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### Cold comfort pharm

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## 1 **Cold Comfort Pharm**

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13

14

### 15 **Abstract**

16 Cooling of the skin has long been thought to be beneficial in pain states but  
17 intense cold is clearly noxious. Does cooling lead to pain or gain? Rapid progress  
18 in this controversy has been made since the discovery of specific ion channels of  
19 the TRP family that are activated by cooling of sensory nerve cells to below body  
20 temperature. This review focuses on the role of one of these, TRPM8, which has  
21 been implicated in cool sensation and cold pain by recent knockout mouse  
22 studies, but remarkably also appears capable of eliciting a novel analgesic gating  
23 control over noxious inputs in chronic pain states. We discuss hypothetical  
24 mechanisms that could bring about this composite profile. It is clear that new and  
25 highly selective agents will need to be developed to further evaluate the potential  
26 therapeutic opportunities offered by low temperature-sensitive TRP channels.

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## 32 **Introduction**

33 It seems to be commonly understood that cooling an area of injury can relieve  
34 pain, but what is the scientific basis? Sports physiotherapists, dentists and  
35 medical practitioners have used cool sprays, mint oil and menthol for quite some  
36 time. Even before this, as far back as classical Greece, in the foundations of  
37 modern civilisation, the ancient Greek physician and 'father of medicine'  
38 Hippocrates (*circa*. 460-370 B.C.) and the personal physician to Marcus Aurelius,  
39 Galen (129-200 A.D.) reported that cutaneous cooling was effective as an  
40 analgesic remedy. Traditional medicine from China and Europe makes use of the  
41 natural cooling agent menthol and mint oils as analgesic therapies. Modern  
42 medicine has continued to make use of peripheral cooling to produce analgesia<sup>1</sup>.  
43 Menthol has also been shown to alleviate thermally-elicited pain in an  
44 experimental setting<sup>2</sup> and to exert an analgesic action in the mouse hot-plate and  
45 acetic acid writhing tests<sup>3</sup>. Despite the apparent success of menthol and cool in  
46 acute pain and inflammation, its effectiveness in chronic pain states has not yet  
47 been substantially established.

48

49 Humans have a highly developed temperature detection system which can  
50 perceive temperature changes as small as 1 deg C. Classic work by Hensel and  
51 Zotterman<sup>4</sup> indicated a role of specific receptors in mediating the transduction of  
52 cold sensation. Recent identification of the TRP (Transient Receptor Potential)  
53 family of ion channels in the nervous system, several of which respond to  
54 changes in temperature, has greatly advanced our understanding of this process.

55

56 Somatosensory systems convert environmental sensations via cutaneous  
57 afferent neurons that have free nerve endings in the skin capable of detecting  
58 physico-chemical stimuli and relay that information to the central nervous system.  
59 Indeed, it seems that distinct sets of such primary afferent neurons (with cell  
60 bodies in dorsal root ganglia, DRG) are specialized to respond to specific  
61 temperatures in the cold/cool to warm/hot range and transmit this information to  
62 the central nervous system.

63

64 Physiological pain detection (nociception) occurs via specialised cutaneous  
65 sensory neurons (nociceptors) which are afferents that are activated by noxious  
66 (painful) stimuli. They are functionally divided into two groups consisting of A $\delta$   
67 mechano-heat nociceptors and C-fibre polymodal nociceptors. Electrical  
68 stimulation of A $\delta$  fibres evokes a rapid, sharp pain sensation (corresponding to  
69 'first pain'), while stimulation of C-fibres produces the dull, diffuse or burning pain  
70 (corresponding to 'second pain'). A $\delta$  and C fibre afferents also contain  
71 subpopulations that respond to cooling to innocuous cool temperatures of 15-  
72 30°C or to noxious cold at 15°C and below<sup>5, 6</sup>. Normal skin temperature is  
73 typically around 32°C and activation of DRG fibers at temperatures of between  
74 32°C and 43°C is perceived as warmth. Temperatures greater than 43°C are  
75 perceived as noxious (painful) heat.

76

77 Chronic neuropathic pain can result from nerve damage of various origins, for  
78 example, by direct constriction, viral infection or due to diabetic or  
79 chemotherapeutic neurotoxicity. Ongoing chronic pain provides no benefit in  
80 terms of redirecting behaviour and severely reduces patients' quality of life.  
81 Laboratory models of peripheral nerve injury allow us to examine the underlying  
82 mechanisms that cause hypersensitive responses with the aim of identifying  
83 novel analgesic targets. Typical models can involve constriction of the sciatic  
84 nerve (chronic constriction injury, CCI or spinal nerve ligation, SNL), which result  
85 in behavioural hyperalgesia (heightened response to a painful stimulus) and  
86 allodynia (pain in response to innocuous stimuli). Models of demyelinating  
87 diseases and chronic inflammation are also used to establish whether there are  
88 common (or distinct) underlying factors that produce pain hypersensitivity  
89 following the different types of injuries.

90

91 This review aims to outline and evaluate the evidence for roles of TRP channel  
92 subtypes in cold sensation, in cold pain and in analgesia for chronic pain states.  
93 The particular focus is on a receptor for mild cooling, TRPM8, which has been

94 implicated in each of these roles from recent mutant mouse and antisense  
95 studies.

96

97

### 98 **TRP channels in temperature detection**

99 Members of the Transient Receptor Potential (TRP) family, the TRPV (vanilloid),  
100 TRPM (melastatin) and TRPA (ankyrin) receptors comprise the temperature-  
101 gated family of ion channels. Identification and cloning of the classic TRPV1  
102 (VR1) vanilloid receptor marked the beginning of the process to identify TRP ion  
103 channels that were gated by warm and hot temperature and substances that elicit  
104 thermal sensations. Prior to this, sensitivity to capsaicin (the agent that produces  
105 the tingling heat perception in response to chili peppers) was a known feature of  
106 nociceptive A $\delta$  and C fibres that correlated with their responsiveness to  
107 moderately raised temperatures of about 43°C. Subsequently, TRPV1 was  
108 identified as the channel responsible, as its activation was demonstrated in  
109 response to both moderate heat (43°C) and capsaicin<sup>7</sup>. This was the first  
110 demonstration of how sensory neurons may detect temperature. TRPV1 is  
111 expressed in the majority of A $\delta$ /C peptidergic afferents and non-peptidergic IB4-  
112 positive afferents in the rat<sup>8</sup>. TRPV1-knockout mice are unresponsive to  
113 capsaicin and have reduced inflammation-induced thermal hyperalgesia<sup>9, 10</sup>.  
114 Although acute thermosensation has been reported as unaffected<sup>10</sup>, thermal  
115 responses of afferent fibres, DRG cells and dorsal horn neurons are clearly  
116 impaired<sup>9</sup>. However, TRPV1 mutant mice retain responsiveness to high threshold  
117 noxious heat and at least some of the alternative thermoreceptors likely to be  
118 responsible have since been identified.

119

120 The closely-related channel, TRPV2 (VRL1), is a capsaicin-insensitive channel  
121 activated by noxious temperatures (activated above 52-55°C) in vitro<sup>11</sup>. TRPV2 is  
122 expressed predominantly by A $\delta$  fibres which could represent a discrete  
123 population of A $\delta$  mechano-heat receptors. TRPV3 (activation range 31-39°C) and  
124 TRPV4 (activated above 25°C) are sensitive to increasing innocuous warm

125 temperatures<sup>12, 13</sup>. TRPV3 is expressed by keratinocytes in the epidermis and its  
126 role in thermosensation is demonstrated by the marked deficits in responses to  
127 innocuous and noxious heat seen in TRPV3-null mice<sup>14</sup>. TRPV4 is found in both  
128 afferents and skin and contributes to both innocuous warm sensation and  
129 inflammation-induced thermal hyperalgesia<sup>15, 16</sup>. Interestingly, TRPV1 has also  
130 been detected in keratinocytes and in bladder epithelial cells<sup>17</sup>. Thus, some of  
131 these channels are not exclusive to sensory neurons and may thus subserve  
132 other as yet unidentified roles possibly involving trans-cellular information transfer  
133 to neurons.

134

135 Cold-responsive primary afferent fibres can be activated either by low threshold  
136 “cool” temperatures (approx 20-35°C)<sup>18, 19</sup> or by high-threshold noxious cold  
137 temperatures (<15°C), which are generally perceived as painful<sup>5, 20</sup>. A similar  
138 differentiation between the encoding of noxious and innocuous cold temperatures  
139 continues in the dorsal horn of the spinal cord. Neurons in the superficial dorsal  
140 horn that are specifically responsive to innocuous cool in primates receive input  
141 mainly from A $\delta$  fibres<sup>21, 22</sup>, whereas noxious cold-responsive cells are generally  
142 multireceptive, being also activated by heat and noxious pinch<sup>23</sup>. The ascending  
143 axons of cool-specific cells and the noxious cold-activated multireceptive cells  
144 have different conduction velocities and different thalamic terminations<sup>24</sup>, as well  
145 as different morphologies. Subpopulations of thalamic neurons have also been  
146 identified that respond to cool, but not noxious cold, or alternatively to noxious  
147 cold<sup>25</sup>. Psychophysical studies further show differences between perception of  
148 cool and noxious cold, suggesting that the two signals are differentially  
149 processed before arrival at cortical levels<sup>26</sup>.

150

### 151 **TRPM8: properties of a molecular sensor of cooling**

152 Major progress in understanding the basis for cool and cold temperature  
153 transduction was provided by the identification of TRPM8 (TRP melastatin family  
154 member 8, formerly known as CMR1), a Ca<sup>2+</sup>-permeable ion channel that can be  
155 activated by cool temperatures (18-24°C)<sup>27, 28</sup>. It is also activated and sensitised

156 by menthol and other chemicals that elicit sensations of cool; e.g. eucalyptol.  
157 Notably, threshold temperatures reported for the activation of recombinant  
158 TRPM8 channels are consistently lower than those in native trigeminal menthol-  
159 sensitive neurons, suggesting that sensitivity is facilitated by endogenous factors  
160 in vivo<sup>29, 30</sup>. TRPM8 is selectively activated by the synthetic cooling agent, icilin,  
161 which is 200 times more potent than menthol<sup>27, 28</sup>, although there is evidence that  
162 responses to icilin, but not menthol, may require concurrent elevation of cytosolic  
163 Ca<sup>2+</sup> concentrations<sup>31</sup>. Icilin can also interact at higher concentrations with other  
164 channels such as TRPA1<sup>32</sup>. The WS series of compounds are derived from  
165 menthol and several such as WS-3 can evoke TRPM8-mediated Ca<sup>2+</sup> entry<sup>33</sup>.  
166 WS-12 is reported as the highest-affinity TRPM8 ligand to date, but it can reduce  
167 the effects of menthol<sup>34</sup>, so may act as a partial agonist. A number of other  
168 pharmacological agents have been described as activators of TRPM8 but their  
169 targeting specificity is not yet entirely clear<sup>33-35</sup>. In addition, it has been shown  
170 that several TRPV1 inhibitors such as BCTC and capsazepine inhibit TRPM8 too  
171 <sup>30, 33, 36</sup>. Some agents that activate other TRP channels such as 2-APB and  
172 URB597 inhibit TRPM8<sup>37, 38</sup>. Whereas some broad-spectrum inhibitors such as  
173 SKF96365 and Cu<sup>2+</sup> - 1, 10-phenanthroline also inhibit TRPM8, others such as  
174 Ruthenium Red do not<sup>30</sup>. Ethanol is also an effective TRPM8 inhibitor<sup>36, 39</sup>.  
175 Natural herbal remedies such as peppermint oil or eucalyptus oil may contain as-  
176 yet-uncharacterised TRPM8 activators, but this remains to be tested. The field  
177 lacks truly selective agents, particularly inhibitors, for TRPM8, and research in  
178 the area will be made more difficult by recent evidence that vanilloid activators of  
179 TRPV1, notably including capsaicin and resiniferatoxin (and also  
180 agonists/antagonists for CB<sub>1</sub> receptors) are highly effective blockers of TRPM8  
181 activation by icilin<sup>40</sup>.

182

183 It is unclear whether there may be an endogenous ligand for TRPM8.  
184 Endogenous and natural exogenous ligands for the TRPV1 receptor have been  
185 identified, eg anandamide and resiniferatoxin (see van der Stelt and di Marzo for  
186 review<sup>41</sup>). Endogenous ligands for TRPM8 in mammals have yet to be identified,

187 although endogenous phospholipid metabolites such as lysophospholipids and  
188 phosphatidylinositol 4,5-bisphosphate have been shown to facilitate TRPM8  
189 channel function<sup>42-44</sup>.

190

### 191 **TRPM8 in cold temperature sensing**

192 TRPM8 is expressed by a subset (~10-15%) of small diameter primary afferents  
193 in DRG and trigeminal ganglia and can be activated by cooling or by menthol<sup>27, 28,</sup>  
194 <sup>45-47</sup>. While low doses of menthol produce a sensation of cooling and analgesia in  
195 chronic pain models<sup>46</sup>, much higher doses produce a noxious burning  
196 sensation<sup>48, 49</sup>, although it is not clear that TRPM8 is being specifically targeted at  
197 such doses<sup>50, 51</sup>. TRPM8 protein is normally co-expressed with peripherin (a  
198 marker of unmyelinated afferents) in the DRG. TRPM8 expression increases  
199 ipsilateral to CCI nerve injury and is also newly expressed in small myelinated  
200 (NF-200-positive), presumed-A $\delta$  fibre cells<sup>46, 52</sup>. However, no alterations in  
201 TRPM8 expression were reported in the SNL model of nerve injury, or in a model  
202 of inflammation<sup>53, 54</sup>. Studies of primary afferent: dorsal horn neuron synapses  
203 found that TRPM8 activation by menthol and/or cooling increased mEPSC  
204 frequency but not amplitude<sup>55-57</sup>, suggesting a location at presynaptic terminals of  
205 the dorsal horn. Indeed, TRPM8 is not thought to be expressed in spinal cord  
206 neurons, and any expression in spinal somatosensory pathways seems to  
207 originate entirely in the periphery<sup>28, 46</sup>.

208

209 Key insights into the physiological roles of TRPM8 in cool sensation and pain  
210 processing have come from a cluster of studies on TRPM8-null mice and other  
211 recent work. The evidence is now overwhelming for a major role of TRPM8 in  
212 cool sensation. Experiments with in vitro skin-nerve preparation<sup>58</sup> showed that  
213 significant subpopulations of C- and A $\delta$ - fibres were activated by cooling from  
214 32°C to 2°C in wild-type mice, but not in TRPM8-knockouts, corresponding to an  
215 earlier in vivo report of C- afferent axons activated by the selective TRPM8  
216 activator, icilin<sup>46</sup>. Furthermore, sensory ganglion cells from knockout mice  
217 showed greatly reduced Ca<sup>2+</sup>-elevation responses to cooling (range 20°C - 10 °C)



218 as well as to menthol and icilin<sup>58-60</sup>. Similarly, the behavioural shakes/jumps  
219 elicited by intraperitoneal injection of a high dose of icilin, suggested to be due to  
220 the cooling sensation of icilin<sup>61</sup>, were greatly reduced in TRPM8-null mice<sup>59, 60</sup>. In  
221 addition, temperature selection studies using either two-plate or multi-range  
222 choice chambers showed a preference of wild-type mice for floor temperatures of  
223 around 30°C rather than those in the range downwards to 15°C<sup>58-60</sup>. Such  
224 preferences were clearly reduced in TRPM8-null mice, indicating that the  
225 avoidance behaviour was TRPM8-mediated. In this temperature range the stimuli  
226 are unlikely to be overtly noxious however, so the behaviours probably reflect  
227 reactions to cool perception rather than cold pain<sup>62</sup>. Similarly, the licking/flinching  
228 responses to skin cooling by acetone were consistently reduced in TRPM8-null  
229 mice<sup>58-60</sup>, but measuring the skin temperature revealed that only innocuous  
230 temperatures > 15°C were reached<sup>59</sup>.

231

232 The question of whether TRPM8 plays a part in the noxious properties of intense  
233 cold stimuli is harder to answer. Paw withdrawal responses from cold surfaces  
234 around 0°C are generally considered to reflect noxious stimulus-evoked  
235 defensive behaviours. However, TRPM8-knockout mice showed prolonged cold  
236 plate paw flick latencies in only one of three reports<sup>59</sup>. Even the TRPM8  
237 knockout mice still find the cold-plate an aversive stimulus and a reduced (but still  
238 significant) subgroup of afferents from their skin respond to cold, indicating that  
239 there are clearly other sensors for noxious cold<sup>58-60</sup>. Possible mediators include  
240 TRPA1, but also other candidates<sup>63</sup>, whose case is supported by evidence of  
241 cool-sensitive, menthol-insensitive afferents that fail to respond to TRPA1  
242 activators<sup>64</sup> (see below). So, the evidence for TRPM8 as a direct mediator of cold  
243 pain per se is not strong, which in fact matches our intuitive understanding that  
244 modest cooling and contact with menthol or icilin at moderate doses really do not  
245 represent noxious experiences. Nevertheless, in CCI or CFA models of  
246 neuropathic or inflammatory pain, acetone application caused greatly increased  
247 behavioural responses that may reflect nociception and these were notably  
248 reduced in TRPM8-null mice<sup>59</sup>. Central sensitisation in these chronic pain models

249 leads to greatly accentuated sensory responses, so this does not necessarily  
250 suggest that the TRPM8-mediated acetone stimulus is in itself overtly noxious,  
251 but rather that it may be interpreted as so in this context. Cool allodynia following  
252 CCI has also been described<sup>46</sup> at skin temperatures less than 16°C but the  
253 involvement of TRPM8 was not investigated. This might suggest that in chronic  
254 pain states, attempts to activate TRPM8 would lead to pain. However, the  
255 selective TRPM8 activators, menthol and icilin consistently do not elicit pain  
256 responses or hypersensitivity at moderate doses, even in established pain  
257 states<sup>3, 46, 65</sup>. Thus TRPM8 activation alone appears insufficient to elicit cold pain.

258

### 259 **TRPM8 activation: pain or gain?**

260 Rather than eliciting cold pain by itself, it seems likely that TRPM8 may play  
261 some auxiliary role in this process. This would be consistent with the pro-  
262 nociceptive effect of intraplantar icilin in the cold plate test in wild-type but not  
263 TRPM8-null mice<sup>60</sup> and the sensitisation of reflex pain behaviours following  
264 topical application of high concentrations of icilin<sup>46</sup>. One hypothetical explanation  
265 might be that explicit cold pain actually requires the activation of dual inputs, both  
266 the direct mediator of noxious cold at <10°C (possibly TRPA1, see below) and  
267 also TRPM8. Noxious cold perception may thus require the activation of two  
268 distinct neural pathways for appropriate interpretation at higher centres. This will  
269 inevitably occur upon cooling from ambient temperatures down to the noxious  
270 temperature range since the procedure will clearly have surpassed the threshold  
271 for TRPM8 activation. Such a dual input logic gate might contribute to explaining  
272 the rather inconsistent observations with TRPM8 and TRPA1 knockouts in terms  
273 of noxious cold withdrawal responses. Dual knockout mice would help to address  
274 this hypothesis. However, the hyperalgesic effects of TRPA1 activation appear to  
275 be attenuated rather than facilitated by TRPM8 activation<sup>46</sup> arguing against this  
276 model, at least in terms of TRPA1 as cold mediator. An alternative hypothesis  
277 would be that there are two physiologically distinct subpopulations of TRPM8-  
278 containing afferents innervating the skin, one reflecting innocuous cool sensation  
279 and a second in which TRPM8 is expressed in nociceptors whose activation may

280 contribute to cold pain (Figure 1). A lower level of TRPM8 expression in the  
281 second group might explain the observation that pro-nociceptive effects of icilin  
282 are seen only at very high concentrations<sup>46, 60</sup>. TRPM8 is largely expressed in  
283 small afferents that are TRPV1-negative but TrkA-positive<sup>28, 32, 66</sup>. However, other  
284 reports describe from 10% up to 29% co-expression with TRPV1<sup>54, 67</sup> and there  
285 are a number of reports of trigeminal and DRG cells responding to both menthol  
286 and capsaicin, implying TRPM8/TRPV1 co-expression<sup>27, 47, 67, 68</sup>. Since TRPV1  
287 characterises heat/acid-sensitive nociceptors, activation of TRPM8 in the same  
288 cells would presumably also be perceived as noxious. This could contribute to  
289 cold pain. The extent of such co-expression may depend on the precise origin of  
290 the afferent cells investigated and the in vivo or in vitro conditions under which  
291 they are studied. Any co-expression of TRPM8 with TRPV1 could further account  
292 for the paradoxical sensations of burning hot pain when a cold stimulus is applied  
293 following experimental A-fibre block or following damage to A-fibres in  
294 demyelinating diseases<sup>69</sup>. Nevertheless, it seems clear that a substantial  
295 proportion of TRPM8-positive cells are not classical nociceptors and may be able  
296 to exert a quite different functional influence on pain processing<sup>46, 62</sup>.

297

298 Indeed, matching ancient and anecdotal descriptions of cooling- and menthol-  
299 induced analgesia, there is now clear evidence that we can gain from TRPM8  
300 activation in chronic sensitised pain states, where it elicits a novel analgesic  
301 influence. Activation of TRPM8 using peripherally (topically) or centrally  
302 (intrathecally) applied TRPM8 activators, menthol and icilin, can prevent the  
303 sensitisation of reflex pain behaviours and the increased responsiveness of  
304 single dorsal horn neurons that are induced in the CCI model of chronic  
305 neuropathic pain<sup>46</sup>. Similarly, analgesia is elicited by mild cooling of the skin  
306 (20°C -16°C range), which would be appropriate temperatures for TRPM8  
307 activation<sup>46</sup>. The icilin-induced analgesia is clearly mediated by TRPM8, as it is  
308 prevented by specific antisense knockdown of TRPM8. Icilin is also capable of  
309 producing analgesia in alternative chronic pain models, for example in Complete  
310 Freund's Adjuvant-induced inflammatory hypersensitivity and following

311 lysolecithin-induced demyelination. No effects on contralateral reflex responses  
312 were observed or in normal animals without a sensitised pain state. This, coupled  
313 with the requirement for only low doses of icilin, suggests that the use of TRPM8  
314 activators for analgesia in chronic pain states may be associated with a good  
315 index of therapeutic specificity. In addition, new evidence from the TRPM8-null  
316 mice further supports the concept of an analgesic role of the channel in pain  
317 states. Modest cooling to 17°C reduced early phase formalin-induced paw licking  
318 behaviours in wild type but not TRPM8-null mice<sup>60</sup>. The fact that late phase  
319 formalin responses were reduced by mild cooling in both wild type and TRPM8-  
320 null mice suggests not only that there may be additional molecular mediators of  
321 modest cooling but also that they too may contribute to cooling-induced  
322 analgesia.

323

#### 324 **Connecting cold inputs and pain processing**

325 If the analgesia produced by cold temperatures is mediated by activation of  
326 specific cold-responsive afferents, what are the possible mechanisms of this  
327 action? How can cold fibres affect central processing of pain? Since icilin can  
328 both activate a subpopulation of fine afferents and can elicit analgesia, there are  
329 likely to be key changes occurring in the central nervous system (CNS). The first  
330 step in central processing of both pain and cold afferents is in the dorsal horn of  
331 the spinal cord. Glutamate receptors are well-established mediators of central  
332 sensitisation; the enhanced spinal synaptic transmission that underlies a wide  
333 range of chronic pain states. Could inhibitory glutamate receptors be underlying  
334 the centrally mediated component of this analgesia? The glutamate receptors are  
335 classed as NMDA, non-NMDA and metabotropic receptors. Although NMDA and  
336 non-NMDA (AMPA, kainate) receptors are exclusively involved in the expression  
337 and enhancement of excitatory transmission, metabotropic receptors can either  
338 be excitatory (Group I, mGluR1, 5) or inhibitory (Group II/III). Thus, it is possible  
339 that cold fibres could inhibit pain messages in the spinal cord by means of  
340 inhibitory metabotropic glutamate receptors. Agonists for inhibitory subtypes of  
341 Group II/III mGluRs cause reversal of CCI-induced mechanical and thermal

342 behavioural sensitisation<sup>46</sup>. So these receptors could potentially mediate an  
343 endogenous analgesic pathway relying on glutamate release.

344

345 Notably, blocking the activation of these receptors with selective mGluR Group II  
346 or III antagonists prevents the ability of icilin to reverse behavioural sensitisation,  
347 suggesting that the central analgesic influence of TRPM8 activation in  
348 neuropathic pain is mediated by mGlu Group II/III receptors. Also, when the  
349 Group II/III antagonist UBP 1112 was applied to dorsal horn neurons, it could  
350 prevent the reduction in noxious stimulus-induced firing that was caused by  
351 peripheral application of icilin<sup>46</sup>. Interestingly, the opioid receptor antagonist,  
352 naloxone had no effect on icilin analgesia, suggesting that this phenomenon is  
353 independent of the classical opioid analgesic system.

354

355 The existence of a modulatory pain system was proposed by Melzack and Wall<sup>70</sup>  
356 in the 'Gate Control' theory of pain, which proposed that spinal nociceptive  
357 transmission could be inhibited by non-nociceptive inputs. In the original 'Gate  
358 Control' theory, this inhibition was proposed to be produced by low-threshold  
359 mechanosensitive A $\beta$  fibres, gating the input from nociceptive C and A $\delta$  fibres.  
360 However, it now seems possible that innocuous cold-sensitive small-diameter  
361 afferents could gate the information from nociceptive afferents. There is evidence  
362 for presynaptic inhibition of nociceptive afferents produced by activity in other  
363 small-diameter afferents: in one recent study repetitive activation of sciatic  
364 A $\delta$  fibres produced a presynaptically-mediated inhibition of saphenous C  
365 afferents<sup>71</sup>, and it has been shown that A $\delta$  fibre stimulation can cause a long-  
366 term depression of C fibre-evoked spinal field potentials<sup>72</sup>. Furthermore, a gating  
367 effect of innocuous cool-sensing afferents could be consistent with earlier  
368 observations, in that blockade of myelinated fibre input by selective conduction  
369 inhibition lowers the threshold for cold-induced pain, and results in the perception  
370 of cold pain as burning heat<sup>73, 74</sup>. Therefore it is possible that innocuous cold-  
371 sensitive fibres, which in humans are myelinated A $\delta$  afferents, suppress the  
372 incoming information from cold-sensitive polymodal nociceptive C fibres and that

373 removal of this inhibition by selective conduction block or demyelination unmasks  
374 cold-induced burning pain. The observation that the hyperalgesia induced by  
375 TRPA1 activation can be attenuated by simultaneous activation of TRPM8<sup>46</sup> is  
376 consistent with this scheme.

377

378 The precise cellular arrangements that might underlie the hypothetical TRPM8-  
379 driven gating system in dorsal horn are of course unclear. TRPM8 activators can  
380 act presynaptically to facilitate excitatory transmitter release<sup>55-57</sup>, although  
381 evidence for TRPM8-independent Ca<sup>2+</sup> mobilisation by menthol<sup>51</sup> may complicate  
382 interpretation. TRPM8 activation can also lead, presumably by indirect means, to  
383 increased postsynaptic excitability of dorsal horn neurons<sup>46</sup>. The inhibitory mGlu  
384 Group II/III receptors that appear to mediate icilin analgesia within the dorsal horn  
385 can be localised both presynaptically and postsynaptically<sup>75-77</sup>. In addition, it  
386 appears that TRPM8-positive afferents, which are also characterised by  
387 cadherin-8 expression, form complex glomerular synapses, in which the core  
388 axonal bouton is surrounded by several dendritic and axonal processes<sup>56</sup>.  
389 Furthermore, the complexity of the different types of synaptic arrangement in  
390 superficial dorsal horn is exemplified in recent work by Lu and Perl<sup>78</sup>. This  
391 illustrates multiple inhibitory and excitatory influences of monoamines observed  
392 in different subpopulations of superficial dorsal horn neurons and emphasises the  
393 principle that TRPM8-induced analgesia may in fact derive from the integration of  
394 a number of diverse polysynaptic processes occurring within this region.

395

396

### 397 **TRPA1 and other possible mediators of cold sensitisation**

398 While TRPM8 and TRPV1 might be expressed in distinct primary afferent  
399 populations, it appears that another putative cold channel, TRPA1 (formerly  
400 known as ANKTM1) that is expressed in about 20% of DRG neurons<sup>79</sup> shows a  
401 97% overlap with expression of TRPV1 but not TRPM8<sup>66</sup>. Thus it is tempting to  
402 suggest that there could be a population of cells that respond to both noxious  
403 heat and noxious cold stimuli, but do not detect innocuous cooling. TRPA1 has

404 been proposed to mediate detection of noxious cold, as TRPA1 is reported to be  
405 activated at temperatures below around 17°C, a temperature approaching pain  
406 for humans<sup>32, 80</sup>. However, there is conflicting evidence as to whether TRPA1 is  
407 the key mediator of noxious cold responses<sup>81</sup>. While TRPA1 knockdown reduced  
408 sensitised noxious cold responses following nerve injury<sup>53</sup>, homozygous TRPA1  
409 knockout mice surprisingly showed only partial (or no) attenuation of noxious cold  
410 withdrawal responses<sup>82, 83</sup>. TRPA1 was also proposed as a mediator of  
411 mechanotransduction in auditory stereocilia but knockout studies have failed to  
412 support this proposed role<sup>82, 83</sup>. TRPA1 is a menthol-insensitive channel that is  
413 activated by strong cold and noxious chemicals such as cinnamaldehyde and  
414 bradykinin<sup>32, 84, 85</sup>. TRPA1 is also a receptor for pungent isothiocyanates (which  
415 are found in wasabi and mustard) and for other natural products found in  
416 cinnamon, wintergreen, clove oil and garlic, such that TRPA1 may be involved in  
417 the inflammatory and vasodilator effects of these compounds<sup>84, 85</sup>. Recent  
418 evidence also indicates that TRPA1 may mediate the nociceptive actions of the  
419 industrial pollutant acrolein, and indeed underlie the formalin inflammatory pain  
420 model<sup>82, 86</sup>. Although there are some reports that TRPA1 does not respond to low  
421 temperatures<sup>85, 87</sup>, the range of thermal and pungent stimuli that have been  
422 described for this channel strongly associate its activation with nociception.  
423 Activators of TRPA1 such as cinnamaldehyde and allicin cause sensitisation of  
424 reflex pain behaviours in naïve animals and enhance the sensitisation already  
425 present following nerve injury<sup>46</sup>, while formalin itself is clearly noxious<sup>86</sup>. TRPA1  
426 can be activated by icilin, although less potently and more slowly than TRPM8,  
427 possibly by an indirect route<sup>32</sup>.

428

429 A number of other channels have also been proposed to be involved in cold  
430 transduction and the function of cold-sensitive afferents<sup>45, 68, 88-92</sup>, Table 1.  
431 Although at present the cold-sensitive TRP channels are the best-studied and  
432 perhaps the most promising candidates for involvement in sensory cold  
433 detection, it seems likely that we still only appreciate a small part of the overall  
434 picture.

435

## 436 **Conclusions**

437 In conclusion, the cloning of cool/cold-sensitive TRP family channels has  
438 provided a clear molecular basis that might explain cool sensation and cold pain.  
439 Different temperature thresholds for the main subject of this review, TRPM8, and  
440 for TRPA1, which is suggested (but disputed) to respond to more intense cold  
441 stimuli, provide a theoretical basis for cooling-induced analgesia in chronic pain  
442 states and cold pain respectively. The differential distribution of TRPM8 in non-  
443 nociceptive thermosensory afferents as well as in some nociceptive cells may go  
444 towards explaining how low doses of TRPM8 activators can cause active  
445 analgesia in chronic pain states, whilst TRPM8 can also contribute to cold pain.  
446 Studies with antisense deletion of TRPM8 and with TRPM8 knockout mice  
447 confirm that TRPM8 activation can elicit analgesia. The underlying mechanism  
448 appears to operate in spinal dorsal horn and rely on inhibitory mGlu Group II/III  
449 receptors, yet be independent of opioids. TRPM8 is not widely expressed, other  
450 than in sensory afferents, but is present in prostate cells and to a lesser extent in  
451 bladder epithelium<sup>93, 94</sup>. The TRPM8 channel is overexpressed in prostate  
452 malignancy<sup>94, 95</sup>, where TRPM8 activation has been shown to lead to increased  
453 apoptosis. The limited distribution of TRPM8 in tissues other than sensory  
454 afferents and the fact that even currently available TRPM8 activators are  
455 effective analgesics by topical cutaneous application as well as local spinal  
456 application support the idea that this may represent a viable therapeutic strategy  
457 for chronic pain states. Furthermore, these findings emphasise the need for the  
458 discovery of more specific TRPM8 agonists/antagonists so that the potential  
459 therapeutic role of this target in chronic pain can be fully evaluated.

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**Table 1: Candidate mediators of primary afferent responses to cooling.**

Channel	Temperature threshold	Reference
TRPM8	19-25°C in heterologous systems  28-30°C in trigeminal ganglion / DRG neurons	27, 28, 29, 30, 45, 47, 92
TRPA1	~17°C	32, 80
Candidates for cold-inhibited K <sup>+</sup> conductances: TREK-1, 2, TRAAK, unidentified background channel	25-31°C	68, 90, 96
Epithelial Na <sup>+</sup> channel (ENaC)	<25°C	88, 92

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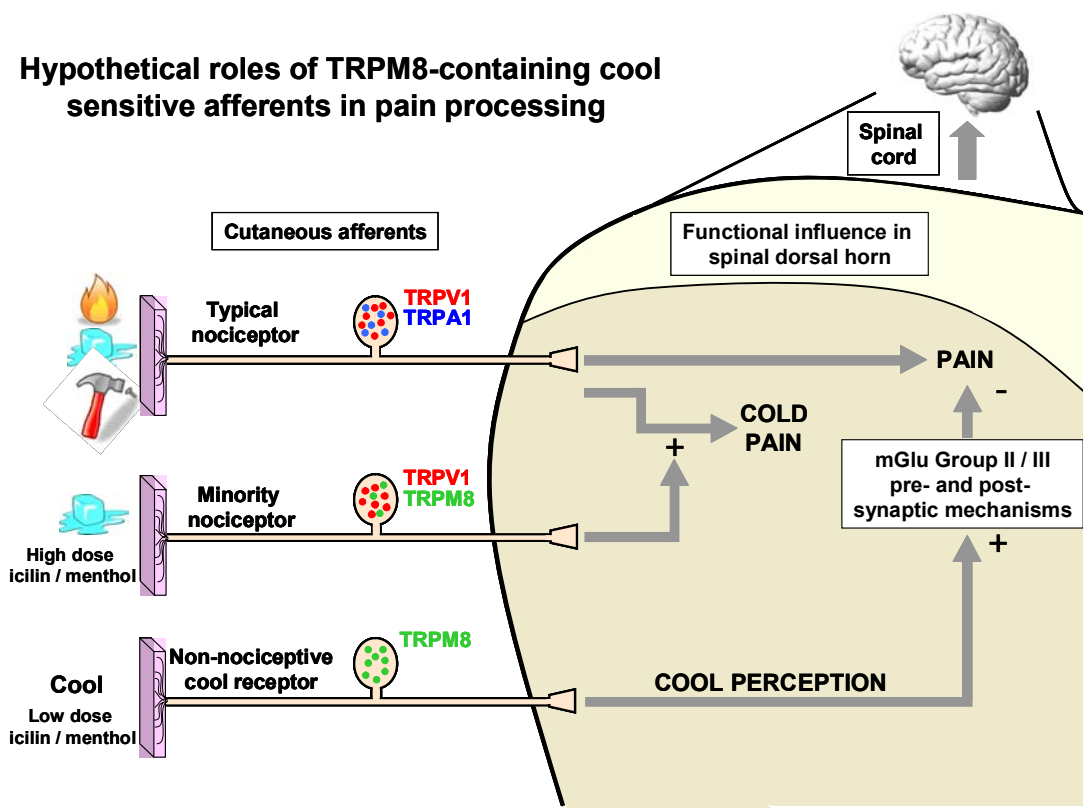
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**Figure 1**

**Schematic plan – hypothetical roles of TRPM8-containing afferents in pain processing**

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744 The diagram illustrates only selected subpopulations of afferents. In chronic pain states, repetitive  
 745 nociceptive input (1) brings about sensitisation of dorsal horn neurons with accentuated  
 746 responsiveness to both noxious and previously innocuous stimuli that is perceived at higher  
 747 centres as pain. This sensitised pain state is subject to powerful analgesia elicited by cooling or  
 748 low dose icilin/menthol through low threshold, non-nociceptive afferents (3) that act centrally  
 749 through mGlu Group II/III receptors. Intense cold leading to cold pain will activate nociceptors  
 750 containing TRPA1 and/or other intense cold detectors (1) and additionally any TRPM8-containing  
 751 afferents that are activated already by even mild cooling. We hypothesise that the minority  
 752 subpopulation of TRPM8-containing nociceptors (2) may contain lower numbers of TRPM8  
 753 channels than the cool afferents (3) and due to this or other factors may require somewhat more  
 754 intense TRPM8-mediated inputs to activate them. In this way the TRPM8-containing nociceptors  
 755 (2) would be activated by cold or perhaps high dose icilin/menthol (as opposed to cool or low  
 756 dose icilin/menthol) and may contribute actively to cold pain.  
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