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Article *in* British Journal of Pharmacology · July 2011

DOI: 10.1111/j.1476-5381.2011.01601.x · Source: PubMed

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DOI:10.1111/j.1476-5381.2011.01601.x
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REVIEW

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

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Keywords
pain; nociceptor; hyperalgesia; sensitization; TRP ion channels; thermo-TRP; TRPV1; neurotrophic factor; NGF; GDNF; artemin

Received
31 August 2010
Revised
13 April 2011
Accepted
1 May 2011

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.

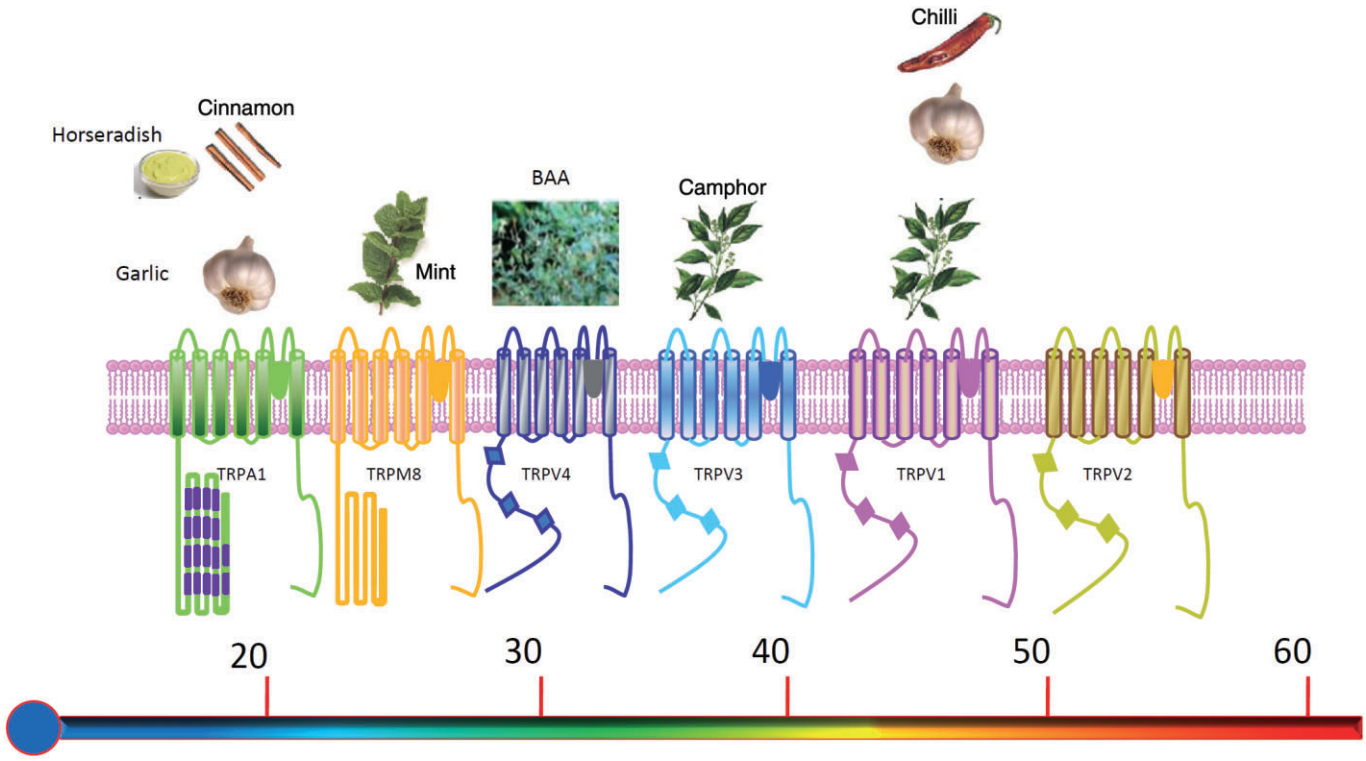

Figure 1

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 activation 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-030031was 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.

Acknowledgements

We thank Dr Michael Fischer for his comments on the manuscript. Work in the authors' lab was supported by grants from the BBSRC, the Human Frontiers Science Program, the Newton Trust, the Helmholtz Foundation, the European Union and the Spanish Ministry of Science and Innovation. PAM thanks the BBVA Foundation, Spain for funding a professorship, and the Professor C. Belmonte and the Instituto de Neurociencias, Alicante, Spain for hospitality during the writing of this review.

Conflict of interest

None of the authors has any conflict of interest.

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