REVIEW

The thermo-TRP ion channel family: properties and therapeutic implications

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The thermo-transient receptor potentials (TRPs), a recently discovered family of ion channels activated by temperature, are expressed in primary sensory nerve terminals where they provide information about thermal changes in the environment. Six thermo-TRPs have been characterised to date: TRP vanilloid (TRPV) 1 and 2 are activated by painful levels of heat, TRPV3 and 4 respond to non-painful warmth, TRP melastatin 8 is activated by non-painful cool temperatures, while TRP ankyrin (TRPA) 1 is activated by painful cold. The thermal thresholds of many thermo-TRPs are known to be modulated by extracellular mediators, released by tissue damage or inflammation, such as bradykinin, PG and growth factors. There have been intensive efforts recently to develop antagonists of thermo-TRP channels, particularly of the noxious thermal sensors TRPV1 and TRPA1. Blockers of these channels are likely to have therapeutic uses as novel analgesics, but may also cause unacceptable side effects. Controlling the modulation of thermo-TRPs by inflammatory mediators may be a useful alternative strategy in developing novel analgesics.

Abbreviations

2-APB, 2-aminoethoxydiphenylborate; AKAP, A-kinase anchoring protein; CaMK, calcium/calmodulin-dependent kinase; CFA, complete Freund's adjuvant; c-Kit, receptor for SCF; DRG, dorsal root ganglia; GDNF, glial cell line-derived nerve growth factor; IGF, insulin-like growth factor; NGF, nerve growth factor; PGE2, prostaglandin E2; PIP$_2$, phosphatidylinositol 4 5-bisphosphate; RET, 'rearranged during transfection' (GDNF receptor); SCF, stem cell factor; Ser, serine; Src ('sarcoma'), a non-receptor tyrosine kinase; Thr, threonine; TM, transmembrane; TrkA, tyrosine receptor kinase type 1 (NGF receptor); TRP, transient receptor potential; TRPA, transient receptor potential ankyrin; TRPC, transient receptor potential canonical; TRPP, transient receptor potential polycystic; TRPM, transient receptor potential melastatin; TRPML, transient receptor potential melastatin-like; TRPN, transient receptor potential NOMPC; TRPV, transient receptor potential vanilloid; Tyr, tyrosine; VR1, vanilloid receptor 1

Introduction

Nociception refers to the initial detection of noxious stimuli at the terminals of primary sensory neurons, first named nociceptors by Sherrington a century ago (Sherrington, 1906). Nociceptive nerve axons are either small-diameter, unmyelinated C fibres or medium-diameter myelinated Aδ fibres (Wood and Perl, 1999). The cell bodies lie within the sensory ganglia, the trigeminal ganglia (TG) innervating the face and head, and the dorsal root ganglia (DRG) for the rest of the body. Nociceptors respond to noxious mechanical, chemical or thermal stimuli, generating action potentials that are propagated via the dorsal horn of the spinal cord to higher brain centres to result in a sensation of pain. In the last decade, roles for several ion channels in the initiation of action potentials in nociceptors have been identified. Cloning and characterization of a thermally sensitive subclass of the transient receptor potential (TRP) channels (the

thermo-TRPs) have opened up our understanding of the molecular basis of thermal sensation. More than 30 members of mammalian TRP channel family have been characterized and can be subdivided into seven main classes according to their sequence homology: TRP ankyrin (TRPA), TRP canonical (TRPC), TRP melastatin (TRPM), TRP melastatin-like (TRPML), TRP NOMPC (TRPN), TRP polycystic (TRPP) and TRP vanilloid (TRPV) (Montell, 2005). Six are recognized as thermo-TRPs, in the sense that they are expressed in primary somatosensory neurons and are activated at specific temperatures in the range from noxious heat to painful cold. TRPV1–4 transduce elevated temperatures ranging from warm (TRPV4 and TRPV3) to noxious heat (TRPV1 and TRPV2). TRPM8 and TRPA1, on the other hand, are activated respectively by moderate and more extreme cooling. All thermo-TRPs are also activated by a wide range of natural compounds present in foodstuffs and in the environment. Three other TRPs (TRPM2, TRPM4 and TRPM5) also show temperature sensitivity but are not usually included in the thermo-TRP family because they are not expressed in primary somatosensory neurons. Note though that TRPM5 is expressed in primary taste receptors where it plays a role in temperature sensitivity of taste (Talavera et al., 2005), and there is recent evidence that these TRPMs may, in fact, be expressed in somatosensory neurons (Lechner et al., 2009; Staaf et al., 2010).

In this review we first briefly present the properties of the thermo-TRP channels. We then present our current knowledge on their modulation by inflammatory mediators or by growth factors, which act via intracellular signalling pathways to alter the thermal thresholds of agonist sensitivity of the thermo-TRPs. Finally we present some recent findings from the rapidly developing field of thermo-TRP agonists and antagonists. We point out that directly blocking these channels may not be the only useful pharmaceutical strategy in controlling pain, and that compounds attacking the modulation of these channels by intracellular signalling pathways may also have value as analgesics.
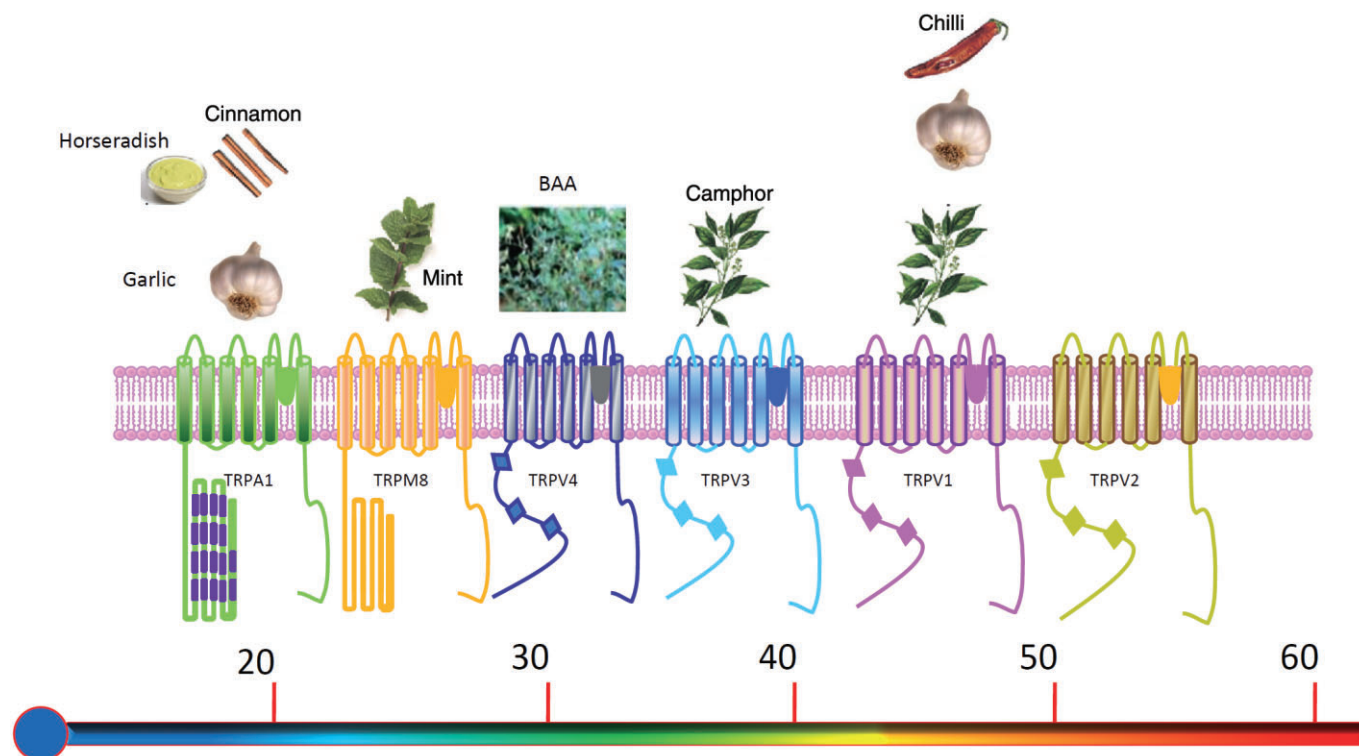
Properties of thermo-TRP channels

The basic architecture of TRP channels is similar to that of the voltage-gated K^+ channel family – four identical or similar subunits, each with six transmembrane (TM) domains (TM1–TM6) and cytosolic N- and C-terminal tails. TM5, TM6 and the connecting loop form the central cation-conducting pore whereas TM1–TM4 and the cytoplasmic N- and C-terminal parts are thought to contain the regulatory domains that control channel gating. Much of what we assume about the structure of the thermo-TRPs is extrapolated from the known structure of other ion channels, but some structural information is now becoming available (Gaudet, 2009; Moiseenkova-Bell and Wensel, 2009). Figure 1 and Table 1 summarize the activation of the thermo-TRPs by temperature and by non-thermal agonists, and outline probable functions of these ion channels.

TRPV1

The activation of heat-sensitive ion channels in sensory neurons was first characterized by work from our group (Cesare and McNaughton, 1996). A heat-sensitive ion channel that seems to be responsible for much of the heat sensitivity of primary sensory neurons (discussed further below) was subsequently cloned using an innovative expression-cloning strategy and named vanilloid receptor 1 (VR1), although this was later changed to TRPV1 to emphasize its membership of the TRP ion channel family (Caterina et al., 1997). TRPV1 is mainly detected in small- to medium-diameter neurons of primary sensory ganglia (DRG, TG and sympathetic ganglia such as the nodose ganglia). TRPV1 is expressed by all the major classes of nociceptive neurons, namely the nociceptive subset of Aδ myelinated-fibre neurons, and the peptidergic and non-peptidergic classes of small unmyelinated C-fibre primary afferents. TRPV1 is the only TRP family member that is activated by vanilloids such as capsaicin, a pungent chemical found in hot chilli peppers. Capsaicin has been known for many years to be a selective activator of unmyelinated and small myelinated nociceptive afferents (Oh et al., 1996). Studies using membrane-permeable and impermeable analogues of capsaicin and the competitive antagonist capsazepine demonstrated that capsaicin binds to an intracellular site of TRPV1 to activate the channel (Jung et al., 1999). TRPV1 is a promiscuous channel that is activated by a wide range of agonists (see Table 1), by low pH and by physical factors such as heat and membrane depolarization (Tominaga et al., 1998; Voets et al., 2004; Zhu, 2007). Noxious heat directly gates the channel with a threshold for activation at >43°C. (Note that the concept of 'threshold' is not strictly applicable to a channel such as TRPV1 where increases in temperature activate the channel by increasing its probability of activation, and where the channel, therefore, has some degree of activity at any temperature. We use here the term 'threshold' as an operational definition to indicate the temperature at which the inward current through a thermo-TRP channel becomes large enough to fire action potentials in an afferent nerve fibre). However, the temperature threshold can be lowered by the action of proinflammatory mediators that are released during tissue injury or inflammation. These mediators can be classified into two broad classes, as discussed further below: those that activate serine–threonine kinases, such as PKA and PKC, via a GPCR; and those which activate tyrosine kinase (TK) via growth factor receptors. The former include bradykinin (Cesare and McNaughton, 1996; Vellani et al., 2001; Bhave et al., 2003), PGs (Bhave et al., 2002; Moriyama et al., 2005), ATP (Moriyama et al., 2003), endothelins (Plant et al., 2007) and prokineticins (Vellani et al., 2006). The second and more recently discovered class of sensitizing agents include nerve growth factor (NGF) (Lewin and Mendell, 1993; Bonnington and McNaughton, 2003; Zhang et al., 2005), insulin and insulin-like growth factor (IGF) (Van Buren et al., 2005; Camprubi-Robles et al., 2009) and stem cell factor (SCF) (Milenkovic et al., 2007).

TRPV1 is directly gated by acidic conditions, pH < 6. Lowering the extracellular pH from 7.6 to 6.4 also sensitized TRPV1 to both capsaicin and heat (Tominaga et al., 1998). TRPV1 can be directly activated by ethanol (Trevisani et al., 2002), and by a variety of endogenous lipids such as anandamide (Ross, 2003) and lipoxygenase products (Hwang et al., 2000). Thus, TRPV1 is viewed as a signalling integrator for many noxious stimuli.

Figure 1

Schematic depiction of the six mammalian thermo-TRP channels. Each subunit consists of six transmembrane domains (S1–S6), a hydrophobic pore loop linking transmembrane segments five (S5) and six (S6), and large cytoplasmic N- and C- terminals (NB: not drawn to scale). All thermo-TRPs have a variable number of ankyrin repeat domains in the N-terminus (except TRPM8, which has none). Thermo-TRPs display distinct thermal thresholds from very hot (TRPV2) to cold (TRPA1). Each thermo-TRP is also activated by specific natural compounds and by synthetic substances, which are also known to induce the relevant thermal and pain sensations in humans. This figure provides a schematic overview of some of the main agonists – see Table 1 for a more complete list. BAA, bisandrographolide A.

Genetic studies showed that TRPV1 is the only receptor for capsaicin, because vanilloids evoked no pain behaviours in TRPV1$^{-/-}$ mice (Caterina et al., 2000). TRPV1$^{-/-}$ mice largely failed to develop thermal hyperalgesia in response to mustard oil, complete Freund's adjuvant (CFA) or carrageenan, showing that TRPV1 is a key element in the generation of thermal hyperalgesia (Caterina et al., 2000; Davis et al., 2000; Moriyama et al., 2005). Mechanical hyperalgesia was little affected by deletion of TRPV1 (Caterina et al., 2000), but pharmacological studies using capsazepine and other more selective TRPV1 antagonists demonstrated reduced mechanical hyperalgesia in different pain models, supporting the hypothesis that TRPV1 contributes at least to some extent to the development of mechanical hyperalgesia (Davis and Perkins, 1996; Hua et al., 1997; Kwak et al., 1998; Caterina, 2003).

Although there is general agreement that TRPV1 mediates thermal hyperalgesia following inflammation, the precise role of TRPV1 in detecting heat stimuli still remains surprisingly elusive. Mice lacking the TRPV1 receptor are relatively normal in their response to noxious heat stimuli (Caterina et al., 2000; Davis et al., 2000). Consistent with this, in culture many isolated sensory neurons (23%) generate an inward current in response to heat but not to the TRPV1 agonist capsaicin (Nagy and Rang, 1999a,b). A heat-detecting

mechanism other than TRPV1 must therefore be present. TRPV2 is the obvious candidate (Caterina et al., 2000), but TRPV2 null mice show no apparent deficit in thermal sensation (Park et al., 2011). There is also evidence at the level of single neurons for heat-detecting mechanisms not mediated by either TRPV1 or TRPV2, because only a minority of heat-sensitive neurons in intact preparations express either of the heat-sensitive ion channels TRPV1 or TRPV2 (Woodbury et al., 2004; Lawson et al., 2008; Koerber et al., 2010). Of particular interest is a recent paper showing that thermal hyperalgesia is communicated via nerve fibres which do not express TRPV1 (Koerber et al., 2010). These studies show that ion channels other than TRPV1 or TRPV2 must play important roles in sensing painful heat, and possibly also in thermal hyperalgesia.

TRPV2

In contrast to TRPV1, TRPV2 is insensitive to capsaicin and responds to higher temperature stimuli, with an activation threshold of 52°C. TRPV2 is highly expressed in a subpopulation of medium to large diameter sensory neurons that predominantly give rise to Aδ fibres (Caterina and Julius, 1999; Bender et al., 2005). TRPV2 is activated in vitro by physical stimuli such as heat, osmotic stress, mechanical stretch (Caterina et al., 1999; Muraki et al., 2003), and non-selective

Table 1

TRP channels and their temperature sensitivity, with examples of agonists acting on each receptor and suggested functions of the receptor in the intact animal

Channel	Temperature sensitivity	Non-thermal agonists	Function
TRPV1	≥42°C	Capsaicin, low pH, ethanol, anadamide, NADA, 12-HPETE, camphor, resiniferatoxin, allicin, 2-APB, lidocaine, gingerol, shogaol, piperine monoacylglycerols, ω-3 fatty acids, membrane stretch	Noxious heat sensor; also involved in inlammatory pain, thermal hyperalgesia, hippocampal long-term depression, diabetes, obesity, bladder function, hypertension, gastrointestinitis, hypothermia, renal excretory function.
TRPV2	≥52°C	2-APB, cannabidiol, membrane stretch	Possible extreme heat sensor ; innate immune system
TRPV3	32°C~39°C	2-APB, camphor, carvacrol (from oregano), incensole acetate, thymol, eugenol	Warmth; possible involvement in noxious heat detection
TRPV4	27°C~34°C	Membrane stretch, phorbol ester, 5,6-EET, anandamide, arachidonic acid, BAA	Warm temperature sensation and volume regulation; possible involvement in noxious mechanical pain and thermal hyperalgesia
TRPM8	25°C~34°C	Menthol, icilin, eucalyptol	Innocuous cold perception, behavioural thermoregulation, cold-mediated analgesia; cold nociception in some neurons
TRPA1	≤17°C	Cinnamaldehyde, acrolein, chlorine, ROS, formalin, fatty acids, mustard oil, allicin, icilin, gingerol, prostanoids, NSAIDs, isoflurane, propofol, etomidate, dihydropyridines, clotrimazole, nicotine, menthol	Cold, mechanical and chemically induced nociception, cold hyperalgesia

NADA, N-Arachidonyl dopamine AA-DA; 12-HPETE, 12- hydroperoxyeicosatetraenoic acid; 2-APB, 2-aminoethoxydiphenyl borate; 5,6-EET, 5,6 epoxyeicosatrienoic acid; BAA, bisandrographolide A; NSAIDs, non-steroidal anti-inflammatory drugs; ROS, reactive oxygen species.

chemical activators such as 2-aminoethoxydiphenyborate (2-APB) (Hu *et al.*, 2004) and cannabidiol (Qin *et al.*, 2008). It has been suggested that TRPV2 is up-regulated in DRG neurons after nerve injury and inflammation (Shimosato *et al.*, 2005; Frederick *et al.*, 2007), which may indicate a potential role for TRPV2 in pain sensation. Compared to TRPV1, TRPV2 has a wider distribution pattern, including lung, spleen, bladder and immune cells, suggesting a broader range of physiological functions than TRPV1. However, the specific physiological and pathological roles of TRPV2 in thermal nociception remain unclear, as TRPV2 null mice showed no evident deficits in thermal sensation (Park *et al.*, 2011; Link *et al.*, 2010).

TRPV3 and TRPV4

These thermo-TRPs are activated by moderate temperatures, with thresholds of 34°C–38°C and 27°C–34°C respectively (Smith *et al.*, 2002; Xu *et al.*, 2002; Peier *et al.*, 2002b; Watanabe *et al.*, 2002b). Mice lacking TRPV3 or TRPV4 have been reported to exhibit deficits in both innocuous and noxious heat sensation indicating an involvement of both channels in thermosensation (Todaka *et al.*, 2004; Lee *et al.*, 2005; Moqrich *et al.*, 2005). In addition to temperature, both are activated by chemical stimuli which in humans give a sensation of pleasant warmth. For example, thymol from thyme, carvacrol from oregano and eugenol, found in cloves, nutmegs, cinnamon, basil and bay leaves, activate TRPV3 (Xu *et al.*, 2006). The synthetic compound 2-aminoethyl diphenylborate (2-APB) is a promiscuous activator of TRPV1,

TRPV2 and TRPV3 (Hu *et al.*, 2004; but note that effects of 2-APB on sensory neurons are mediated via TRPV1, see Zimmermann *et al.*, 2005). Hypotonic stress and phorbol esters activate many TRPV channels including TRPV4 (Watanabe *et al.*, 2002a; Fan *et al.*, 2009). TRPV4 is activated by the compound bisandrographolide from the Chinese herbal plant *Andrographis paniculata* (Smith *et al.*, 2006). Both TRPV3 and TRPV4 are found to be predominantly expressed in skin keratinocytes, suggesting that warm stimuli might be detected initially in keratinocytes and then excitation transferred to primary afferent terminals by an extracellular mediator, which has been proposed to be ATP (Peier *et al.*, 2002b; Mandadi *et al.*, 2009) or PGE2 (Huang *et al.*, 2008). TRPV4 is also expressed in mouse and rat urothelium and vascular endothelium, and studies with knockout animals demonstrated a role for TRPV4 in voiding behaviour, raising the possibility that TRPV4 plays a critical role in urothelium-mediated transduction of intravesicular mechanical pressure (Gevaert *et al.*, 2007). Knockout studies suggest that TRPV4 plays a role in thermal hyperalgesia (Todaka *et al.*, 2004), and studies in which TRPV3 was overexpressed in keratinocytes suggest a possible similar role for TRPV3 (Huang *et al.*, 2008). TRPV4 has been suggested to play a potential role in mechanosensation (Alessandri-Haber *et al.*, 2005) and in normal hearing (Nilius and Voets, 2005).

TRPM8 and TRPA1

Soon after the discovery of TRPV1, two cold-sensitive TRP channels, TRPM8 and TRPA1, were cloned and characterized

(McKemy *et al*., 2002; Peier *et al*., 2002a; Story *et al*., 2003). Like TRPV1, both are Ca^{2+}-permeable cation channels. TRPM8 is activated by menthol, which produces a sensation of pleasant cooling in human subjects, and responds to non-noxious cool temperatures, being first activated on cooling at 25°C in expression systems, though the activation threshold of TRPM8 in native sensory neurons is ~6°C higher than in HEK cells (De la Pena *et al*., 2005). TRPM8 is activated by chemicals that mimic cooling, such as menthol, icilin and eucalyptol (McKemy *et al*., 2002; Peier *et al*., 2002a; Chuang *et al*., 2004; Voets *et al*., 2004). Low levels of menthol elevate the threshold for TRPM8 activation to warmer temperatures, and repeated menthol stimulation rapidly desensitises TRPM8 in a Ca^{2+}-dependent manner (McKemy *et al*., 2002; Reid *et al*., 2002). The expression of TRPM8 in small-diameter sensory neurones lacking nociceptor markers, such as TRPV1 and calcitonin gene-related peptide (CGRP), supports the idea that TRPM8 acts as a non-noxious cool thermosensor *in vivo* (McKemy *et al*., 2002; Peier *et al*., 2002a), although TRPM8 is also co-expressed with TRPV1 in probable nociceptive neurons, for reasons that are currently unclear (Babes *et al*., 2004; Okazawa *et al*., 2004). *In vivo* studies of TRPM8$^{-/-}$ mice showed deficits in sensation of non-noxious cool temperatures, demonstrating that this channel is a major sensor of peripheral innocuous coolness (Bautista *et al*., 2007; Colburn *et al*., 2007; Dhaka *et al*., 2007). However, these mice are not deficient in responses to noxious cold, and therefore, other receptors must exist to detect more extreme cold.

TRPA1 is activated by cooling to the noxious cold range of temperatures (~17°C), suggesting that this channel may be responsible for detecting painful levels of cold (Story *et al*., 2003). TRPA1 is directly activated by intracellular Ca^{2+} binding to an EF-hand domain in its N-terminal region, suggesting that the apparent cold sensitivity may result not from direct temperature-sensitive gating but instead from a cold-induced increase in intracellular Ca^{2+} (Doerner *et al*., 2007; Zurborg *et al*., 2007). Against this idea, more recent data show that the response to cold is still seen in the absence of extracellular Ca (Karashima *et al*., 2009).

There is some difference of opinion as to whether TRPA1 does actually fulfil a physiological function as a cold detector (Bautista *et al*., 2006; Kwan *et al*., 2006; 2009), but evidence in favour of this idea has accumulated in recent years (Fajardo *et al*., 2008; Belmonte *et al*., 2009; Karashima *et al*., 2009). In experiments on isolated sensory neurons, several groups have found that a substantial fraction of cold-sensitive DRG neurons does not express either TRPM8 or TRPA1, implying the existence of cold-sensing mechanisms not dependent on either TRPM8 or TRPA1 (Babes *et al*., 2006; Munns *et al*., 2007; Karashima *et al*., 2009). TRPA1-deficient mice were initially found to exhibit normal cold sensitivity (Bautista *et al*., 2006), and double TRPM8/TRPA1 knockout mice are not very different in their responses to cold from TRPM8$^{-/-}$ mice (Knowlton *et al*., 2010); significantly, both still show aversive responses to cold stimuli below 15°C. Other groups have since found that TRPA1-deficient mice do in fact show some deficits in painful cold sensation (Kwan *et al*., 2006; Karashima *et al*., 2009), and TRPA1 does appear to play a role in cold hyperalgesia or allodynia after injuries, because TRPA1 mRNA is up-regulated after tissue inflammation and peripheral nerve injury, and TRPA1 knock-down mice have reduced

cold hyperalgesia (Obata *et al*., 2005; Katsura *et al*., 2006). Taken overall, these experiments agree in showing a role for TRPM8 in mild cold sensation and in some aspects of more extreme cold sensation, and a role for TRPA1 in some aspects of painful cold sensation, but all experiments agree that extreme cold sensation is not completely accounted for by either TRPM8 or TRPA1. The idea that TRPA1 can act as a physiological temperature sensor is given credence by a recent report showing that snake TRPA1 channels are highly temperature-sensitive (Gracheva *et al*., 2010).

There is little doubt, however, that TRPA1 acts as a detector of environmental irritants such as acrolein, a pollutant found in smoke (Bautista *et al*., 2005). TRPA1 is also activated by pungent compounds of the isothiocyanate family, such as mustard oil and allicin from garlic (Bandell *et al*., 2004; Bautista *et al*., 2005). Many TRPA1 agonists irreversibly activate the channel by covalently modifying N-terminal cysteines (Hinman *et al*., 2006; Macpherson *et al*., 2007) but it has recently been shown that non-electrophilic non-steroidal anti-inflammatory drugs can also selectively activate TRPA1 by conventional non-covalent reversible ligand–receptor interactions (Hu *et al*., 2010). Menthol can also activate TRPA1 by a non-covalent action (Karashima *et al*., 2007; Xiao *et al*., 2008). Unlike TRPM8, TRPA1 is found to be expressed exclusively in nociceptive TRPV1 and CGRP-positive neurons, consistent with an exclusive role for this channel as a sensor of noxious stimuli (Dhaka *et al*., 2006). This also raises the possibility that the expression of TRPA1 in some but not all TRPV1-expressing neurons may delineate subsets of polymodal nociceptors (Bautista *et al*., 2006; Kwan *et al*., 2006). TRPA1 is also strongly expressed in hair cells of the inner ear (Corey *et al*., 2004), but TRPA1$^{-/-}$ mice have normal hearing, making a role for TRPA1 in auditory transduction unlikely (Bautista *et al*., 2006; Kwan *et al*., 2006).

Modulation of thermo-TRP ion channels by GPCRs

Many inflammatory mediators released during tissue injury or inflammation, such as PGs, bradykinin, ATP and proteinases, bind to GPCRs and cause downstream activation of PKs such as PKA and PKC. These serine/threonine kinases phosphorylate thermo-TRP ion channels, modulating their thermal thresholds and also influencing their gating by a wide range of agonists.

TRPV1
TRPV1 contains multiple phosphorylation sites for serine/threonine PKs such as PKC, PKA, calcium/calmodulin-dependent kinase (CaMK)II and sarcoma (Src) kinase, all of which regulate TRPV1 activity by a dynamic interplay between receptor phosphorylation and dephosphorylation (Huang *et al*., 2006). Several sites on TRPV1 are known to be phosphorylated by PKC, but only Ser502 and Ser800 play important functional roles in modulation of TRPV1 (Numazaki *et al*., 2002; Bhave *et al*., 2003; Mandadi *et al*., 2006). PKC-dependent phosphorylation of TRPV1 reduces the temperature threshold for TRPV1 activation and potentiates the capsaicin-induced channel open probability (Studer

and McNaughton, 2010). PKA is also important in regulating the sensitivity of TRPV1 to many activators, including heat (Lopshire and Nicol, 1998; Bhave et al., 2002; Rathee et al., 2002). The critical residues have been reported to be Ser116 and Thr370 (Bhave et al., 2002), although more recent evidence shows that Ser502 is also important (Zhang et al., 2008). Recent studies have demonstrated that the scaffolding protein A-kinase anchoring protein (AKAP)79 (AKAP150 in rodents) is critical in phosphorylation of TRPV1 by both PKA and PKC. AKAP79 binds PKA and PKC and recognizes a C-terminal domain on TRPV1, thus bringing the kinases into proximity to TRPV1 to form a signalling complex, without which phosphorylation, and therefore sensitization, is completely abolished (Zhang et al., 2008). In vivo studies have confirmed that PKC-induced sensitization to a thermal stimulus is abrogated in AKAP150 knockout mice (Jeske et al., 2009).

The normal activation threshold of TRPV1 is around 43°C, but following sensitization by phosphorylation, the threshold can fall below body temperature, suggesting that part or all of the pain associated with inflammation may be due to the activation of TRPV1 by the normal body temperature (Cesare and McNaughton, 1996; Premkumar and Ahern, 2000; Vellani et al., 2001; Bhave et al., 2003). There is also a substantial increase in the sensitivity of TRPV1 to protons (Tominaga et al., 1998), which may also contribute to ongoing pain in the presence of elevated H^+ levels in inflamed tissues. The possible involvement of TRPV1 in inflammatory pain has led to a huge effort to develop blockers of TRPV1 as analgesic therapies (discussed later).

TRPV2/3/4

TRPV4 is regulated by Ser/Thr phosphorylation in a basically similar manner to TRPV1. Both PKC and PKA enhance activation of the TRPV4 ion channel by phosphorylation at specific residues (PKC phosphorylates TRPV4 at Ser162, Thr175, and Ser189 and PKA phosphorylates TRPV4 at Ser824), and the phosphorylation depends on the assembly of PKC and PKA by AKAP79 into a signalling complex with TRPV4 (Fan et al., 2009). Xu et al. (2003a,b) suggested that hypotonic stress increases the Tyr phosphorylation level of TRPV4 via Tyr253, while Wegierski et al. (2009) identified Tyr110 to be the critical phosphorylation site of TRPV4 by Src in inflammatory hyperalgesia.

Information on the regulation of TRPV2 and TRPV3 by PKs is very limited. Some evidence suggests a role for PKA in modulation of TRPV2 function by phosphorylation (Stokes et al., 2004) and other studies showed PI3-kinase (PI3K) may regulate TRPV2 gating (Penna et al., 2006). In addition, it has been suggested that TRPV3 activity is enhanced by PLC-dependent GPCR activation, but no in vivo studies have shown a role for TRPV3 in the sensitization of nociceptors (Xu et al., 2006).

TRPM8

In comparison with TRPV1, whose activation is enhanced by phosphorylation, modulation of TRPM8 by PKs appears to function as a negative regulator. Activity of TRPM8 was found to be down-regulated by PKC activation, which has been proposed to initiate dephosphorylation of TRPM8 by activa-

tion of a phosphatase (Premkumar et al., 2005). Recent evidence also shows that TRPM8 is desensitized by phosphorylation by PKA (Linte et al., 2007; Bavencoffe et al., 2010).

TRPA1

It has been suggested that mustard oil activation of TRPA1 is sensitized by bradykinin in a mechanism involving PKA and PLC both in vitro (Wang et al., 2008) and in vivo (Schmidt et al., 2009). PLC-induced degradation of phosphatidylinositol bisphosphate (PIP_2) may reduce desensitization of TRPA1, suggesting that PIP_2 inhibits TRPA1 activity (Dai et al., 2007; Karashima et al., 2008; Kim et al., 2008). Besides bradykinin, protease-activated receptor 2 was also shown to sensitize TRPA1 through PLC/PIP_2 signalling in sensory neurons (Dai et al., 2007). A recent paper has shown that inflammatory signals or acute activation of TRPA1 by mustard oil induces translocation of TRPA1 to the membrane in sensory neurons, and that this incorporation of new channels is PKA- and PLC-dependent (Schmidt et al., 2009). However, the molecular mechanisms mediated by PKA, PLC and PIP_2, and leading to TRPA1 sensitization and translocation, still remain to be elucidated.

Modulation of thermo-TRP ion channels by growth factors

Growth factors such as neurotrophins of the NGF and glial cell line-derived nerve growth factor (GDNF) families typically bind to and activate receptors coupled to downstream TK. Sensory neurons express receptors for many growth factors, and it has become clear that these receptors perform different functions in the developing embryo, where they determine the survival of neurons, and in the adult, where neurons are no longer dependent on growth factors for survival but where their properties are subject to modulation by growth factors released during infection and inflammation, and acting much more like inflammatory mediators than growth factors in the conventional sense (Lewin and Barde, 1996; Farinas, 1999).

NGF

NGF is released from a range of cell types following inflammation or injury (Weskamp and Otten, 1987). Injection of NGF in animals produces a rapid hyperalgesic effect, and also causes a sustained hyperalgesia lasting for days or weeks (Lewin and Mendell, 1993; Lewin et al., 1994; Pezet and McMahon, 2006). Two receptors [Tyr receptor kinase type 1 (TrkA) and p75[NTR]] interact with NGF, but the effect of NGF in thermal hyperalgesia is predominantly mediated via TrkA receptors (McMahon et al., 1994). In rat DRG neurons, TRPV1 is expressed in ~50% of TrkA-positive cells, and ~50% of TRPV1-positive cells express TrkA. TRPM8 is found in ~40% of TrkA-positive cells, and virtually all TRPM8-positive cells express TrkA (Kobayashi et al., 2005).

NGF has a long-term effect in up-regulating TRPV1 expression via the Ras-MAPK pathway. In particular, retrograde transport of NGF from a peripheral site of inflammation acts via p38MAPK activation to increase translation of TRPV1 in the cell body, resulting in an increased transport of

TRPV1 to peripheral terminals (Ji *et al.*, 2002). A short-term effect of NGF is also important, and acts via two distinct signalling pathways present in the nerve terminal (Bonnington and McNaughton, 2003; Zhang *et al.*, 2005). A major pathway is initiated by autophosphorylation of the Tyr760 site of TrkA and, subsequently, the activation of PI3K. The non-receptor TK Src is activated downstream and directly phosphorylates TRPV1 at Tyr200, triggering the translocation of TRPV1 from a subcellular vesicular store and its insertion into the cell surface membrane (Zhang *et al.*, 2005; Stein *et al.*, 2006). The specific Src inhibitor PP2 eliminated both NGF-induced phosphorylation of TRPV1 and translocation to the surface membrane (Zhang *et al.*, 2005). Tyr200 is well conserved among TRPV1s of various species and also other TRPV family members, TRPV2–4, suggesting that Tyr200 may play an important role in regulating the surface membrane expression of other thermo-TRPs. In addition to the PI3K–Src pathway, the PKCε pathway, as outlined above, plays a more minor role in NGF-induced sensitization of TRPV1 (Zhang *et al.*, 2005). In addition, NGF has also been reported to alter the activation threshold of TRPV1 via the p42/44 MAPK pathway (Zhu and Oxford, 2007). NGF was initially proposed to enhance TRPV1 function via activation of PLCγ and consequent depletion of PIP_2, which some experiments suggested acts as an inhibitor of TRPV1 activation (Chuang *et al.*, 2001), but there is now strong evidence against this idea, as follows: (i) all of the regulation of TRPV1 by NGF can be explained by the PIP_2-independent pathways outlined above (Zhang *et al.*, 2005; Zhang and McNaughton, 2006); (ii) the supposed C-terminal TRPV1 modular PIP_2-binding domain thought to underlie the effect (Prescott and Julius, 2003) in fact promotes TRPV1 activation by enhancing by Src-dependent phosphorylation rather than by binding PIP_2 (Zhang *et al.*, 2005); and (iii) PIP_2 actually promotes TRPV1 activation, rather than inhibiting it as required by the original PIP_2 hypothesis (Stein *et al.*, 2006; Sowa *et al.*, 2010).

GDNF family

GDNF was originally purified from the supernatant of a rat glial cell line lysate, and was later found to be synthesized and secreted by a variety of tissues. The four GDNF family members (GDNF, neurturin, artemin and persephin) share the common receptor TK 'rearranged during transfection' (RET), and specificity is conferred by the RET-binding partners GFRα1–4 as follows: GDNF–GFRα1, neurturin–GFRα2, artemin–GFRα3 and persephin–GFRα4 (Airaksinen *et al.*, 1999). Although initial studies of sensory neurotrophic factors focused on their importance for neuronal survival and the regulation of differentiation during development, we now know that, as in the case of NGF, they also regulate functional properties of adult sensory neurons (Stucky *et al.*, 2002; Albers *et al.*, 2006). Like NGF, artemin potentiates TRPV1 activity in over 60% of capsaicin responsive sensory neurons *in vitro* and application of both NGF and artemin potentiate of the TRPV1 response fourfold greater than that seen with either growth factor alone (Malin *et al.*, 2006). When injected into the hind paw of adult mice, artemin produced acute thermal hyperalgesia that lasted for up to 4 h. Using reverse transcriptase-PCR, the mRNA expression of artemin was found to be significantly up-regulated in response to inflammation induced by hindpaw injection of

complete Freund's adjuvant (CFA) (Malin *et al.*, 2006). These experiments show that GDNF family members regulate the sensitivity of thermal nociceptors and moreover that they play a role in inflammatory hyperalgesia.

The mechanism of sensitization of TRPV1 by artemin was investigated by observing the effects of inhibitors of artemin-activated second messenger signalling pathways (unpublished data from our lab). Pharmacological blockade of PKC, PI3K and Src kinase all prevented enhancement of TRPV1 by artemin, whereas inhibition of PKA, MAPK and Akt had no effect. These data support the hypothesis that sensitization of TRPV1 by artemin is mediated by two pathways, much as was the case for NGF. A signalling cascade involving PKC, PI3K and Src kinase and resulting in translocation of TRPV1 to the surface membrane is the major mediator of artemin-induced TRPV1 sensitization, whereas the PLCδ/PKCε signalling pathway plays a more minor role.

Insulin and IGFs

Insulin and IGFs play a pivotal role in regulating nutrient metabolism and maintain vital neuronal functions. Insulin and IGF-I potentiate TRPV1-mediated membrane currents both in DRG neurons and in heterologous expression systems. Enhancement of membrane current is dependent on the activation of PI3K and, as in the case of NGF, results in enhanced translocation of TRPV1 to the plasma membrane (Van Buren *et al.*, 2005). TRPV2 activity is also regulated by IGF, which promotes, via the PI3K pathway, a dynamic and transient translocation of the TRPV2 from the cytosol to the plasma membrane (Kanzaki *et al.*, 1999; Boels *et al.*, 2001). However, some recent data show that PI3K directly enhances TRPV2 activity instead of regulating surface expression of TRPV2 (Penna *et al.*, 2006). Other studies also showed TRPV2 is regulated by insulin in an autocrine manner in pancreatic beta cells (Hisanaga *et al.*, 2009).

SCF

SCF has recently been shown to bind to and activate a receptor TK, c-Kit, in DRG neurons, leading to a reduced thermal threshold and a potentiation of heat-activated currents mediated by TRPV1 in a similar way to the factors discussed in the preceding paragraphs (Milenkovic *et al.*, 2007).

Recycling of TRPV receptors from the cell membrane

Pathways mediating recovery of TRPV channels from the membrane have been little studied. An interesting recent paper (Shukla *et al.*, 2010) showed that internalization of TRPV4 is promoted by β-arrestin, which, following binding of angiotensin to its receptor, is recruited to a complex of TRPV4 and the angiotensin receptor where it binds to and activates a ubiquitin ligase, which ubiquitinates TRPV4 and so mediates its down-regulation.

Thermo-TRP ion channels: therapeutic implications

Members of the thermo-TRP family are attractive targets for novel analgesics effective in a wide range of pathophysiologi-

cal conditions (reviewed in Nilius *et al.*, 2007), but in view of the recent discovery of the channels, the development of clinically effective agonists and antagonists of these ion channels as analgesic compounds is in its infancy. Many potent agonists are present in natural compounds used for herbal and folk remedies and in culinary flavourings, and are often found in over-the-counter analgesic preparations. Examples include the TRPV1 agonist capsaicin, the active ingredient of chilli peppers, which is used in topical creams to stimulate blood flow and thus to relieve mild muscle pain (Hautkappe *et al.*, 1998; Chrubasik *et al.*, 2010); the TRPM8 agonist menthol, used as a topical analgesic (Proudfoot *et al.*, 2006); and agonists of TRPV3, such as camphor, cajuput oil and extracts of oregano, which are used in proprietary topical analgesic preparations.

TRPV1 agonists

Potent TRPV1 agonists, such as capsaicin or resiniferatoxin, produce pain but also profoundly desensitize the receptor (Novakova-Tousova *et al.*, 2007). This inactivation reduces the sensitivity to heat and other ligands and can be used to reduce pain, albeit with the problem of the initial pain produced before desensitization (which can be alleviated by lidocaine pretreatment). Pain reduction was seen in patients with human immunodeficiency virus-associated distal sensory polyneuropathy, in patients with postherpetic neuralgia and in patients with urinary tract inflammation (Simpson *et al.*, 2008; Backonja *et al.*, 2009).

TRPV1 antagonists

Knockout studies provided clear evidence for an involvement of TRPV1 in inflammatory heat hyperalgesia, and pharmacological studies showed some effect of antagonists in reducing the clinically more important inflammatory mechanical hyperalgesia (see earlier discussion). Most efforts to date have therefore focussed on the development of antagonists to this member of the thermo-TRP family. Several TRPV1-selective antagonists have recently been found to be particularly potent and efficacious in acute and chronic inflammatory pain models associated with thermal hyperalgesia (recently reviewed by Szallasi *et al.*, 2007; Gomtsyan and Faltynek, 2010). The behavioural effects of TRPV1 antagonists are in broad agreement with the phenotype observed in TRPV1 knockout mice when challenged with agonists such as capsaicin or inflammatory agents such as carrageenan and CFA. However, in the initial TRPV1 genetic deletion experiments, no change in susceptibility of null mice to neuropathic pain was seen (Caterina *et al.*, 2000), but some TRPV1 antagonists have, in fact, been found to reduce neuropathic pain, for reasons not currently understood. A possible action in counteracting neuropathic pain, a common condition poorly treated by existing pharmaceuticals, has attracted considerable attention. Several TRPV1 antagonists have entered clinical trials, including ABT-102 (Abbott; IC_{50} = 3–4 nM for block of activation by capsaicin and N-arachidonoyl dopamine, 0.7 nM for block of activation by pH 5.5; Gomtsyan *et al.*, 2008), AMG-517 (Amgen; IC_{50} 1 nM for block of activation by capsaicin, acid or heat; Doherty *et al.*, 2007), SB-705498 (GSK), GRC-6211 (Lilly/Glenmark) and MK 2295 (Merck; Crutchlow, 2009).

There are two major drawbacks to the systemic use of TRPV1 antagonists. First, it has become apparent that at least some TRPV1-expressing afferent nerve fibres are partially activated at normal bodily temperature. Blocking TRPV1 removes this ongoing afferent input and, thus, causes a mild hyperthermia (Gavva *et al.*, 2008), but one that could be dangerous in a patient already suffering from hyperthermia for other reasons. Antagonist-induced hyperthermia was not observed in TRPV1 knockout mice, showing that it is an on-target effect, and it is seen with both brain penetrant (AMG8163) and peripherally restricted (AMG1692 and AMG3731) TRPV1 antagonists in rats (Gavva *et al.*, 2007) and with AMG 517 in humans (Gavva *et al.*, 2008), showing that it is a peripheral rather than a central effect. The main site of action in inducing hyperthermia appears to be the viscera, because desensitizing TRPV1 in the viscera by prior application of the potent TRPV1 agonist resiniferatoxin blocks the hyperthermic effect of subsequent antagonist administration (Steiner *et al.*, 2007). Hyperthermia may not be a fatal weakness for the use of TRPV1 antagonists, provided they are administered under careful supervision, as the hyperthermia lessens after a few days (Honore *et al.*, 2009). There have been suggestions that hyperthermia is less obvious with some TRPV1 antagonists than with others, raising the possibility that hyperthermia may arise from a differential block of only one of the three main modes of activation of TRPV1 (Gavva *et al.*, 2007; Lehto *et al.*, 2008; Kym *et al.*, 2009). For instance, if hyperthermia is caused by a block of the action of endogenous agonists on TRPV1, while the relevant modality for analgesia is thermal activation of TRPV1, then an antagonist that blocks thermal but not agonist activation of TRPV1 may get round the problem of hyperthermia. With these thoughts in mind, a number of companies are currently seeking to develop modality-selective antagonists (Wong and Gavva, 2009).

Secondly, the threshold for detection of noxious heat is somewhat elevated following genetic deletion of TRPV1 in mice (Caterina *et al.*, 2000; Davis *et al.*, 2000). In humans treated with TRPV1 antagonists, the thermal threshold is also elevated, although the elevation seems to be more pronounced for some antagonists than for others (Chizh *et al.*, 2007; Crutchlow, 2009). The elevation in thermal threshold raises the possible complication that block of TRPV1 may make patients susceptible to accidental burns.

Modulation of TRPV1 threshold

The problems arising from a direct block of TRPV1 suggest that an indirect action on the modulation of TRPV1 may be a more fruitful avenue to explore. Inflammatory mediators cause heat hyperalgesia by lowering the thermal threshold of TRPV1 (Cesare and McNaughton, 1996; Tominaga *et al.*, 1998; Vellani *et al.*, 2001), raising the interesting possibility that hyperalgesia could be counteracted without affecting the normal thermal threshold of TRPV1 by blocking processes leading to its sensitization. The scaffolding protein AKAP79 has recently been shown to be crucial for the actions of PKA and PKC by binding to TRPV1 and promoting its sensitization by anchoring these kinases adjacent to critical phosphorylation sites on TRPV1 (Jeske *et al.*, 2008; 2009; Schnizler *et al.*, 2008; Zhang *et al.*, 2008). The interaction between AKAP79/150 and TRPV1 occurs at a small region in the TRPV1 C-terminal cytoplasmic tail, and the introduction of a

peptide with the same sequence is able to displace AKAP79/150 from TRPV1 and, thus, completely switch off sensitization by inflammatory mediators acting through both PKA and PKC (Zhang *et al.*, 2008). This finding suggests that small-molecule antagonists could be developed that would antagonize binding between AKAP79/150 in a similar manner, and thus abolish heat hyperalgesia without affecting the underlying heat sensitivity of TRPV1.

Analgesia using TRPV1 as a Trojan horse

TRPV1 shows a time and agonist-dependent increase in permeability to large cations owing to changes in the TRPV1 selectivity filter that reflect increases in pore diameter (Chung *et al.*, 2008). This property allows cationic molecules with molecular masses up to 500 Da to enter the cell through TRPV1. In an exciting recent development, it has been shown that QX-314, a charged quaternary amine derivative of the local anaesthetic lidocaine (ineffective in blocking sodium channels when administered extracellularly because it cannot gain access to the inner face of the channels) can produce a long-lasting analgesia specific to TRPV1-expressing neurones when applied in conjunction with a TRPV1 agonist (Binshtok *et al.*, 2007). To use the technology successfully in patients, a non-pungent activator of TRPV1 would be ideal to minimize patient discomfort until sodium channel blockade is achieved on entry of the charged membrane-impermeant sodium channel blocker, and this requirement appears to be met by the use of the local anaesthetic lidocaine (Binshtok *et al.*, 2009; Roberson *et al.*, 2011). This simple but elegant idea is currently being tested as a possible basis for the potential development of next generation local anaesthetics.

TRPM8

TRPM8 is often thought of as an ion channel giving rise only to non-painful sensations but it is co-expressed with TRPV1 in some sensory neurones and there is evidence that TRPM8 channels may play a role in noxious cold sensation and cold allodynia (Proudfoot *et al.*, 2006; Fleetwood-Walker *et al.*, 2007; Belmonte *et al.*, 2009). The possibility that antagonists of TRPM8 may have analgesic effects in particular situations, for example in the cornea where TRPM8 is the dominant thermo-TRP expressed in sensory nerve endings, deserves further investigation. Recently it has been shown that several TRPM8 antagonist compounds act as negative allosteric modulators of channel gating, shifting rather than abolishing activation by voltage and cold (Malkia *et al.*, 2007).

TRPA1

TRPA1 is specifically expressed in a subclass of TRPV1-expressing nociceptors and is activated by both noxious cold (Story *et al.*, 2003) and by a wide range of environmental irritants (Bautista *et al.*, 2006). NGF evokes the functional up-regulation of TRPA1 (Obata *et al.*, 2005; Diogenes *et al.*, 2007), and various knock-down or knockout studies in rodents have demonstrated reduced nociceptive behaviour in a variety of pain models (Bautista *et al.*, 2006; Kwan *et al.*, 2006; McNamara *et al.*, 2007) and attenuated airway inflammation in models of asthma (Caceres *et al.*, 2009). Cold and pollutants, such as those present in smog, can cause cough and reflex bronchconstriction in asthmatics (Colsoul *et al.*,

2009), and a number of TRPA1 antagonists are currently under investigation as antitussives and to counteract airway constriction (reviewed by Viana and Ferrer-Montiel, 2009). Pharmacological inhibition of TRPA1 significantly attenuated CFA-induced mechanical hypersensitivity in wild-type but not TRPA1 knockout mice (Petrus *et al.*, 2007). A selective TRPA1 antagonist, HC-030031 was effective in reducing leucocyte infiltration and airway hyperreactivity in a model of lung inflammation (Caceres *et al.*, 2009) and pain behaviours in inflammatory and neuropathic models (Eid *et al.*, 2008).

Conclusion

Several TRPV1 antagonists are in advanced clinical trials and it seems likely that at least some of these will enter the clinic. The two main problems currently identified are hyperthermia, which could lead to dangerously elevated core temperatures particularly if combined with a pre-existing pyrexia, and reduced sensitivity to painful or damaging heat stimuli, which could lead to damaging accidental burns. The bright spot is that these features do not seem to be invariable accompaniments of TRPV1 antagonism – some antagonists cause less hyperthermia than others, and, in a similar way, the elevation of heat threshold seems to be less pronounced for some antagonists than for others. Several companies are working to develop antagonists that will selectively block some modes of activation of TRPV1 while sparing others, and these may provide useful solutions to the problems faced by antagonists that block all modes with approximately equal affinity. This search is currently proceeding in a heuristic manner, but it could be assisted by gaining a better understanding at a fundamental level of how the different modes of activation lead to channel opening. Do all modes of activation act simply as triggers of a single pathway leading to channel opening or do the different pathways of activation converge on channel opening at a late stage? Recent evidence suggests that it is possible to separate activation of TRPV1 by heat, which produces structural changes in the extracellular domain adjacent to the pore region, from activation by agonists that activate the channel without producing these structural changes (Grandl *et al.*, 2008; Yang *et al.*, 2010).

Antagonists for other thermo-TRPs are at earlier stages of development. The most promising target is likely to be TRPA1, which is exclusively expressed in a subset of nociceptive nerve endings. This thermo-TRP is a highly sensitive detector of environmental irritants, such as are present in smog, and of cold, and its activation probably contributes to the brochospasm that many asthmatics experience as a particularly distressing symptom on cold, smoggy mornings. TRPA1 antagonists, either oral or inhaled along with antiasthmatic pharmaceuticals, may provide effective relief.

Direct roles for other thermo-TRPs in pain are less well established. The cool receptor TRPM8 is typically expressed in non-nociceptive sensory neurons and its activation has an analgesic action, as is exploited by many folk and non-prescription remedies. In some sensory neurons (for instance in the cornea) it is co-expressed with TRPV1 and, therefore, is likely to serve a nociceptive function. Antagonists may therefore have value as analgesics in (for instance) eye drops to treat the dry eye, which is often an after effect of corneal

surgery. TRPV3, in a similar way, is activated by non-noxious warm temperatures and seems largely to have an analgesic function, and is exploited by traditional anti-inflammatory preparations. There have been some preliminary indications that TRPV3 may also contribute to inflammatory heat pain, and if this is confirmed, then antagonists may be of value. Little is known about whether the other thermo-TRPs play important roles in inflammatory pain, though knockout studies and development of antagonists are currently being reported, and the picture will no doubt become clearer in the near future.

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Conflict of interest

None of the authors has any conflict of interest.

References

Airaksinen MS, Titievsky A, Saarma M (1999). GDNF family neurotrophic factor signaling: four masters, one servant? Mol Cell Neurosci 13: 313–325.

Albers KM, Woodbury CJ, Ritter AM, Davis BM, Koerber HR (2006). Glial cell-line-derived neurotrophic factor expression in skin alters the mechanical sensitivity of cutaneous nociceptors. J Neurosci 26: 2981–2990.

Alessandri-Haber N, Joseph E, Dina OA, Liedtke W, Levine JD (2005). TRPV4 mediates pain-related behavior induced by mild hypertonic stimuli in the presence of inflammatory mediator. Pain 118: 70–79.

Babes A, Zorzon D, Reid G (2004). Two populations of cold-sensitive neurons in rat dorsal root ganglia and their modulation by nerve growth factor. Eur J Neurosci 20: 2276–2282.

Babes A, Zorzon D, Reid G (2006). A novel type of cold-sensitive neuron in rat dorsal root ganglia with rapid adaptation to cooling stimuli. Eur J Neurosci 24: 691–698.

Backonja MM, Walk D, Edwards RR, Sehgal N, Moeller-Bertram T, Wasan A et al. (2009). Quantitative sensory testing in measurement of neuropathic pain phenomena and other sensory abnormalities. Clin J Pain 25: 641–647.

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ et al. (2004). Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41: 849–857.

Bautista DM, Movahed P, Hinman A, Axelsson HE, Sterner O, Hogestatt ED et al. (2005). Pungent products from garlic activate the sensory ion channel TRPA1. Proc Natl Acad Sci USA 102: 12248–12252.

Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J et al. (2006). TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 124: 1269–1282.

Bautista DM, Siemens J, Glazer JM, Tsuruda PR, Basbaum AI, Stucky CL et al. (2007). The menthol receptor TRPM8 is the principal detector of environmental cold. Nature 448: 204–208.

Bavencoffe A, Gkika D, Kondratskyi A, Beck B, Borowiec AS, Bidaux G et al. (2010). The transient receptor potential channel TRPM8 is inhibited via the alpha 2A adrenoreceptor signaling pathway. J Biol Chem 285: 9410–9419.

Belmonte C, Brock JA, Viana F (2009). Converting cold into pain. Exp Brain Res 196: 13–30.

Bender FL, Mederos YS, Li Y, Ji A, Weihe E, Gudermann T et al. (2005). The temperature-sensitive ion channel TRPV2 is endogenously expressed and functional in the primary sensory cell line F-11. Cell Physiol Biochem 15: 183–194.

Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RW (2002). cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 35: 721–731.

Bhave G, Hu HJ, Glauner KS, Zhu W, Wang H, Brasier DJ et al. (2003). Protein kinase C phosphorylation sensitizes but does not activate the capsaicin receptor transient receptor potential vanilloid 1 (TRPV1). Proc Natl Acad Sci USA 100: 12480–12485.

Binshtok AM, Bean BP, Woolf CJ (2007). Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. Nature 449: 607–610.

Binshtok AM, Gerner P, Oh SB, Puopolo M, Suzuki S, Roberson DP et al. (2009). Coapplication of lidocaine and the permanently charged sodium channel blocker QX-314 produces a long-lasting nociceptive blockade in rodents. Anesthesiology 111: 127–137.

Boels K, Glassmeier G, Herrmann D, Riedel IB, Hampe W, Kojima I et al. (2001). The neuropeptide head activator induces activation and translocation of the growth-factor-regulated Ca(2+)-permeable channel GRC. J Cell Sci 114: 3599–3606.

Bonnington JK, McNaughton PA (2003). Signalling pathways involved in the sensitisation of mouse nociceptive neurones by nerve growth factor. J Physiol 551: 433–446.

Caceres AI, Brackmann M, Elia MD, Bessac BF, del Camino D, D'Amours M et al. (2009). A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. Proc Natl Acad Sci USA 106: 9099–9104.

Camprubi-Robles M, Planells-Cases R, Ferrer-Montiel A (2009). Differential contribution of SNARE-dependent exocytosis to inflammatory potentiation of TRPV1 in nociceptors. FASEB J 23: 3722–3733.

Caterina MJ (2003). Vanilloid receptors take a TRP beyond the sensory afferent. Pain 105: 5–9.

Caterina MJ, Julius D (1999). Sense and specificity: a molecular identity for nociceptors. Curr Opin Neurobiol 9: 525–530.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997). The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389: 816–824.

Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D (1999). A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 398: 436–441.

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR et al. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288: 306–313.

Cesare P, McNaughton PA (1996). A novel heat-activated current in nociceptive neurons, and its sensitization by bradykinin. Proc Natl Acad Sci USA 93: 15435–15439.

Chizh BA, O'Donnell MB, Napolitano A, Wang J, Brooke AC, Aylott MC et al. (2007). The effects of the TRPV1 antagonist SB-705498 on TRPV1 receptor-mediated activity and inflammatory hyperalgesia in humans. Pain 132: 132–141.

Chrubasik S, Weiser T, Beime B (2010). Effectiveness and safety of topical capsaicin cream in the treatment of chronic soft tissue pain. Phytother Res 24: 1877–1885.

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI et al. (2001). Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411: 957–962.

Chuang HH, Neuhausser WM, Julius D (2004). The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. Neuron 43: 859–869.

Chung MK, Guler AD, Caterina MJ (2008). TRPV1 shows dynamic ionic selectivity during agonist stimulation. Nat Neurosci 11: 555–564.

Colburn RW, Lubin ML, Stone DJ Jr, Wang Y, Lawrence D, D'Andrea MR et al. (2007). Attenuated cold sensitivity in TRPM8 null mice. Neuron 54: 379–386.

Colsoul B, Nilius B, Vennekens R (2009). On the putative role of transient receptor potential cation channels in asthma. Clin Exp Allergy 39: 1456–1466.

Corey DP, Garcia-Anoveros J, Holt JR, Kwan KY, Lin SY, Vollrath MA et al. (2004). TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. Nature 432: 723–730.

Crutchlow M Pharmacologic inhibition of TRPV1 impairs sensation of potentially injurious heat in healthy subjects. ASCPT 2009 annual meeting, 69. 2009.

Dai Y, Wang S, Tominaga M, Yamamoto S, Fukuoka T, Higashi T et al. (2007). Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. J Clin Invest 117: 1979–1987.

Davis AJ, Perkins MN (1996). Substance P and capsaicin-induced mechanical hyperalgesia in the rat knee joint; the involvement of bradykinin B1 and B2 receptors. Br J Pharmacol 118: 2206–2212.

Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P et al. (2000). Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405: 183–187.

De la Pena E, Malkia A, Cabedo H, Belmonte C, Viana F (2005). The contribution of TRPM8 channels to cold sensing in mammalian neurones. J Physiol 567: 415–426.

Dhaka A, Viswanath V, Patapoutian A (2006). Trp ion channels and temperature sensation. Annu Rev Neurosci 29: 135–161.

Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A (2007). TRPM8 is required for cold sensation in mice. Neuron 54: 371–378.

Diogenes A, Akopian AN, Hargreaves KM (2007). NGF up-regulates TRPA1: implications for orofacial pain. J Dent Res 86: 550–555.

Doerner JF, Gisselmann G, Hatt H, Wetzel CH (2007). Transient receptor potential channel A1 is directly gated by calcium ions. J Biol Chem 282: 13180–13189.

Doherty EM, Fotsch C, Bannon AW, Bo Y, Chen N, Dominguez C et al. (2007). Novel vanilloid receptor-1 antagonists: 2. Structure-activity relationships of 4-oxopyrimidines leading to the selection of a clinical candidate. J Med Chem 50: 3515–3527.

Eid SR, Crown ED, Moore EL, Liang HA, Choong KC, Dima S et al. (2008). HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. Mol Pain 4: 48.

Fajardo O, Meseguer V, Belmonte C, Viana F (2008). TRPA1 channels mediate cold temperature sensing in mammalian vagal sensory neurons: pharmacological and genetic evidence. J Neurosci 28: 7863–7875.

Fan HC, Zhang X, McNaughton PA (2009). Activation of the TRPV4 ion channel is enhanced by phosphorylation. J Biol Chem 284: 27884–27891.

Farinas I (1999). Neurotrophin actions during the development of the peripheral nervous system. Microsc Res Tech 45: 233–242.

Fleetwood-Walker SM, Proudfoot CW, Garry EM, Allchorne A, Vinuela-Fernandez I, Mitchell R (2007). Cold comfort pharm. Trends Pharmacol Sci 28: 621–628.

Frederick J, Buck ME, Matson DJ, Cortright DN (2007). Increased TRPA1, TRPM8, and TRPV2 expression in dorsal root ganglia by nerve injury. Biochem Biophys Res Commun 358: 1058–1064.

Gaudet R (2009). Divide and conquer: high resolution structural information on TRP channel fragments. J Gen Physiol 133: 231–237.

Gavva NR, Bannon AW, Surapaneni S, Hovland DN Jr, Lehto SG, Gore A et al. (2007). The vanilloid receptor TRPV1 is tonically activated in vivo and involved in body temperature regulation. J Neurosci 27: 3366–3374.

Gavva NR, Treanor JJ, Garami A, Fang L, Surapaneni S, Akrami A et al. (2008). Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain 136: 202–210.

Gevaert T, Vriens J, Segal A, Everaerts W, Roskams T, Talavera K et al. (2007). Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. J Clin Invest 117: 3453–3462.

Gomtsyan A, Faltynek CR (2010). Vanilloid Receptor TRPV1 in Drug Discovery. John Wiley & Sons: Hoboken, NJ.

Gomtsyan A, Bayburt EK, Schmidt RG, Surowy CS, Honore P, Marsh KC et al. (2008). Identification of (R)-1-(5-tert-butyl-2, 3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)urea (ABT-102) as a potent TRPV1 antagonist for pain management. J Med Chem 51: 392–395.

Gracheva EO, Ingolia NT, Kelly YM, Cordero-Morales JF, Hollopeter G, Chesler AT et al. (2010). Molecular basis of infrared detection by snakes. Nature 464: 1006–1011.

Grandl J, Hu H, Bandell M, Bursulaya B, Schmidt M, Petrus M et al. (2008). Pore region of TRPV3 ion channel is specifically required for heat activation. Nat Neurosci 11: 1007–1013.

Hautkappe M, Roizen MF, Toledano A, Roth S, Jeffries JA, Ostermeier AM (1998). Review of the effectiveness of capsaicin for painful cutaneous disorders and neural dysfunction. Clin J Pain 14: 97–106.

Hinman A, Chuang HH, Bautista DM, Julius D (2006). TRP channel activation by reversible covalent modification. Proc Natl Acad Sci USA 103: 19564–19568.

Hisanaga E, Nagasawa M, Ueki K, Kulkarni RN, Mori M, Kojima I (2009). Regulation of calcium-permeable TRPV2 channel by insulin in pancreatic beta-cells. Diabetes 58: 174–184.

Honore P, Chandran P, Hernandez G, Gauvin DM, Mikusa JP, Zhong C et al. (2009). Repeated dosing of ABT-102, a potent and selective TRPV1 antagonist, enhances TRPV1-mediated analgesic activity in rodents, but attenuates antagonist-induced hyperthermia. Pain 142: 27–35.

Hu HZ, Gu Q, Wang C, Colton CK, Tang J, Kinoshita-Kawada M et al. (2004). 2-aminoethoxydiphenyl borate is a common activator of TRPV1, TRPV2, and TRPV3. J Biol Chem 279: 35741–35748.

Hu H, Tian J, Zhu Y, Wang C, Xiao R, Herz JM et al. (2010). Activation of TRPA1 channels by fenamate nonsteroidal anti-inflammatory drugs. Pflugers Arch 459: 579–592.

Hua XY, Chen P, Hwang J, Yaksh TL (1997). Antinociception induced by civamide, an orally active capsaicin analogue. Pain 71: 313–322.

Huang J, Zhang X, McNaughton PA (2006). Modulation of temperature-sensitive TRP channels. Semin Cell Dev Biol 17: 638–645.

Huang SM, Lee H, Chung MK, Park U, Yu YY, Bradshaw HB et al. (2008). Overexpressed transient receptor potential vanilloid 3 ion channels in skin keratinocytes modulate pain sensitivity via prostaglandin E2. J Neurosci 28: 13727–13737.

Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J et al. (2000). Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc Natl Acad Sci USA 97: 6155–6160.

Jeske NA, Diogenes A, Ruparel NB, Fehrenbacher JC, Henry M, Akopian AN et al. (2008). A-kinase anchoring protein mediates TRPV1 thermal hyperalgesia through PKA phosphorylation of TRPV1. Pain 138: 604–616.

Jeske NA, Patwardhan AM, Ruparel NB, Akopian AN, Shapiro MS, Henry MA (2009). A-kinase anchoring protein 150 controls protein kinase C-mediated phosphorylation and sensitization of TRPV1. Pain 146: 301–307.

Ji RR, Samad TA, Jin SX, Schmoll R, Woolf CJ (2002). p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 36: 57–68.

Jung J, Sun WH, Kwak J, Lee S-Y, Kang C-J, Won BK et al. (1999). Capsaicin binds to the intracellular domain of the capsaicin-activated ion channel. J Neurosci 19: 529–538.

Kanzaki M, Zhang YQ, Mashima H, Li L, Shibata H, Kojima I (1999). Translocation of a calcium-permeable cation channel induced by insulin-like growth factor-I. Nat Cell Biol 1: 165–170.

Karashima Y, Damann N, Prenen J, Talavera K, Segal A, Voets T et al. (2007). Bimodal action of menthol on the transient receptor potential channel TRPA1. J Neurosci 27: 9874–9884.

Karashima Y, Prenen J, Meseguer V, Owsianik G, Voets T, Nilius B (2008). Modulation of the transient receptor potential channel TRPA1 by phosphatidylinositol 4,5-biphosphate manipulators. Pflugers Arch 457: 77–89.

Karashima Y, Talavera K, Everaerts W, Janssens A, Kwan KY, Vennekens R et al. (2009). TRPA1 acts as a cold sensor in vitro and in vivo. Proc Natl Acad Sci USA 106: 1273–1278.

Katsura H, Obata K, Mizushima T, Yamanaka H, Kobayashi K, Dai Y et al. (2006). Antisense knock down of TRPA1, but not TRPM8, alleviates cold hyperalgesia after spinal nerve ligation in rats. Exp Neurol 200: 112–123.

Kim D, Cavanaugh EJ, Simkin D (2008). Inhibition of transient receptor potential A1 channel by phosphatidylinositol-4, 5-bisphosphate. Am J Physiol Cell Physiol 295: C92–C99.

Knowlton WM, Bifolck-Fisher A, Bautista DM, McKemy DD (2010). TRPM8, but not TRPA1, is required for neural and behavioral responses to acute noxious cold temperatures and cold-mimetics in vivo. Pain 150: 340–350.

Kobayashi K, Fukuoka T, Obata K, Yamanaka H, Dai Y, Tokunaga A et al. (2005). Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. J Comp Neurol 493: 596–606.

Koerber HR, McIlwrath SL, Lawson JJ, Malin SA, Anderson CE, Jankowski MP et al. (2010). Cutaneous C-polymodal fibers lacking TRPV1 are sensitized to heat following inflammation, but fail to drive heat hyperalgesia in the absence of TPV1 containing C-heat fibers. Mol Pain 6: 58.

Kwak JY, Jung JY, Hwang SW, Lee WT, Oh U (1998). A capsaicin-receptor antagonist, capsazepine, reduces inflammation-induced hyperalgesic responses in the rat: evidence for an endogenous capsaicin-like substance. Neuroscience 86: 619–626.

Kwan KY, Allchorne AJ, Vollrath MA, Christensen AP, Zhang DS, Woolf CJ et al. (2006). TRPA1 contributes to cold, mechanical, and chemical nociception but is not essential for hair-cell transduction. Neuron 50: 277–289.

Kwan KY, Glazer JM, Corey DP, Rice FL, Stucky CL (2009). TRPA1 modulates mechanotransduction in cutaneous sensory neurons. J Neurosci 29: 4808–4819.

Kym PR, Kort ME, Hutchins CW (2009). Analgesic potential of TRPV1 antagonists. Biochem Pharmacol 78: 211–216.

Lawson JJ, McIlwrath SL, Woodbury CJ, Davis BM, Koerber HR (2008). TRPV1 unlike TRPV2 is restricted to a subset of mechanically insensitive cutaneous nociceptors responding to heat. J Pain 9: 298–308.

Lechner SG, Frenzel H, Wang R, Lewin GR (2009). Developmental waves of mechanosensitivity acquisition in sensory neuron subtypes during embryonic development. EMBO J 28: 1479–1491.

Lee H, Iida T, Mizuno A, Suzuki M, Caterina MJ (2005). Altered thermal selection behavior in mice lacking transient receptor potential vanilloid 4. J Neurosci 25: 1304–1310.

Lehto SG, Tamir R, Deng H, Klionsky L, Kuang R, Le A et al. (2008). Antihyperalgesic effects of (R,E)-N-(2-hydroxy-2,3-dihydro-1H-inden-4-yl)-3-(2-(piperidin-1-yl)-4-(tri fluoromethyl)phenyl)-acrylamide (AMG8562), a novel transient receptor potential vanilloid type 1 modulator that does not cause hyperthermia in rats. J Pharmacol Exp Ther 326: 218–229.

Lewin GR, Barde YA (1996). Physiology of the neurotrophins. Annu Rev Neurosci 19: 289–317.

Lewin GR, Mendell LM (1993). Nerve growth factor and nociception. Trends Neurosci 16: 353–359.

Lewin GR, Rueff A, Mendell LM (1994). Peripheral and central mechanisms of NGF-induced hyperalgesia. European. J Neurosci 6: 1903–1912.

Link TM, Park U, Vonakis BM, Raben DM, Soloski MJ, Caterina MJ (2010). TRPV2 has a pivotal role in macrophage particle binding and phagocytosis. Nat Immunol 11: 232–239.

Linte RM, Ciobanu C, Reid G, Babes A (2007). Desensitization of cold- and menthol-sensitive rat dorsal root ganglion neurones by inflammatory mediators. Exp Brain Res 178: 89–98.

Lopshire JC, Nicol GD (1998). The cAMP transduction cascade mediates the prostaglandin E2 enhancement of the capsaicin-elicited current in rat sensory neurons: whole-cell and single-channel studies. J Neurosci 18: 6081–6092.

McKemy DD, Neuhausser WM, Julius D (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 416: 52–58.

McMahon SB, Armanini MP, Ling LH, Phillips HS (1994). Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. Neuron 12: 1161–1171.

McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M et al. (2007). TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci USA 104: 13525–13530.

Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF et al. (2007). Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. Nature 445: 541–545.

Malin SA, Molliver DC, Koerber HR, Cornuet P, Frye R, Albers KM et al. (2006). Glial cell line-derived neurotrophic factor family members sensitize nociceptors in vitro and produce thermal hyperalgesia in vivo. J Neurosci 26: 8588–8599.

Malkia A, Madrid R, Meseguer V, De La PE, Valero M, Belmonte C et al. (2007). Bidirectional shifts of TRPM8 channel gating by temperature and chemical agents modulate the cold sensitivity of mammalian thermoreceptors. J Physiol 581: 155–174.

Mandadi S, Tominaga T, Numazaki M, Murayama N, Saito N, Armati PJ et al. (2006). Increased sensitivity of desensitized TRPV1 by PMA occurs through PKCepsilon-mediated phosphorylation at S800. Pain 123: 106–116.

Mandadi S, Sokabe T, Shibasaki K, Katanosaka K, Mizuno A, Moqrich A et al. (2009). TRPV3 in keratinocytes transmits temperature information to sensory neurons via ATP. Pflugers Arch 458: 1093–1102.

Milenkovic N, Frahm C, Gassmann M, Griffel C, Erdmann B, Birchmeier C et al. (2007). Nociceptive tuning by stem cell factor/c-Kit signaling. Neuron 56: 893–906.

Moiseenkova-Bell VY, Wensel TG (2009). Hot on the trail of TRP channel structure. J Gen Physiol 133: 239–244.

Montell C (2005). The TRP superfamily of cation channels. Sci STKE 272: re3.

Moqrich A, Hwang SW, Earley TJ, Petrus MJ, Murray AN, Spencer KS et al. (2005). Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science 307: 1468–1472.

Moriyama T, Iida T, Kobayashi K, Higashi T, Fukuoka T, Tsumura H et al. (2003). Possible involvement of P2Y2 metabotropic receptors in ATP-induced transient receptor potential vanilloid receptor 1-mediated thermal hypersensitivity. J Neurosci 23: 6058–6062.

Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y et al. (2005). Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. Mol Pain 1: 3.

Munns C, Alqatari M, Koltzenburg M (2007). Many cold sensitive peripheral neurons of the mouse do not express TRPM8 or TRPA1. Cell Calcium 41: 331–342.

Muraki K, Iwata Y, Katanosaka Y, Ito T, Ohya S, Shigekawa M et al. (2003). TRPV2 is a component of osmotically sensitive cation channels in murine aortic myocytes. Circ Res 93: 829–838.

Nagy I, Rang H (1999a). Noxious heat activates all capsaicin-sensitive and also a sub-population of capsaicin-insensitive dorsal root ganglion neurons. Neuroscience 88: 995–997.

Nagy I, Rang HP (1999b). Similarities and differences between the responses of rat sensory neurons to noxious heat and capsaicin. J Neurosci 19: 10647–10655.

Nilius B, Voets T (2005). TRP channels: a TR(I)P through a world of multifunctional cation channels. Pflugers Arch 451: 1–10.

Nilius B, Owsianik G, Voets T, Peters JA (2007). Transient receptor potential cation channels in disease. Physiol Rev 87: 165–217.

Novakova-Tousova K, Vyklicky L, Susankova K, Benedikt J, Samad A, Teisinger J et al. (2007). Functional changes in the vanilloid receptor subtype 1 channel during and after acute desensitization. Neuroscience 149: 144–154.

Numazaki M, Tominaga T, Toyooka H, Tominaga M (2002). Direct phosphorylation of capsaicin receptor VR1 by protein kinase Cepsilon and identification of two target serine residues. J Biol Chem 277: 13375–13378.

Obata K, Katsura H, Mizushima T, Yamanaka H, Kobayashi K, Dai Y et al. (2005). TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. J Clin Invest 115: 2393–2401.

Oh U, Sun WH, Kim D (1996). Capsaicin activates a nonselective cation channel in cultured neonatal rat dorsal root ganglion neurons. J Neurosci 16: 1659–1667.

Okazawa M, Inoue W, Hori A, Hosokawa H, Matsumura K, Kobayashi S (2004). Noxious heat receptors present in cold-sensory cells in rats. Neurosci Lett 359: 33–36.

Park U, Vastani N, Guan Y, Srinivasa NR, Koltzenburg M, Caterina MJ (2011). TRP Vanilloid 2 knock-out mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. J Neurosci 31: 11425–11436.

Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM et al. (2002a). A TRP channel that senses cold stimuli and menthol. Cell 108: 705–715.

Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, Hergarden AC et al. (2002b). A heat-sensitive TRP channel expressed in keratinocytes. Science 296: 2046–2049.

Penna A, Juvin V, Chemin J, Compan V, Monet M, Rassendren FA (2006). PI3-kinase promotes TRPV2 activity independently of channel translocation to the plasma membrane. Cell Calcium 39: 495–507.

Petrus M, Peier AM, Bandell M, Hwang SW, Huynh T, Olney N et al. (2007). A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. Mol Pain 3: 40.

Pezet S, McMahon SB (2006). Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci 29: 507–538.

Plant TD, Zollner C, Kepura F, Mousa SS, Eichhorst J, Schaefer M et al. (2007). Endothelin potentiates TRPV1 via ETA receptor-mediated activation of protein kinase C. Mol Pain 3: 35.

Premkumar LS, Ahern GP (2000). Induction of vanilloid receptor channel activity by protein kinase C. Nature 408: 985–990.

Premkumar LS, Raisinghani M, Pingle SC, Long C, Pimentel F (2005). Downregulation of transient receptor potential melastatin 8 by protein kinase C-mediated dephosphorylation. J Neurosci 25: 11322–11329.

Prescott ED, Julius D (2003). A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. Science 300: 1284–1288.

Proudfoot CJ, Garry EM, Cottrell DF, Rosie R, Anderson H, Robertson DC et al. (2006). Analgesia mediated by the TRPM8 cold receptor in chronic neuropathic pain. Curr Biol 16: 1591–1605.

Qin N, Neeper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM (2008). TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. J Neurosci 28: 6231–6238.

Rathee PK, Distler C, Obreja O, Neuhuber W, Wang GK, Wang SY et al. (2002). PKA/AKAP/VR-1 module: a common link of Gs-mediated signaling to thermal hyperalgesia. J Neurosci 22: 4740–4745.

Reid G, Babes A, Pluteanu F (2002). A cold- and menthol-activated current in rat dorsal root ganglion neurones: properties and role in cold transduction. J Physiol 545: 595–614.

Roberson D, Binshtok A, Blasl F, Bean B, Woolf C (2011). Targeting of sodium channel blockers into nociceptors to produce long-duration analgesia: a systematic study and review. Br J Pharmacol 164: 48–58.

Ross RA (2003). Anandamide and vanilloid TRPV1 receptors. Br J Pharmacol 140: 790–801.

Schmidt M, Dubin AE, Petrus MJ, Earley TJ, Patapoutian A (2009). Nociceptive signals induce trafficking of TRPA1 to the plasma membrane. Neuron 64: 498–509.

Schnizler K, Shutov LP, Van Kanegan MJ, Merrill MA, Nichols B, McKnight GS et al. (2008). Protein kinase A anchoring via AKAP150 is essential for TRPV1 modulation by forskolin and prostaglandin E2 in mouse sensory neurons. J Neurosci 28: 4904–4917.

Sherrington CS (1906). The Integrative Action of the Nervous System. Scribner: New York.

Shimosato G, Amaya F, Ueda M, Tanaka Y, Decosterd I, Tanaka M (2005). Peripheral inflammation induces up-regulation of TRPV2 expression in rat DRG. Pain 119: 225–232.

Shukla AK, Kim J, Ahn S, Xiao K, Shenoy SK, Liedtke W et al. (2010). Arresting a transient receptor potential (TRP) channel: beta-arrestin 1 mediates ubiquitination and functional down-regulation of TRPV4. J Biol Chem 285: 30115–30125.

Simpson DM, Brown S, Tobias J (2008). Controlled trial of high-concentration capsaicin patch for treatment of painful HIV neuropathy. Neurology 70: 2305–2313.

Smith GD, Gunthorpe MJ, Kelsell RE, Hayes PD, Reilly P, Facer P et al. (2002). TRPV3 is a temperature-sensitive vanilloid receptor-like protein. Nature 418: 186–190.

Smith PL, Maloney KN, Pothen RG, Clardy J, Clapham DE (2006). Bisandrographolide from Andrographis paniculata activates TRPV4 channels. J Biol Chem 281: 29897–29904.

Sowa NA, Street SE, Vihko P, Zylka MJ (2010). Prostatic acid phosphatase reduces thermal sensitivity and chronic pain sensitization by depleting phosphatidylinositol 4,5-bisphosphate. J Neurosci 30: 10282–10293.

Staaf S, Franck MC, Marmigere F, Mattsson JP, Ernfors P (2010). Dynamic expression of the TRPM subgroup of ion channels in developing mouse sensory neurons. Gene Expr Patterns 10: 65–74.

Stein AT, Ufret-Vincenty CA, Hua L, Santana LF, Gordon SE (2006). Phosphoinositide 3-Kinase Binds to TRPV1 and Mediates NGF-stimulated TRPV1 Trafficking to the Plasma Membrane. J Gen Physiol 128: 509–522.

Steiner AA, Turek VF, Almeida MC, Burmeister JJ, Oliveira DL, Roberts JL et al. (2007). Nonthermal activation of transient receptor potential vanilloid-1 channels in abdominal viscera tonically inhibits autonomic cold-defense effectors. J Neurosci 27: 7459–7468.

Stokes AJ, Shimoda LM, Koblan-Huberson M, Adra CN, Turner H (2004). A TRPV2-PKA signaling module for transduction of physical stimuli in mast cells. J Exp Med 200: 137–147.

Story GM, Peier AM, Reeve AJ, Eid SR, Mosbacher J, Hricik TR et al. (2003). ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 112: 819–829.

Stucky CL, Rossi J, Airaksinen MS, Lewin GR (2002). GFR alpha2/neurturin signalling regulates noxious heat transduction in isolectin B4-binding mouse sensory neurons. J Physiol 545: 43–50.

Studer M, McNaughton PA (2010). Modulation of single-channel properties of TRPV1 by phosphorylation. J Physiol 588: 3743–3756.

Szallasi A, Cortright DN, Blum CA, Eid SR (2007). The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. Nat Rev Drug Discov 6: 357–372.

Talavera K, Yasumatsu K, Voets T, Droogmans G, Shigemura N, Ninomiya Y et al. (2005). Heat activation of TRPM5 underlies thermal sensitivity of sweet taste. Nature 438: 1022–1025.

Todaka H, Taniguchi J, Satoh J, Mizuno A, Suzuki M (2004). Warm temperature-sensitive transient receptor potential vanilloid 4 (TRPV4) plays an essential role in thermal hyperalgesia. J Biol Chem 279: 35133–35138.

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K et al. (1998). The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21: 531–543.

Trevisani M, Smart D, Gunthorpe MJ, Tognetto M, Barbieri M, Campi B et al. (2002). Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci 5: 546–551.

Van Buren JJ, Bhat S, Rotello R, Pauza ME, Premkumar LS (2005). Sensitization and translocation of TRPV1 by insulin and IGF-I. Mol Pain 1: 17.

Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA (2001). Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J Physiol 534: 813–825.

Vellani V, Colucci M, Lattanzi R, Giannini E, Negri L, Melchiorri P et al. (2006). Sensitization of transient receptor potential vanilloid 1 by the prokineticin receptor agonist Bv8. J Neurosci 26: 5109–5116.

Viana F, Ferrer-Montiel A (2009). TRPA1 modulators in preclinical development. Expert Opin Ther Pat 19: 1787–1799.

Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B (2004). The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 430: 748–754.

Wang YY, Chang RB, Waters HN, McKemy DD, Liman ER (2008). The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. J Biol Chem 283: 32691–32703.

Watanabe H, Davis JB, Smart D, Jerman JC, Smith GD, Hayes P *et al*. (2002a). Activation of TRPV4 channels (hVRL-2/mTRP12) by phorbol derivatives. J Biol Chem 277: 13569–13577.

Watanabe H, Vriens J, Suh SH, Benham CD, Droogmans G, Nilius B (2002b). Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. J Biol Chem 277: 47044–47051.

Wegierski T, Lewandrowski U, Muller B, Sickmann A, Walz G (2009). Tyrosine phosphorylation modulates the activity of TRPV4 in response to defined stimuli. J Biol Chem 284: 2923–2933.

Weskamp G, Otten U (1987). An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and in peripheral tissues. J Neurochem 48: 1779–1786.

Wong GY, Gavva NR (2009). Therapeutic potential of vanilloid receptor TRPV1 agonists and antagonists as analgesics: recent advances and setbacks. Brain Res Rev 60: 267–277.

Wood JN, Perl ER (1999). Pain. Curr Opin Genet Dev 9: 328–332.

Woodbury CJ, Zwick M, Wang S, Lawson JJ, Caterina MJ, Koltzenburg M *et al*. (2004). Nociceptors lacking TRPV1 and TRPV2 have normal heat responses. J Neurosci 24: 6410–6415.

Xiao B, Dubin AE, Bursulaya B, Viswanath V, Jegla TJ, Patapoutian A (2008). Identification of transmembrane domain 5 as a critical molecular determinant of menthol sensitivity in mammalian TRPA1 channels. J Neurosci 28: 9640–9651.

Xu H, Ramsey IS, Kotecha SA, Moran MM, Chong JA, Lawson D *et al*. (2002). TRPV3 is a calcium-permeable temperature-sensitive cation channel. Nature 418: 181–186.

Xu F, Satoh E, Iijima T (2003a). Protein kinase C-mediated Ca2+ entry in HEK 293 cells transiently expressing human TRPV4. Br J Pharmacol 140: 413–421.

Xu H, Zhao H, Tian W, Yoshida K, Roullet JB, Cohen DM (2003b). Regulation of a transient receptor potential (TRP) channel by tyrosine phosphorylation. SRC family kinase-dependent tyrosine phosphorylation of TRPV4 on TYR-253 mediates its response to hypotonic stress. J Biol Chem 278: 11520–11527.

Xu H, Delling M, Jun JC, Clapham DE (2006). Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat Neurosci 9: 628–635.

Yang F, Cui Y, Wang K, Zheng J (2010). Thermosensitive TRP channel pore turret is part of the temperature activation pathway. Proc Natl Acad Sci USA 107: 7083–7088.

Zhang X, McNaughton PA (2006). Why pain gets worse: the mechanism of heat hyperalgesia. J Gen Physiol 128: 491–493.

Zhang X, Huang J, McNaughton PA (2005). NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 24: 4211–4223.

Zhang X, Li L, McNaughton PA (2008). Proinflammatory mediators modulate the heat-activated ion channel TRPV1 via the scaffolding protein AKAP79/150. Neuron 59: 450–461.

Zhu MX (2007). Understanding the role of voltage gating of polymodal TRP channels. J Physiol 585: 321–322.

Zhu W, Oxford GS (2007). Phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways mediate acute NGF sensitization of TRPV1. Mol Cell Neurosci 34: 689–700.

Zimmermann K, Leffler A, Fischer MM, Messlinger K, Nau C, Reeh PW (2005). The TRPV1/2/3 activator 2-aminoethoxydiphenyl borate sensitizes native nociceptive neurons to heat in wildtype but not TRPV1 deficient mice. Neuroscience 135: 1277–1284.

Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA (2007). Direct activation of the ion channel TRPA1 by Ca2+. Nat Neurosci 10: 277–279.