Review

Mechanisms of Cold Pain

Thomas Foulkes
John N. Wood

Molecular Nociception Group, University College London, London, UK
*Correspondence to: Thomas Foulkes, University College London, Gower Street, London WC1E 6BT United Kingdom, Tel.: 44.207.679.2118; Fax: 44.207.679.3519; Email: t.foulkes@ucl.ac.uk

Original manuscript submitted: 07/05/07
Manuscript accepted: 07/06/07

This manuscript was previously published online as a Channels E-publication.

KEY WORDS
cold, pain, nociception, neuropathic, Na, 1.8, TRPM8, TRPA1

ABBREVIATIONS
DRG Dorsal root ganglia
Na, 1.8 Voltage-gated sodium channel 1.8
TTX Tetrodotoxin
TRPM8 Transient receptor potential channel M8
TRPA1 Transient receptor potential channel A1
VGSC Voltage-gated sodium channel
TREK-1 TWIK 1-related K+ channel

ACKNOWLEDGEMENTS
We thank Ramin Raouf and Katharina Zimmermann for helpful criticism, and the BBSRC and Vernalis Pharmaceuticals for funding Thomas Foulkes.

ABSTRACT

Avoidance of cold pain is an important survival mechanism. Intriguingly, whilst cooling can cause numbness, damage sensing mechanisms still seem to operate at low temperatures, and pain can be perceived from cooled damaged tissue. Recent studies have identified two cold-activated Transient Receptor Potential (TRP) channels present in sensory neurons as transducers of cold stimuli. TRPM8 seems to mediate responses to cooling whilst TRPA1 is activated, possibly indirectly, by more extreme cold conditions. The existence of cold-responsive neurons that do not express these channels suggests that other transducers of cold stimuli remain to be discovered. Subsequent action potential electrogenesis and probably propagation from sensory neurons innervating cold tissues depends upon the presence of Na, 1.8, the sole voltage-gated sodium channel that fails to inactivate at low temperatures. This may explain the remarkable specificity of Na, 1.8 expression in nociceptive neurons, where it plays an important role in pain pathways.

INTRODUCTION

Noxious stimuli remain detectable in cooled tissue and noxious cold can produce a sensation of pain, suggesting that a mechanism for the transduction of noxious cold exists in damage-sensing neurons (nociceptors). Both the ability to detect painful stimuli at low temperatures and the perception of noxious cold are required for survival-related responses, and thus nociceptive neurons are subject to evolutionary pressure to continue to function at sub-physiological temperatures. Aberrant perception of cold is also associated with peripheral nerve damage, and cold hypersensitivity can occur in patients suffering from neuropathic pain syndromes. For example, cold hypersensitivity has been reported in 9% of patients suffering from a spectrum of neuropathies, and cold pain is present in 23% of post-stroke central pain patients. While the transduction of noxious thermal stimuli has been investigated extensively, insights into the molecular mechanism of noxious cold detection have arisen more recently, together with insights into the mechanisms by which nociceptors continue to function at low temperatures at which other sensory neurons are inactive. This review examines the current understanding of the transduction of noxious cold by sensory neurons, and the ability of nociceptive neurons to continue to function at low temperatures. The roles of recently identified cold transducers, particularly TRPM8 and TRPA1, are considered along with the unique cold resistance of the voltage-gated sodium channel Na, 1.8.

DETECTION OF NOXIOUS COLD

Psychophysical studies in humans describe the perception of cold pain at temperatures of around 15°C and below. In contrast to the precise thresholds recorded for the detection of noxious heat, the transition from cool to painfully cold appears to be less well-defined. This uncertainty means that it is difficult to define a cold stimulus as noxious or innocuous, making the distinction between cold hyperalgesia and allodynia problematic. In this review, we use the term “cold hypersensitivity” to encompass both states. Subpopulations of both Aδ- and C-fiber neurons are responsive to cooling, although different studies report different proportions of cold-sensitive neurons. Whole-animal electrophysiological studies in the rat suggest that all nociceptive neurons are cold-sensitive, although some require very low temperatures (<0°C) for activation. This may be explained, however, by a non-specific response to tissue damage caused by freezing. Other investigations have reported lower proportions of cold-sensitive nociceptors, although this discrepancy may
Cold Pain

be attributed to the use of different temperature ranges or rates of cooling. Reid and Flonta used Ca$^{2+}$ imaging in primary cultures of dorsal root ganglia (DRG) neurons to identify those that were cold-responsive. Only ~7% of DRG neurons were sensitive to cooling to ~18°C, shown as a robust increase in intracellular Ca$^{2+}$, compared to 14% of trigeminal ganglia neurons. These proportions are similar to those reported from skin-nerve preparation studies in the rat.

The cyclic terpene alcohol menthol, which occurs naturally in mint oils, produces a cooling sensation when applied to the skin. Like capsaicin, the topical application of small doses produces a mild analgesic effect. Higher doses cause burning, irritation and pain. Since cold-sensitive fibers are directly activated or sensitised by menthol (Hensel and Zotterman, 1951), it has proved to be a useful tool in the search for transducers of noxious cold. Icilin is a synthetic related compound, substantially more potent than menthol. Icilin, but not menthol, can also activate an alternative channel, TRPA1.

MECHANISMS OF COLD TRANSDUCTION

Several mechanisms for the transduction of cold stimuli have been proposed: direct activation of a cold-gated ion channel, inhibition of background (“leak”) K+ channel or voltage-gated Ca$^{2+}$ channel function, and inhibition of the Na+/K+ ATPase. These are shown in Figure 1. It is likely that all of these events occur upon cooling; the debate revolves around the relative contribution of each mechanism to the transduction of noxious cold. Indeed, the involvement of several mechanisms of transduction may account for the wide range of activation thresholds of cold-sensitive neurons. It seems logical to expect a cold transducer to be expressed at high levels only in cold-sensitive neurons, and to be regulated by menthol or icilin, providing tools for the search for the molecular identity of the transducers of noxious cold.

Direct activation of a cold-gated ion channel is a simple mechanism for cold transduction, and is thus an appealing hypothesis. Reid and

Figure 1. Mechanisms of cold transduction and transmission. Upper panel shows primary afferent neuron activity in the presence of noxious cold (note larger Na,1.8 current due to increased membrane resistance), while lower panel shows action potential generation at physiological temperature in response to mustard oil, a specific activator of TRPA1. Left side represents the peripheral terminal of the neuron, while the right shows the main axon (which is not exposed to decreases in temperature). Inset boxes show the shape of action potential observed in each environment (adapted from Zimmerman et al., 2007). TTX-S represents a group of tetrodotoxin-sensitive channels, including Na,1.1, 1.7 etc. Note the inactivation of these channels in cold conditions, shown as ball-and-chain pore block. VGSC represents a selection of TTX-sensitive and TTX-resistant channels.
Flonta first described an inward current activated by cooling in DRG neurons. All cold-sensitive neurons were found to express a cold-activated mixed cationic (Erev ≈ 13 mV) current, which was potentiated by menthol via increased amplitude and a shift in activation threshold to higher temperatures. This current was insensitive to amiloride but sensitive to changes in Ca2+ concentration, with increased external Ca2+ reducing current. Menthol was also shown to activate this current directly, providing a mechanism for neuronal activation. In a later paper, Reid et al. described properties of cold-sensitive DRG neurons distinct from cold-insensitive nociceptors. Cold-sensitive nociceptors had shorter action potentials, smaller AHPs and more rapidly decaying Na+ currents. The majority of the cold/menthol-activated current was carried by Na+ ions, and was blocked by high concentrations of the TRPV1 antagonist capsazepine, although this is known to act at a variety of channels at high concentrations, including inhibiting the Ih current.

Ionen channel inhibition is an alternative mechanism for the transduction of cold. Inhibition of K+ channels by cooling would reduce K+ efflux at resting potential, increasing neuronal excitability. Cooling of cultured rat DRG neurons was found to inhibit a background K+ current by 49–72% (32–20°C) that was resistant to tetraethylammonium (TEA) and 4-aminopyridine (4-AP). Subsequently, Viana et al. found that cold-sensitive trigeminal neurons (~9%) differed from those which were cold-insensitive in K+ current expression profile. In cold-sensitive neurons, cooling and menthol inhibited K+ leak channels, causing depolarisation and firing, which is then limited by a slower reduction in inward rectification through Ih, a cationic inward current. In cold-insensitive neurons this inhibition also occurred, but did not cause depolarisation due to the expression of another K+ current, IKdp. This current acts as an "excitability brake" in cold-insensitive neurons. Selective inhibition of IKdp by 4-AP-induced cold-sensitivity in previously unresponsive neurons, leading to the suggestion that cold hypersensitivity in neuropathic pain states could be caused by changes in K+ channel distribution in sensory neuron termini. For this mechanism of cold transduction to be physiologically relevant, the relative distributions of the K+ channels observed here (in the soma) must be similar to that at the nerve termini, the site of cold transduction.

Inhibition of the Na+/K+ ATPase would be predicted to shift membrane potentials to more positive values, but does not explain the lack of cold sensitivity observed in many neurons. Inhibition by ouabain elicited a depolarisation of only 10–15% of that induced by cooling, and did not produce action potentials in DRG neurons, implying a secondary role in cold transduction.

**MOLECULAR IDENTITY OF COLD-GATED CHANNELS**

While much is known about the electrophysiological consequences of cooling, the molecular identities of the underlying proteins have only recently come under scrutiny. Considerable progress has been made in establishing the channels responsible for the cold-sensitive cation current, while the molecular identity of cold-transducing K+ channels has remained elusive.

The 2-pore domain K+ channel TREK-1 was proposed as a detector of cold by Reid and Flonta. Temperature sensitivity in related channels has been reported, and TREK-1 itself is opened by heat and closed upon cooling. It is highly expressed in primary sensory neurons, colocalising with TRPV1. In cultured trigeminal neurons from the rat, however, Gd3+ inhibition of TREK-1 did not mimic the effect of cold in cold-responsive cells. Ba2+ block of TRAAK and TWIK-1 was likewise insufficient to account for cold transduction. 

**Cold Pain**

2-pore domain K+ channel TREK-1 was found to inhibit a cooling, and did not produce action potentials in DRG neurons, leading to the suggestion that cold hypersensitivity in neuropathic pain states could be caused by changes in K+ background currents described by Viana et al. The molecular identity of a channel capable of conferring cold sensitivity to DRG neurons was discovered by expression cloning, using Fura-2 Ca2+ imaging to identify cells responding to menthol. A trigeminal ganglia (TG) cDNA library, chosen due to the high proportion of cold-sensitive neurons compared to DRG, was expressed in HEK293 cells for screening. A single cDNA clone conferring cold sensitivity was identified, and termed CMR1. When expressed in Xenopus oocytes, electrophysiological analysis revealed currents elicited by menthol and icilin (a super-cooling agent), but not by analogous compounds or capsaicin. In transfected HEK293 cells, menthol- and cold-evoked currents displayed properties similar to those observed in trigeminal neurons. CMR1 was found to have significant homology to certain members of the TRP channel family, most notably those of the TRPM class, and was found to be the rat orthologue of the human channel TRPM8. Transcripts for this protein were found in a subset of small-diameter sensory neurons in DRG and TG, similar to those expressing TRPV1, and representing C and possibly Aδ-fibers. Transcripts were not found in larger diameter (cold-insensitive) neurons, but were more prevalent in TG than DRG, consistent with the greater proportion of cold-sensitive cells in this structure. Peier et al. identified TRPM8 as a cold sensor at the same time as the work by McKemy et al., using an alternative approach. A bioinformatic search based on TRP channel homology identified TRPM8 was found to be expressed in a subset of small diameter sensory neurons, and not coexpressed with CGRP and TRPV1. A cold- and menthol-activated current was produced upon expression in CHO cells, with similar properties to the cold-activated current in DRG.

TRPM8 is activated at a threshold of ~25°C, and is expressed in a subset of small-diameter neurons that do not coexpress known markers of nociceptors. This suggests that TRPM8 may not be a transducer for noxious cold stimuli. Consistent with this hypothesis, populations of cold-sensitive but menthol-insensitive (therefore TRPM8-negative) neurons have been identified. The role of TRPM8 in cold sensation was examined recently by several groups, each using independently-derived TRPM8-null mice. The studies reported deficits in responses to non-noxious cooling following TRPM8 deletion, using skin-nerve preparations and behavioural tests, confirming the role of this protein as a transducer of environmental cooling. The contribution of TRPM8 to the detection of noxious cold, however, was less clear. Neither group...
found an effect of TRPM8 deletion on responses to noxious cold stimuli, measured using nociceptive responses on a cold plate. Dhaka et al., 26 however, described a markedly reduced response to topical acetone application, thought to produce an unpleasant cold stimulus, in the TRPM8-null animals. A reduction in acetone-evoked cold hypersensitivity following nerve damage was also reported. 28 On balance it appears that TRPM8 plays a role in the detection of cool temperatures. At noxious cold temperatures (<15°C), however, other mechanisms of cold transduction seem to become more significant.

A bioinformatic approach was used again by Story et al. 14 to identify ANKTM1, another TRP-like channel expressed in nociceptors and activated by cold temperatures. ANKTM1 was shown to be expressed in a peptidergic population of nociceptors expressing TRPV1 (a distinct population from those expressing TRPM8). Expression in CHO cells identified a responsiveness to low temperatures (≤17°C), resulting in Ca2+ influx, sensitive to icilin but not menthol. The cold sensitivity of this channel in cultured sensory neurons was confirmed in a later paper. 29 A subpopulation of DRG neurons was found to possess properties consistent with ANKTM1 expression. This channel was later shown to be responsible for currents evoked by pain-causing mustard oil. 30 ANKTM1 was later termed TRPA1, and found to be upregulated by nerve damage and inflammation, contributing to the cold hypersensitivity observed under neuropathic and inflammatory conditions. 31 This paper reported TRPA1 (but not TRPM8) upregulation by administration of NGF, via the p38 MAPK pathway.

Two independent studies analysed the effect of TRPA1 deletion from mice. Kwan et al. 32 found deficits in behavioural responses to noxious cold, assessed using cold plate (0°C) and acetone tests. This effect was found to be gender-dependent: a trend towards diminished sensitivity in TRPA1-null males was greater in female mice, adding an additional layer of complexity to the role of TRPA1 in the detection of noxious cold. Wild-type females are also more sensitive to noxious cold stimulation; 32 responses to moderate cooling, however, do not appear to be affected by gender. 28 Bautista et al. 33 however, found no difference from control in either cold plate or acetone tests, and further found no differences in electrophysiological assays of cold sensitivity in DRG neurons. These differences may arise from differences in methodology; for example, Kwan et al. 32 used mice of both genders, whereas Bautista et al. 33 only tested males, in which smaller differences were reported by the former group. Additionally, different constructs for TRPA1 deletion were used in each study. In both cases, a truncated fragment was expressed, but Kwan et al. 32 included an ER retention signal to retard fragment trafficking. Since TRP channels are known to form heteromultimers, it is possible that truncated TRPA1 fragments could exert dominant-negative effects on a variety of other channels, confounding phenotypic analysis. Alternatively, difficulties in assessing cold-induced pain behaviour (for example, some mice show no response to very cold temperatures 39) may explain these discrepancies.

A recent report from Zurborg et al. 35 provides an explanation for the differences between cellular and whole-animal studies on the role of TRPA1 in cold sensing. They used a heterologous expression system to show that intracellular Ca2+ activates TRPA1 via an EF-hand domain, and that it is increased intracellular Ca2+ concentration during cooling that activates the channel in these systems, rather than gating by cold. This study used a logical series of experiments to demonstrate that this indirect (and perhaps non-physiological) mechanism is responsible for previous observations of TRPA1 gating by cold. Responses to cold were seen in TRPA1-expressing cells but also in TRPA1-negative neurons, with almost identical thresholds, while Ca2+-buffered whole cell patch-clamp experiments found no activation of TRPA1 channels by cold. The possibility arises, however, that indirect activation of TRPA1 by Ca2+ is a physiological mechanism of cold detection. That et al. 22 described a significant cold-induced rise in intracellular Ca2+ concentration in a nominally Ca2+-free extracellular environment, probably from intracellular stores, although other studies did not observe this response. 36 The activation of TRPA1 by intracellular Ca2+ has been reported previously 30 and means that TRPA1 is highly likely to be activated under cold conditions by inward Ca2+ currents, 7 independent of whether it is intrinsically cold sensitive. A view of TRPA1 as a general integrator of noxious stimuli is supported by its broad range of activators, including a wide variety of pungent cysteine-modifying reagents (mustard oil, garlic, cinnamaldehyde etc.) and the inflammatory mediator bradykinin. 29 The search for a novel specific transducer of noxious cold therefore continues.

Several studies report populations of cold-sensitive DRG neurons expressing neither TRPM8 nor TRPA1. Munns et al. 24 found that a third of cold-responsive DRG neurons did not respond to any TRP channel agonist, including menthol (TRPM8) and mustard oil (TRPA1). Another group reported a novel cold-sensitive DRG neuron subpopulation, sensitive to cold but with rapid adaptation, expressing neither TRPA1 nor TRPM8, and possibly corresponding to the rapidly-adapting cold sensors described in vivo. 25 Bautista et al. 33 also observed two distinct populations of cold-sensitive neurons in DRG culture, one responding to menthol but neither to mustard oil. Additionally, cold-sensitive neurons in the TRPM8-null mouse were reported not to express TRPA1. 27 This suggests the presence of an additional transduction mechanism (possibly another cold-activated ion channel, or inhibition of K+ channels) for noxious cold in DRG neurons. Potential cold transducers are illustrated in Figure 1.

**TRANSMISSION IN COLD CONDITIONS**

The detection of innocuous stimuli by sensory neurons is inhibited by cooling, as exemplified by the numbness that accompanies cold limbs. Motor function is also attenuated, demonstrating that this is likely a general effect on neuronal activity. Nociceptors, however, remain functional at low temperatures. In some cases, cold may even sensitise nociceptors, particularly with respect to mechanical stimuli, resulting in hyperalgesia or allodynia. The reason for this unique cold-resistance of nociceptors has remained elusive until recently, when a novel insight into sodium channel function provided an explanation for this phenomenon.

Voltage-gated sodium channels (VGSCs), responsible for the upstroke of the action potential in neurons, can be grouped according to sensitivity to the puffer fish poison tetrodotoxin (TTX). The majority of channels are TTX-sensitive; that is, they are inhibited by nM concentrations of TTX. The channels Na1.8 and Na1.9, however, are TTX-resistant. A selection of TTX-sensitive channels are expressed in all neurons, where they are required for excitability. 38 Na1.8, in contrast, is expressed exclusively in a subset of small- to medium-diameter sensory neurons, over 85% of which are nociceptors. 39,40 At ambient temperatures (~30°C), mechanically- or electrically-induced action potentials in the isolated
skin-nerve preparation are blocked by the application of TTX, indicating that TTX-sensitive channels are required for action potential generation. At lower temperatures (-10°C), however, most C-fibers are not inhibited by TTX, regaining mechanical and electrical excitability and behaving almost as if TTX were absent.38 This implies that a TTX-resistant channel becomes increasingly important for the physiological function of nociceptive C-fibers at low temperatures. Whole-cell patch-clamp studies on cultured DRG neurons found that the TTX-sensitive channels are temperature sensitive, with cooling slowing both activation and inactivation kinetics and reducing peak current amplitudes. TTX-resistant channels, however, were affected to a much smaller extent. Cold-induced shut-down of the TTX-sensitive channels was observed and ascribed to a shift in slow inactivation to more hyperpolarised potentials. At a resting potential of -80 mV (in the physiological range), however, the slow inactivation of the TTX-resistant channel Na\textsubscript{1.8} is affected to a much smaller extent than those that are TTX-sensitive. Indeed, reduced temperatures were found to facilitate activation of Na\textsubscript{1.8} by shifting the voltage-dependence of activation to more negative potentials. These effects were reproduced in heterologous expression systems using recombinant Na\textsubscript{1.7} and Na\textsubscript{1.8} to produce TTX-sensitive and -resistant currents, respectively. The differential sensitivity to cold of these currents is thus a result of properties inherent to distinct VGSC subunits. These results were verified in a series of experiments using Na\textsubscript{1.8-null} mice. Na\textsubscript{1.8-null} C-fibers did not regain excitability in the presence of TTX at low temperatures, in contrast to wild-type neurons. Na\textsubscript{1.8-null} animals were almost entirely insensitive to noxious cold in behavioural tests, demonstrating conclusively the key role of this channel in action potential propagation at low temperatures. Action potential generation at low temperatures is thus entirely dependent on Na\textsubscript{1.8}.38 It is interesting to note that it is only the extremities that experience significant drops in temperature, and that Na\textsubscript{1.8} expression is localized to free nerve endings (i.e., the peripheral termini of nociceptors).41

Na\textsubscript{1.8} has an unusually positive activation threshold compared to the TTX-sensitive channels, meaning that only strongly depolarising stimuli are capable of activating nociceptors in the presence of TTX. The congenital insensitivity to pain observed in humans with loss-of-function mutations in SCN9A, the gene encoding Na\textsubscript{1.7},32,33 would imply that this channel is essential for action potential generation in nociceptive neurons. Upon cooling, however, Na\textsubscript{1.8} appears to be sufficient for the generation of action potentials in response to more moderate stimulation. This change is mediated by the effects of reduced temperature on neuronal membrane properties.38 As temperatures decrease, the conductance of background (“leak”) K\textsuperscript{+} channels decreases,17,18 and the activity of the Na\textsuperscript{+}/K\textsuperscript{+} ATPase is reduced.44 These changes effectively increase the membrane resistance, augmenting the voltage change achieved by a given depolarising current (charge transfer). Consistent with this, the charge transfer at the nerve terminal required to generate an action potential was reduced by cooling. This means that a given generator current (for example through a cold-activated ion channel) is more likely to reach the activation potential of Na\textsubscript{1.8}, enhancing excitability. In addition to effects on membrane properties, low temperatures act directly on Na\textsubscript{1.8} to shift its voltage-dependence of activation to more negative potentials, again increasing excitability.38

The unique cold-resistance of the nociceptor-specific VGSC Na\textsubscript{1.8} thus ensures that nociceptive sensory neurons continue to function at low temperatures, allowing both the avoidance of noxious cold stimuli and the detection of noxious stimuli of other modalities in cold conditions. The survival value inherent in these abilities is reflected by the high evolutionary conservation of Na\textsubscript{1.8}, particularly in cold-blooded animals. Additionally, the tissue specificity of Na\textsubscript{1.8} expression means that only putative nociceptors are active at low temperatures: other sensory neurons are not functional. This provides a mechanism for the selective activation of nociceptors by noxious cold. While at first this may not seem significant, it means that the expression of the transducer of noxious cold may not be restricted to nociceptors: it may occur in other neurons, where its activity is not capable of generating action potentials at low temperatures due to a lack of available VCSCs. In other words, it is possible that the transduction of noxious cold is not specific to nociceptors, but transmission is. These findings are summarised in Figure 1.

**COLD HYPERSENSITIVITY AND COOLING AS AN ANALGESIC**

Pain induced by mild cold temperatures is a relatively common feature of neuropathic pain syndromes,1,2 and may have a significant impact on quality of life. The mechanisms behind this change have been illuminated by recent work. While a general increase in nociceptor excitability may explain multi-modality hypersensitivity, abnormal sensitivity to a particular modality is likely to be due to more specific mechanisms. Cold hypersensitivity is mediated by capsaicin-sensitive, unmyelinated or thinly-myelinated primary afferents.45-48

Interestingly, TRPA1 appears to be heavily implicated in cold hypersensitivity following inflammation and nerve damage. Obata et al.29 reported TRPA1 (but not TRPM8) upregulation in TrkA-expressing sensory neurons under inflammatory conditions or after nerve damage. This was shown to be induced by NGF through the p38 MAPK pathway, and to contribute to cold hypersensitivity under these conditions. Intrathecally-administered anti-NGF, p38 MAPK inhibitor, and antisense TRPA1 all decreased the induction of TRPA1 and reduced cold hypersensitivity. A subsequent paper from the same group confirmed this observation, presenting data showing that antisense knockdown of TRPA1, but not TRPM8, alleviates cold hypersensitivity after spinal nerve ligation in the rat.49 TRPA1 upregulation was shown to follow the time-course of cold hypersensitivity, further supporting a mechanistic link. The extra-cellular signal-regulated big MAP kinase ERK5 also contributes to inflammatory cold hypersensitivity through TRPA1 upregulation,50 while activation of the channel by bradykinin provides another mechanism for this phenomenon.29

In contrast to TRPA1, TRPM8 does not appear to contribute to cold hypersensitivity. As mentioned above, cold hypersensitivity is mediated by capsaicin-sensitive (therefore TRPV1-expressing) neurons. TRPM8 is not colocalized with TRPV1 in vivo,23 although a degree of joint expression is observed in cultured sensory neurons,15 implying that TRPM8 is not required for cold hypersensitivity (unless upregulated in capsaicin-sensitive neurons upon nerve damage). Inflammatory conditions result in a functional downregulation of TRPM8 currents, implying that this channel does not mediate cold hypersensitivity. Decreased pH, in the physiological range occurring during inflammation, reduces activation of TRPM8 in response to cold and icilin, but not menthol,51 (suggesting differential mechanisms of activation, consistent with previous reports13) while...
the inflammatory mediator bradykinin significantly downregulates TRPM8 through the action of protein kinase C. Phospholipase C-mediated PI(4,5)P(2) depletion also desensitises TRPM8. Surprisingly, cold hypersensitivity in models of neuropathic pain was attenuated in TRPM8-null mice, although this was not reproduced by antisense TRPM8 administration.

While nerve injury can lead to cold hypersensitivity, non-noxious cooling has long been used as an analgesic (example in ref. 54). Menthol and icilin, activators of TRPM8, can suppress mechanical and thermal hypersensitivity induced by nerve damage when administered peripherally or intrathecally. This effect was abolished by antisense knockdown of TRPM8, confirming this as the site of action for menthol/icilin-induced analgesia. Central mechanisms were invoked to explain this analgesia, with evidence suggesting that glutamate released by icilin-sensitive primary afferents may act on metabotropic glutamate receptors in the dorsal horn (particularly mGluRII/III), resulting in the inhibition of nociceptive transmission. In contrast to previous studies, an upregulation of TRPM8 protein was reported following nerve injury, although the functional consequence of this is not clear. Dhaka et al. also report that TRPM8 mediates the analgesic effect of moderate cooling after formalin administration (a painful, tissue-damaging stimulus), supporting a central role for TRPM8 in general cooling-induced analgesia, and providing a rationale for the relief obtained upon cooling injured or inflamed body parts.

Distinct cold-sensitive channels appear to play defined roles in cold hypersensitivity. Current evidence suggests that TRPA1 is primarily responsible for cold hypersensitivity observed following inflammation and nerve damage, increasing the probability that this represents the main noxious cold receptor. TRPM8, in contrast, appears to mediate cooling-induced analgesia, supporting a role as a detector of innocuous cooling.

COLD-INDUCED HYPERALGESIA

While certain modalities of pain, such as that of exercise-induced ischemia, are attenuated by cooling, increased sensitivity to noxious mechanical stimulation is often observed at very low temperatures. Cold fingers banged on a door, for example, can be particularly painful. The mechanisms behind this are unclear, but may involve summation of ongoing, low-level cold-induced nociceptor activity with the mechanically-induced event. TRPA1 is activated by low temperatures, either directly or indirectly. This channel has also been proposed as a candidate (noxious) mechanotransduser, supported by observations in TRPA1-null mice. It is therefore possible that cold and mechanical stimuli may integrate through this receptor, allowing cold potentiation of mechanotransduction. Additionally, many noxious stimuli result in cellular Ca\(^{2+}\) influx (example in ref. 56). TRPA1 is activated by increases in intracellular Ca\(^{2+}\) meaning that cold-induced Ca\(^{2+}\) entry below the action potential threshold would add to Ca\(^{2+}\) levels raised by other stimuli, decreasing the stimulus size required for TRPA1 activation and subsequent action potential generation. Essentially, TRPA1 would be acting as a molecular site of integration of noxious stimuli, as proposed by Story et al. and Kwan et al. Alternatively, cold-induced facilitation of Na\(_{1.8}\) activation, in combination with cold-induced changes in physical membrane properties may explain the mechanical hypersensitivity observed.

Cold-induced changes in protein expression and function in primary sensory neurons are likely to play a significant role in cold-induced hypersensitivity. For example, cold-induced activation of p38 MAPK pathways results in the functional upregulation of TRPA1, and thus cold hypersensitivity. Phosphorylation by p38 MAPK also increases Na\(_{1.8}\) current density, resulting in increased neuronal excitability.

More speculatively, the lack of background innocuous sensory activity may sensitize nociceptive pathways at the level of the spinal cord. Non-nociceptive sensory neurons may exert inhibitory control over nociceptor-specific ascending pathways. In the absence of non-noxious input, as occurs under very cold conditions, nociceptor-specific pathways may be released from this inhibition (dissociated), and thus sensitised.

CONCLUSIONS

Several mechanisms have been proposed for the transduction of noxious cold stimuli, including direct activation of specific TRP channels, inhibition of K\(^{+}\) currents, and reduction of electrogentic pump activity. While it is clear that all of these occur to some extent upon cooling, the relative contribution of each mechanism remains to be defined. Recent work suggests that TRPM8 and TRPA1 may play important roles in the detection of cooling and noxious cold respectively, with a role for TRPA1 in cold hypersensitivity, and that the inhibition of an as yet unidentified background 2-pore domain K\(^{+}\) channel may be important. In addition to current research focusing on transduction by primary sensory neurons, recent reports have suggested that epidermal cells, particularly keratinocytes, may be responsible for the transduction of noxious stimuli, signalling indirectly to primary sensory neurons. Novel cold activated molecular sensors, both in sensory neurons and perhaps on non-neuronal cells thus remain to be defined. Cold transduction, like heat transduction seems to involve both TRP and other channels. The attractive notion of single thermo-transducers for distinct temperature ranges is thus a considerable oversimplification.

The discovery that Na\(_{1.8}\) is not inactivated by cooling, in contrast to all other voltage-gated sodium channels, provides an insight into the mechanisms behind noxious cold sensation. It provides a clear explanation for the observation that while non-nociceptive sensory neurons become inactive upon cooling, resulting in numbness, nociceptors are sensitised. The TTX-resistant channels, including Na\(_{1.8}\), are the evolutionary precursors of the TTX-sensitive channels, which arose through gene duplication. The conservation of Na\(_{1.8}\) reflects the vital importance of the ability to detect noxious stimuli in cold environments.

References

1. Verdugo R, Ochoa JL. Quantitative somatosensory thermotest: A key method for functional evaluation of small calibre afferent channels. Brain 1992; 115:935-931.
2. Greenspan JD, Ohara S, Sarelani E, Lenz FA. Alldynia in patients with post-stroke central pain (CPSD) studied by statistical quantitative sensory testing within individuals. Pain 2004; 109:357-66.
3. Morin C, Bushnell MC. Temporal and qualitative properties of cold pain and heat pain: A psychophysical study. Pain 1998; 74:67-73.
4. Hensel H, Zotterman Y. The effect of menthol on the thermoreceptors. Acta Physiol Scand 1951; 24:27-34.
5. Simone DA, Kajander KC. Responses of cutaneous A-fiber nociceptors to noxious cold. J Neurophysiol 1997; 77:2049-60.
6. Simone DA, Kajander KC. Excitation of rat cutaneous nociceptors by noxious cold. Neurosci Lett 1996; 213:53-6.
7. Reid G, Flonta ML. Physiology: Cold current in thermoreceptive neurons. Nature 2001; 413:480.

www.landesbioscience.com Channels 159
13. Chuang HH, Neuhausser WM, Julius D. The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. Neuron 2004; 43:839-69.

20. Maingret F, Lauritzen I, Patel AJ, Heurteaux C, Reyes R, Lesage F, Lazdunski M, Honoré Koerber HR, Davis BM. Nociceptors lacking TRPV1 and TRPV2 have normal heat sensitivity in mice. Neuron 2003; 40:371-8.

25. Babes A, Zorzon D, Reid G. A novel type of cold-sensitive neuron in rat dorsal root ganglia neurons: Properties and role in cold transduction. J Physiol 2002; 545:595-614.

26. Dhaka A, Murray AN, Mathur J, Earley TJ, Petrus MJ, Patapoutian A. TRPM8 is required for cold sensation in mice. Neuron 2007; 54:379-86.

40. Djouhri L, Fang X, Okuse K, Wood JN, Berry CM, Lawson SN. The TTX-resistant sodium channel Nav1.8 is important for pain at low temperatures. Neuropeptides 2001; 35:239-52.

43. Goldberg YP, MacFarlane J, MacDonald ML, Thompson J, Dube MP, Martice M, Fraser Y, Young D, Liu Y, Flores CM, Qin N. Attenuated cold sensitivity in TRPM8 null mice. Neuron 2007; 54:371-8.

49. Katsura H, Obata K, Mizushima T, Yamanaka H, Kobayashi K, Dai Y, Fukushima T, Tokunaga A, Sakagami M, Noguchi K. Antisense knock down of TRPA1, but not TRPM8, alleviates cold hyperalgesia after spinal nerve ligation in rats. Exp Neurol 2006; 200:112-23.

50. Katsura H, Obata K, Mizushima T, Sakurai J, Kobayashi K, Yamada M, Yamanaka H, Dai Y, Fukushima T, Sakagami M, Noguchi K. Activation of extracellular signal-regulated protein kinase 5 in primary afferent neurons contributes to heat and cold hyperalgesia after inflammation. J Neurochem 2007; in press.

54. Proudfoot CJ, Garry EM, Cottrell DF, Rosie R, Anderson H, Robertson DC, Andria G, Tiscano E, Kerdraon J, Bowsher D, Pimstone SN, Samuels ME, Sherrington R, Catterall WA, Goldin AL, Waxman SG. International union of pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacol Rev 2005; 57:397-409.

Cold Pain

---

1.8. McKey DD, Neuhauser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 2002; 416:52-8.

2.2. Gee MD, Lynn B, Basile S, Pierau FK, Consell B. The relationship between axonal spike shape and functional modality in cutaneous C-fibers in the pig and rat. Neuroscience 1999; 90:509-18.

3.3. Green BG. The sensory effects of l-menthol on human skin. Somatosens Mot Res 1992; 9:235-44.

4.4. Walter G, Schachtenthaler J, Binder A, Baron R. Topical menthol—a human model for cold pain by activation and sensitization of C nociceptors. Brain 2004; 127:1159-71.

5.5. Wei ET, Seid DA. AG-3-5: A chemical producing sensations of cold. J Pharm Pharmacol 1974; 26:545-52.

6.6. Green BG. The sensory effects of l-menthol on human skin. Somatosens Mot Res 1992; 45:250-63.