in reduced anxiety and conditioned fear [38]. These hippocampal effects might be related to the ability of TRPV1 to act as an ionotropic receptor for endocannabinoids such as anandamide or *N*-arachidonyldopamine [38]. Given that most TRPV1 literature states that the channel has a threshold for activation of ~43°C, a role for TRPV1 in central thermosensation and thermoregulation seems unlikely. Yet, upon membrane depolarisation [39] or in the presence of sensitisers (see above), TRPV1 can carry significant current at 37°C, and thus potentially sense fluctuations in core body temperature. This might explain why TRPV1 antagonists, which are widely pursued as novel analgesics [7], evoke hyperthermia in rodents, dogs and primates [40]. Alternatively, TRPV1 antagonists could affect body temperature by inhibiting the tonic activity of TRPV1 in sensory neurons that regulate vascular tone [40]. Clearly, a better understanding of the mechanisms underlying this unwanted hyperthermia is an essential step in the development of TRPV1 antagonists for clinical use.

Hippocampal neurons also express TRPV4, which allows influx of cations at 37 °C and thereby increases the excitability of these neurons. Consequently, TRPV4-deficient hippocampal neurons have a more negative resting membrane potential and thus require larger depolarisations to evoke action potential firing than WT cells. When hippocampal cells are brought to 25 °C, a significant hyperpolarisation of WT neurons is observed, and the difference in resting membrane potential between WT and TRPV4-deficient cells vanishes [41]. Such TRPV4-dependent modulation of excitability could underlie known effects of cooling on hippocampal function and even learning. Strong TRPV4 expression is also found in the preoptic/anterior hypothalamic region, which is involved in body temperature control. However, there is currently little evidence suggesting a direct involvement of TRPV4 in central thermoregulation [5].

Expression of TRPV2 and to a lesser extent of TRPV3, TRPA1 and TRPM8 has also been found in different parts of the brain [42], but the impact of these thermoTRPs on brain function is currently elusive.

Insights into the mechanism of thermo- and chemosensing

A fundamental question remains: how do thermoTRPs sense temperature? Based on a detailed analysis of the whole-cell gating kinetics of two prototype thermoTRPs, the cold-activated TRPM8 and the heat-activated TRPV1, we have put forward a simple principle for temperature-sensitive channel gating (Box 1) [1,39]. This principle was based on the fundamental finding that TRPM8 and TRPV1 are voltage-gated channels activated upon membrane depolarisation, whose voltage dependence of activation is highly sensitive to temperature [39]. A two-state model was shown to accurately reproduce the temperature-dependent activation of TRPM8, TRPV1, TRPM4 and TRPM5, and further thermodynamic analysis allowed calculation of the enthalpies and entropies associated with the gating of these thermoTRPs [1,18,39].

Theoretical analysis predicted that the strong temperature dependence of thermoTRPs is correlated with the low gating charge (<1 equivalent charge per channel, compared to ~14 equivalent charges in shaker-type voltage-gated K^+ channels) of their voltage sensors (Box 1). This was addressed in detail in a recent study aimed at identifying the voltage-sensing residues in TRPM8 [43]. Charge-neutralising mutations in a region encompassing TM4 and the TM4-TM5 linker were found to affect the voltage dependence of channel activation, reflected in parallel changes in the apparent thermal threshold. Moreover, as predicted by the two-state model, a single point mutation (K856A) leading to reduced gating charge resulted in significantly large temperature-induced shifts of the activation curve [43].

The two-state model is arguably the simplest scheme to describe temperature effects on channel opening, and obviously represents an oversimplification of the full gating intricacies of thermoTRPs. Nevertheless, it provides for a quite accurate and highly instrumental description of the voltage-, temperature- and time-dependent gating of TRPV1, TRPM8, TRPM4 and TRPM5. Several recent studies have used a more complex eight-state model to describe the temperature-dependent gating of thermoTRPs [44,45]. In these 'allosteric' or 'modular' models, distinct sensors for voltage and temperature are coupled to an intrinsically voltage- and temperature-independent channel gate. In our opinion, a strictly allosteric mechanism for thermosensing is fundamentally incorrect, as changes in temperature are known to affect every single atom in a system, and thus thermal effects cannot simply be compartmentalised into a delineated region of a thermoTRP. Using the two-state formalism does not, however, exclude that some regions in thermoTRPs have a more prominent contribution to the overall temperature sensitivity. Moreover, recent analysis revealed that, when fitted to steady-state TRPM8 data, the allosteric eight-state model essentially reverts to the two-state model [43].

Several thermoTRP agonists including capsaicin (TRPV1) and menthol (TRPM8) mimic changes in temperature by acting as gating modifiers that shift voltage-dependent gating toward negative voltages [39]. Interestingly, mutations in TM4 and the TM4-TM5 linker of TRPM8 were found to affect the affinity of the channel for menthol, suggesting a direct interaction of ligands with the voltage sensor region [43] (Box 1). A growing number of studies indicate that agonists of thermoTRPs such as menthol and icilin (TRPM8), capsaicin (TRPV1) or

Box 1. Modelling thermo- and chemosensing by thermoTRPs

In the absence of a ligand, temperature- and voltage-dependent gating of thermoTRPs including TRPM8 and TRPV1 can be reduced to a two-state model [18,39] where the transition between the closed and open states is determined by the voltage-dependent activation and deactivation rates α and β:

$$Closed \underset{\beta(V,T)}{\overset{\alpha(V,T)}{\rightleftarrows}} Open.$$

To introduce the effects of ligand binding on thermoTRP channel gating, we have extended the two-state model to a Monod-Wyman-Changeux-type (MWC) model [43]. Each of the four channel subunits is able to bind one ligand molecule, and all four subunits undergo a concerted voltage-dependent transition between the closed (C) and open (O) states (Figure I).

Thus

$$P_{open} = \frac{1}{1 + \exp\left(\frac{(V_{1/2,0} - \Delta V_{1/2}) - V}{RT/zF}\right)}.$$

$V_{1/2,0}$ represents the voltage for half-maximal activation in the absence of ligand and is given by

$$V_{1/2,0} = \frac{1}{zF}(\Delta H_0 - T\Delta S_0),$$

where ΔH_0 and ΔS_0 represent the difference in enthalpy and entropy between O_0 and C_0, respectively ($\Delta H_0 = \Delta H_{open,0} - \Delta H_{close,0}$; $\Delta S_0 = \Delta S_{open,0} - \Delta S_{close,0}$). $\Delta V_{1/2}$ represents the effect of ligand binding on the voltage for half-maximal activation and is given by

$$\Delta V_{1/2} = \frac{RT}{zF} ln \frac{\Sigma_{i=0}^{4}\left(\frac{4}{i}\right)\left(\frac{L}{K_d}\right)^i}{\Sigma_{i=0}^{4}\left(\frac{4}{i}\right)\left(\frac{L}{K_d}\right)^i \exp\left(\frac{i\frac{1}{4}\Delta\Delta H_{max}}{RT}\right)},$$

where L is the ligand concentration, $K_d = k_{off}/k_{on}$ is the ligand affinity of a single menthol binding site when the channel is open and $\Delta\Delta H_{max}$ is the maximal change in enthalpy obtained at saturating menthol concentrations. Assuming that each ligand binding event has an equivalent effect on ΔH, $\Delta H_i = \Delta H_0 + i\Delta\Delta H_{max}/4$. The affinity of a single ligand binding site differs between the open and closed state of the channel, according to the relation

$$K_d^* = K_d \exp\left(\frac{-\Delta\Delta H_{max}}{4RT}\right).$$

For example, in the case of TRPM8, the affinity for menthol in the closed state is ~6.5-fold lower than in the open state.

This model implies that P_{open} in the function of voltage can be described by a single Boltzmann-type function, and that menthol changes the position of the activation curve along the voltage axis, without altering the slope factor, which matches our experimental observations for TRPM8. Moreover, changes in $V_{1/2}$ induced by temperature or ligand binding are inversely correlated to the gating charge z, as has been confirmed for TRPM8 voltage sensor mutants [43]. Figure I shows some predictions of the model for TRPM8 and TRPV1 using published parameters [39,43].

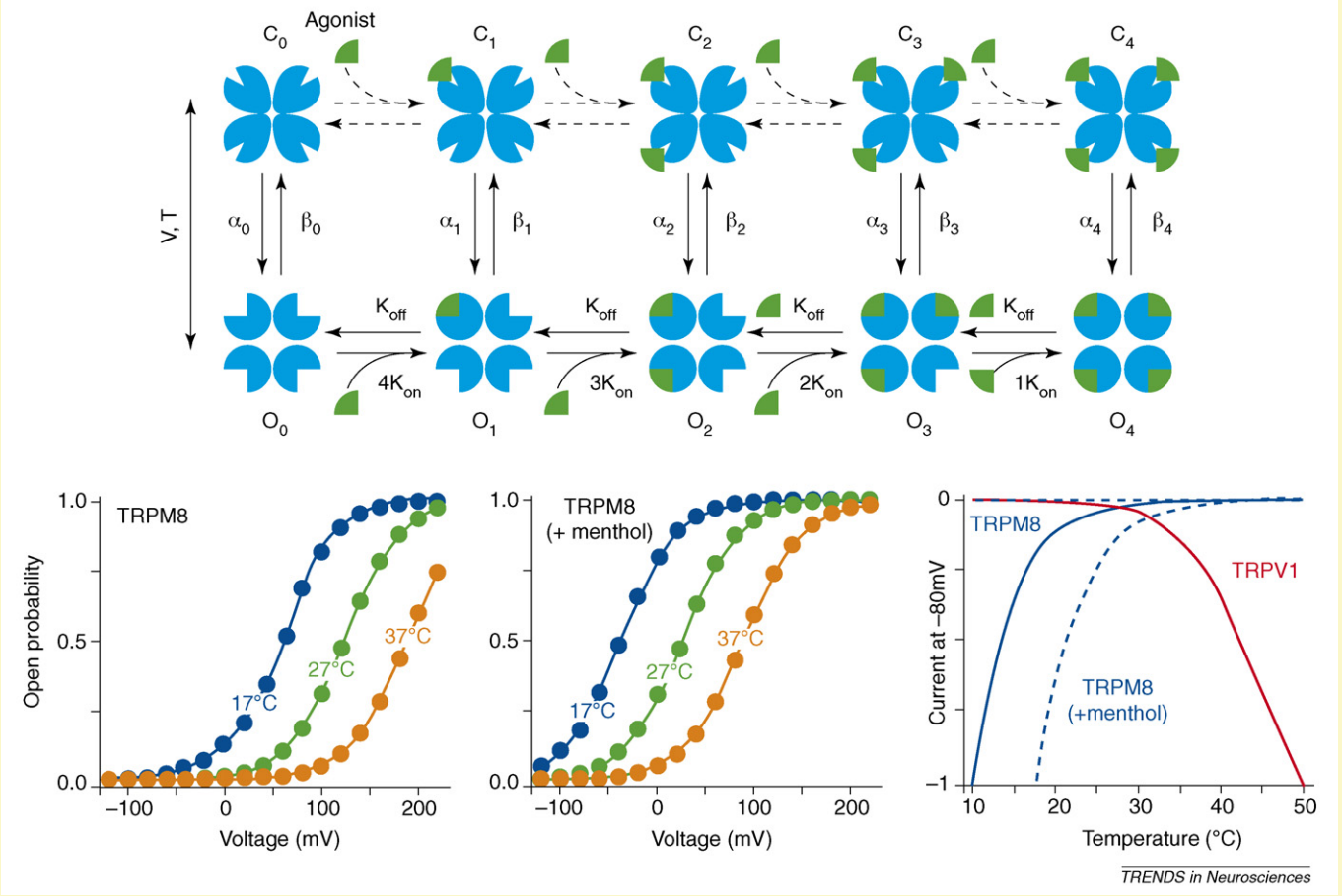

Figure I. The MWC-type gating model. (Top) Schematic representation of the MWC model. (Bottom left and center) Apparent open probability of TRPM8 at different voltages and temperatures in the absence and presence of 30 μM menthol. (Bottom right) Normalised inward current at –80 mV for TRPM8 and TRPV1.

4α-PDD (TRPV4) activate these channels through interaction with TM1–4 [43,46–49].

A strikingly different mechanism for chemosensing was recently proposed for TRPA1 [50,51]. It was recognised that many pungent compounds known to activate TRPA1, including (but not limited to) mustard oil, cinnamaldehyde, acrolein and formalin, have little structural similarity but share the ability to covalently bind cysteine residues. Using click chemistry [50] and site-directed mutagenesis [50,51], several cytosolic cysteines were shown to be critically involved in binding these agonists, resulting in long-lasting channel activity. Thus, TRPA1 can act as a sensor of a broad range of chemicals that cause protein modification and tissue damage.

Brain TRPC channels in pathfinding and cell survival

Analogous to the role of sensory neuron TRP channels in detecting environmental signals, TRP channels in the developing brain appear to play an important role in detecting internal chemical cues. In particular, recent evidence implicates several members of the TRPC subfamily in the responses of brain neurons to neurotrophic factors (neurotrophins) and chemoattractants such as BDNF or Netrin-1 [52]. The response of neurons to neurotrophic factors and guidance cues is closely associated with changes in the intracellular Ca^{2+} concentration. Whereas older studies focussed on Ca^{2+} signals evoked by voltage-gated Ca^{2+} channels, NMDA receptors and Ins(1,4,5)P_3 receptors, recent findings also suggest an important role for TRPC channels [52]. In a seminal study demonstrating a link between TRPC channels and neurotrophin signalling in the brain, it was found that TRPC3 and TrkB are widely coexpressed in the brain and that BDNF activates a TRPC3-dependent nonselective cation current (I_{BDNF}) in pontine neurons [53]. Based on these findings, the possibility was raised that TRPC channel activity could mediate some of the neurotrophin-induced effects in the nervous system [54]. Indeed, several recent papers have provided compelling evidence that TRPC channels are required for the morphological changes at growth cones and dendritic spines in response to neurotrophins or Netrin-1.

In cultured *Xenopus* spinal neurons, Netrin-1 and BDNF evoked Ca^{2+} influx and a depolarising, TRPC-like current in both soma and growth cones [55]. Inhibition of the *Xenopus* homologue of mammalian TRPC1 (XTRPC1) prevented Ca^{2+} influx, TRPC-like current activation and the chemotropic turning of the growth cone in response to a gradient of Netrin-1 or BDNF [55]. Moreover, *in vivo* studies demonstrated that XTRPC1 is critically involved in Netrin-1-dependent processes such as the formation of commissural interneuron axon tracts by and for attraction of these axons to the CNS midline [56].

A similar role for TRPC channels was also reported in rat cerebellar granule neurons (CNGs), in which downregulation of TRPC3/TRPC6 function prevented BDNF-induced Ca^{2+} influx and growth-cone turning [57]. In addition to its effects on growth-cone turning, BDNF-induced influx of Ca^{2+} through TRPC3/TRPC6 also promotes the survival of CNGs, and this prosurvival effect depends on activation of the cAMP/Ca^{2+} response element binding protein CREB [58]. Correspondingly, RNAi-mediated knockdown of TRPC3 or TRPC6 significantly reduced granule-cell survival *in vivo*, specifically in the window between postnatal days 10 and 12 when expression of TRPC3 and TRPC6 is at its peak [58].

In hippocampal CA1 pyramidal neurons, BDNF is known to evoke a fast Na^+ current through $Na_V1.9$ as well as a slower nonselective cation current (I_{BDNF}). I_{BDNF} was found to depend on TRPC3, as it was inhibited by intracellular application of anti-TRPC3 antibodies as well as by siRNA-mediated knockdown of TRPC3 [59]. Moreover, BDNF is known to induce an increase in the density of dendritic spines in these cells, and this remodelling is also inhibited upon TRPC3 knockdown [59]. A plausible model proposes that BDNF-induced Ca^{2+} influx through TRPC3 provokes changes in spine morphology through Ca^{2+}-dependent activation of the MAPK/ERK pathway.

Mechanisms of TRPC activation in the brain

An important yet unresolved question is how chemical cues such as BDNF or Netrin-1 lead to TRPC channel activation. In contrast to ligand activation of different thermoTRPs, which are known to interact directly with ligands such as capsaicin (TRPV1) or menthol (TRPM8) [39,43,46], the TRPC channels involved in the detection of neurotrophic or chemoattractant cues do not seem to directly bind these factors. Instead, these extracellular signalling molecules bind to their respective metabotropic receptors, which initiate intracellular signalling pathways that eventually cause TRPC channel gating.

The neurotrophin receptors of the Trk family are receptor tyrosine kinases, in which ligand binding leads to autophosphorylation of tyrosine residues. These phosphotyrosines act as interaction sites for SH2 or PTB domain-containing proteins in an activated receptor, which leads to the recruitment and activation of several key signal transduction pathways, including phospholipase C_γ (PLC_γ), phosphatidylinositol-3 kinase (PtdIns[3]K) and the small G protein Ras [60]. Figure 2 illustrates a few possible TRPC activation mechanisms downstream of Trk activation.

First, PLC_γ activation catalyses the hydrolysis of PtdIns(4,5)P_2, generating diacylglycerol (DAG) and Ins(1,4,5)P_3. DAG activates TRPC3 and TRPC6 in a direct, membrane-delimited manner (Figure 2a), but is without effect on TRPC1/C4/C5 channels [61].

Second, TRPC channel activation might be a consequence of Ins(1,4,5)P_3-induced depletion of ER Ca^{2+} (Figure 2b). In fact, all TRPC channels have been put forward as store-dependent channels, although in most cases this store dependence has later been disputed. Recently, two other families of proteins have been implicated in store-dependent Ca^{2+} entry, the Stim and Orai (also called CRACM) proteins [62]. The current view is that Stim proteins acts as ER Ca^{2+} sensors that redistribute toward the plasma membrane upon store depletion, whereas Orai proteins in the plasma membrane make up the Ca^{2+}-selective pore of the store-operated channel [62]. Intriguingly, there are indications that both Stim1 and Orai proteins can functionally interact with TRPC channels and thereby modulate their function as store-dependent channels [63,64]. In this respect, it would be of great importance to investigate the role of Stim and Orai

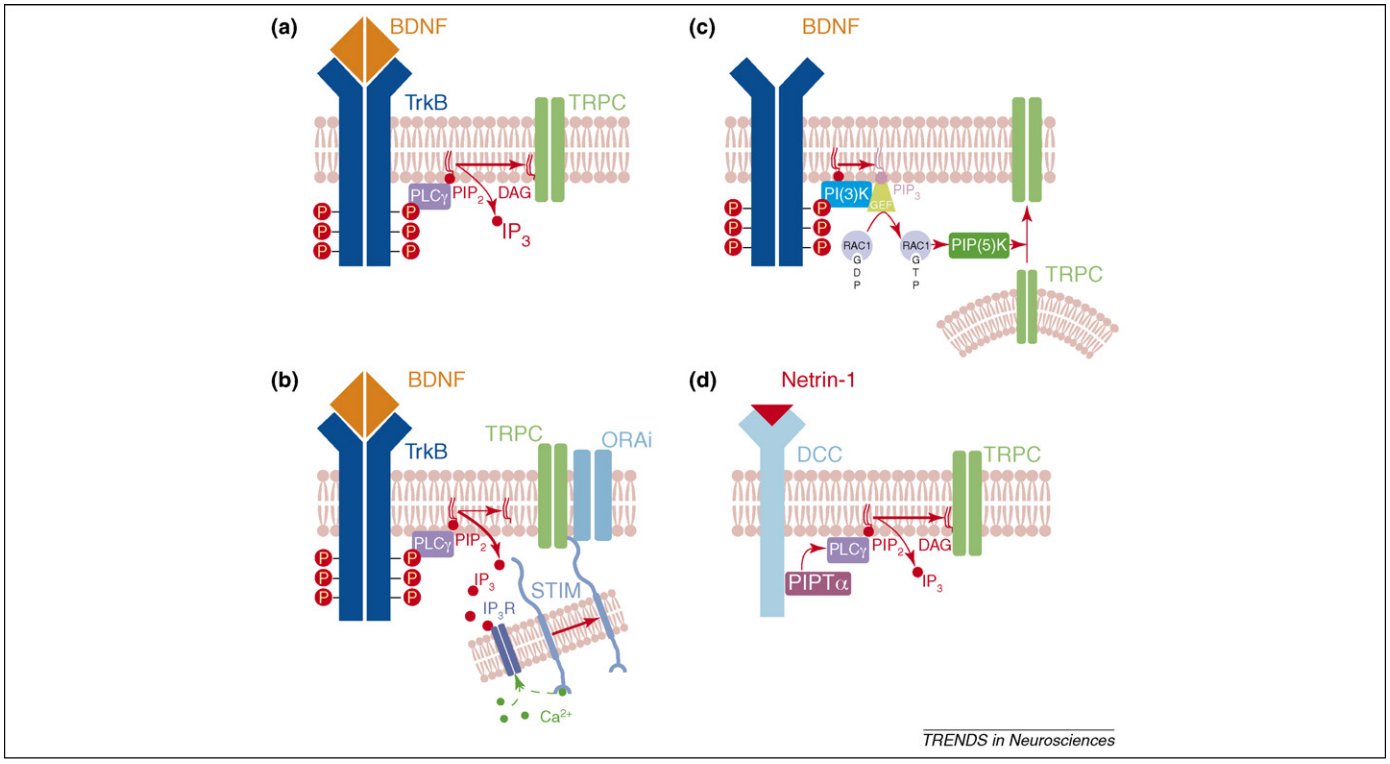

Figure 2. Potential mechanisms for receptor-mediated activation of TRPC channels involved in the actions of neurotrophins or Netrin-1. **(a–c)** BDNF induces dimerisation and autophosphorylation of its receptor TrkB, a receptor tyrosine kinase. This can lead to gating of TRPC channels via activation of PLCγ, which leads to formation of DAG (a) and depletion of intracellular stores (b). Alternatively, TrkB-mediated activation of PtdIns(3)K can induce rac1- and PIP(5)K-dependent trafficking and fusion of TRPC-containing vesicles. **(d)** Netrin-1 activates TRPC channels through activation of its receptor DCC (deleted in colon cancer). This can lead to activation of PLCγ and subsequent activation of TRPC channels via DAG and/or store depletion. See text for details.

proteins in growth-cone turning and cell survival in the developing brain.

Third, activation of neurotrophin receptors might affect TRPC channels through activation of PtdIns(3)K (Figure 2c). PtdIns(3)K converts plasma membrane PtdIns(4,5)P_2 into PtdIns(3,4,5)P_3, which recruits and activates specific pleckstrin homology (PH) domain-containing proteins such as protein kinase B (Akt). Current evidence indicates that neurotrophin-induced activation of PtdIns(3)K modulates Ca^{2+} influx through TRP channels by promoting their expression at the plasma membrane rather than by influencing their gating. This has been well documented in hippocampal neurons, where stimulation with growth factors such as NGF or BDNF causes a very rapid translocation of TRPC5 from vesicles to the plasma membrane, thereby inhibiting neurite outgrowth [65,66]. Rapid translocation of TRPC5 requires PtdIns(3)K, as well as the downstream signalling elements Rac1 (a Rho-GTPase) and phosphatidylinositol 4-phosphate 5-kinase (PIP[5]Kα) (Figure 2c) [66].

The signal transduction pathways activated by the known Netrin-1 receptors, deleted in colorectal cancer (DCC) and Unc5a-c, are less well understood. One possibility is that Netrin-1 receptor signalling to TRPC channels is mediated via hydrolysis of PtdIns(4,5)P_2, which then causes TRPC channel activity through a DAG- or store-dependent mechanism. It has been shown that DCC binds and activates the phosphatidylinositol transfer protein PIPTα, a protein involved in the transfer of phosphatidylinositol between different membranes and known regulators of PLC_γ [67,68], and that this interaction is strengthened in the presence of Netrin-1 PIPTα activity (Figure 2d).

Brains sans TRPCs

Despite the striking effects of TRPC channel inhibition using (nonspecific) pharmacological tools and siRNA- or morpholino-based knockdown on neuronal pathfinding and survival, studies describing different TRPC knockout mice have not yet reported any phenotype that would suggest crucial defects in brain development. In particular, knockout mice for TRPC1, TRPC2, TRPC4 and TRPC6 appear healthy and do not exhibit obvious signs of gross CNS deficits [69,70]; mice deficient for TRPC3, TRPC5 or TRPC7 have not yet been reported. A recent study reported that Δ202 mice, a mouse line carrying a transgene coding for the SV40 T antigen, lack TRPC3 expression owing to insertion of the transgenic construct in the promoter region of the *trpc3* gene [71]. Interestingly, homozygous Δ202 mice develop progressive paralysis, which has been attributed to a defect in the postnatal development of the CNS [71]. It would be of great interest to investigate whether classical or inducible TRPC3 knockout mice develop similar neurological disorders. Clearly, more definitive answers will require a detailed analysis of brain anatomy and function in single and combined TRPC channel knockout mice.

Conclusions

This review of neuronal TRP channels illustrates a recurring theme in TRP research, namely their function as sensors or parts of sensory complexes involved in the detection of environmental or endogenous stimuli.

Box 2. Outstanding questions

- How do TRPV3- and TRPV4-expressing keratinocytes convey thermal information to sensory neurons?
- Is TRPA1 cold sensitive and does it contribute to cold sensation *in vivo*?
- Does the temperature sensitivity of thermoTRPs arise from a distinct modular thermosensor region?
- What is the role of thermoTRPs in the brain?
- What are the cellular mechanisms that couple receptor activation in central neurons to TRP channel gating, growth-cone guidance and neuronal survival?

ThermoTRPs act as versatile thermometers that allow us to assess the environmental temperature, and warn us of life-threatening thermal and chemical danger. Future work should not only solve some important outstanding questions concerning the *modus operandi* of these channels and their relative contribution to different thermosensory processes (Box 2) but also further explore the use of thermoTRPs as targets for novel treatments of pain and sensory disorders.

Equally intriguing but less well understood is the role of TRP channels in the brain (Box 2). We have highlighted some important new developments that point toward a crucial involvement of TRPC channels in the detection of chemical cues in the brain. Future research in this field is expected to establish the contribution of TRPC channels in brain development and wiring, and their involvement in the etiology of various neurological diseases.

Acknowledgements

We thank all the members of our laboratory for comments and discussions. Support from the Belgian Federal Government (IUAP P6/28), the Research Foundation–Flanders (G.0136.00, G.0172.03 and G.0565.07) and the Research Council of KU Leuven (GOA2004/07 and EF/95/010) is gratefully acknowledged.

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