TRPA1 as an Analgesic Target

Christopher M. Fanger, Donato del Camino, and Magdalene M. Moran*

Hydra Biosciences, 790 Memorial Drive, Cambridge, MA, 02139, USA

Abstract: Humans have cultivated chili peppers for over 5,000 years. For more than 1,000 years extracts from these plants have been used medicinally in the treatment of various forms of pain. As a result, pain researchers have had a long-standing interest in the molecular identity of the receptor for capsaicin, the chemical that produces the sensation of heat from "hot" peppers. When the Julius lab published that TRPV1 is the protein that confers capsaicin responsiveness [1], there was already significant investigation of this target in the pharmaceutical community. The future of TRPV1 as an analgesic target is currently uncertain because inhibiting TRPV1 attenuates patients' ability to sense damaging heat [2, 3] and because concerns have arisen that TRPV1 antagonists cause a transient hyperthermia. However, this interest in TRPV1 has spurred interest in a variety of other TRP channels in the pain area. As the receptor for mustard oil and other noxious compounds that cause pain, TRPA1 in particular has emerged as a promising target. Recent data suggest that TRPA1 is a broad chemosensor, activated by reactive chemicals that are encountered exogenously and by compounds such as hydrogen peroxide that are endogenously produced during inflammation or tissue damage. In this review, we explore the rationale surrounding the use of TRPA1 as an analgesic target and discuss the unique challenges that face those developing antagonists.

Keywords: TRP channel, TRPA1, HC-030031, AP18, AITC, formalin, pain, analgesic, neurogenic, inflammation, asthma.

TRP CHANNELS

The first Transient Receptor Potential (TRP) ion channel was identified in *Drosophila* in a screen for fly blindness. *Trp* mutant flies displayed a transient receptor potential in response to prolonged light [4]. Since then, dozens of structurally related proteins have been identified in numerous branches of the evolutionary tree including yeast, *C. elegans*, and mammals. In mammals, the TRP superfamily of cation channels consists of 28 members with a wide range of physiological functions. Six families comprise the superfamily: TRPV, TRPM, TRPP, TRPC, TRPML and TRPA [5]. TRPA1 distinguishes itself from other TRP channels with a large, cysteine-rich N-terminus that contains 18 predicted ankyrin repeats [6]. Because of its unique structure, it comprises the entirety of the mammalian TRPA family [5], though there are multiple TRPA family members in invertebrates [7].

Mammalian TRPA1 was originally cloned as ANKTM1, a 1119 amino acid protein whose expression was lost from human fibroblasts following oncogenic transformation [6]. Later work revealed that TRPA1 is a non-selective, calcium-permeable cation channel that is highly expressed by nociceptors [8-10]. In particular, a subset of small diameter sensory neurons with cell bodies in the dorsal root, trigeminal, nodose and jugular ganglia that also express the capsaicin receptor, TRPV1, show high levels of TRPA1 mRNA [8-11]. TRPA1 expression increases with age in the mouse, indicating that the transcript is developmentally regulated [12].

*Address correspondence to this author at the Hydra Biosciences, 790 Memorial Drive, Cambridge, MA, 02139, USA; Tel: 617-494-5230, Ext. 3324; Fax: 617-494-5230; E-mail: mmoran@hydrabiosciences.com

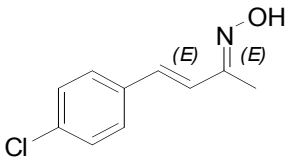
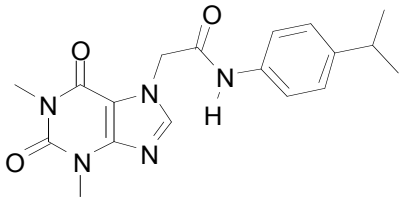
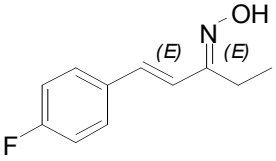
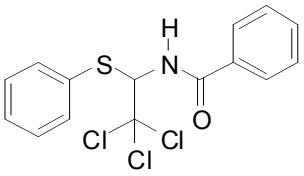
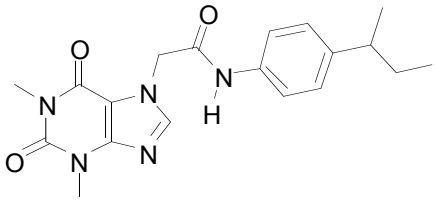
Some recent work suggests the potential for broader TRPA1 expression. TRPA1 expression is reported in keratinocytes and some epithelial cells [13, 14], but definitive results showing functional TRPA1 currents have been hard to obtain because these cells change in culture and often do not respond to mustard oil. Similarly, there have been a variety of conflicting reports regarding the expression of TRPA1 in pathological conditions, both inflammatory and neuropathic. While some groups report an increase in TRPA1 levels in a variety of pain models [15-17], a number of groups see no such change and sometimes even report a decrease [18, 19]. The lack of an antibody that robustly stains TRPA1 in normal tissue but does not stain tissue from *Trpa1*$^{-/-}$ animals complicates the interpretation of data concerning TRPA1 expression. It is hoped that new reagents [20] will be useful in clarifying these issues.

STATE OF TRPA1 ANTAGONISTS

Because so much of the data that established TRPA1 as a pain target come from pharmacological agents, it is important to understand the available tools. Active drug discovery efforts have resulted in the production of a few commercially available, selective inhibitors of TRPA1. Promiscuous channel blockers such as ruthenium red and gadolinium are known to block TRPA1. Amiloride [9], camphor [21], and menthol [22] have all been shown to antagonize TRPA1 in the millimolar range. Gentamycin and other antibiotics are somewhat more potent [9], but the first available selective blockers of TRPA1 that worked in the range of 1 micromolar and below were HC-030031 [23] and AP-18 [24]. These have been the basis for the vast majority of the *in vivo* pharmacology done on TRPA1. Other structurally related compounds CHEM-5861528 [25] and A-967079 (CHI Presentation, Regina Reilly [26]) have also recently been reported. Though many studies employing

1877-3818/10

Table 1. TRPA1 Antagonist Chart

Compound	Structure	Potency (hTRPA1)	Reference	Rat Activity
AP-18		3.1 microM in calcium flux assay (Cinnamaldehyde agonist)	Petrus *et al.,* 2007 *Mol Pain*	Antagonist, similar potency
HC-030031		1 microM in whole cell patch clamp (AITC agonist)	McNamara *et al*., 2007 *Proc Natl Acad Sci USA*	Antagonist, similar potency
A-967079		51 nM in whole cell patch (AITC agonist)	Reilly *et al*., 2009 *CHI Presentation, WO2009/089082*	Antagonist, similar potency
AMG-9090		21 nM in Ca^{45} assay (AITC agonist)	Klionsky *et al*., 2007 *Mol Pain*	Agonist in rat
CHEM-5861528		14.3 microM in calcium flux assay (AITC agonist)	Wei *et al*., 2009 *Anaesthesiology*	Antagonist, similar potency

these tools have demonstrated the significance of TRPA1 in analgesia, none of these compounds seems immediately suitable as a drug in humans. One factor that may contribute to the relative dearth of useful TRPA1 antagonists is the degree of divergence in TRPA1 sequence between species. This difficulty has been highlighted by both the Amgen [27] and Abbott [28] groups, who have published on compounds that are antagonists of the human TRPA1, but are either inactive or are agonists against the rat version of the channel. Consequently, these compounds can not be tested in pre-clinical rat models. Though TRPA1 presents some unique challenges, a great deal of effort in the pharmaceutical industry continues to focus on finding more potent, drug-like inhibitors of this channel (Table **1**).

TRPA1 AS IRRITANCY RECEPTOR

The physiological role for TRPA1 has been controversial. In 2003, it was proposed to be the noxious cold receptor and the first electrophysiological recordings were published [8]. Though it now seems clear that cold increases current of recombinantly expressed TRPA1, whether TRPA1 serves this function *in vivo* is unclear. The role that invertebrate TRPA channels play in mechanosensation and the observation that an anti-TRPA1 antibody stained hair cells led to the hypothesis that TRPA1 is the elusive mechanosensitive channel that translates soundwaves into neural signals in the ear [29]. However, hearing was unaffected in subsequent gene deletion experiments [30, 31] and the specificity of the antibody was called into question (personal communication, David Corey). Since TRPA1 expression was also detected in the cochlea using in situ hybridization [29, 32], the possibility remains that the channel plays a more subtle role in hearing.

Taking a candidate gene approach based on the expression pattern of TRPA1 and the knowledge that the chemosensitive neurons are a subset of the capsaicin-sensitive population, the Julius lab demonstrated in 2004 that mustard oil and THC activated the recombinant channel [10]. Numerous other chemically reactive ligands emerged over the next few years, including cinnamaldehyde [33], allicin [34, 35], and acrolein [31]. The chemical diversity of TRPA1 activators combined with the preponderance of cysteines in the N-terminus led to the exploration of the mechanism of TRPA1 activation. An elegant series of studies demonstrated that reactive chemicals can covalently bind to the N-terminal cysteines, resulting in channel

activation [36, 37]. The mechanism by which cysteine modification is translated into channel activity remains unclear, but a recent report suggests dynamic translocation of TRPA1 to the plasma membrane may be partially responsible [20]. It is TRPA1's responsiveness to reactive chemicals that makes channel activation independent of a traditional "lock and key" mechanism of activation, and that positions it as a sensor for diverse chemical agents that can cause tissue damage.

A surprisingly broad role for TRPA1 in chemosensation is revealed by the combination of results from experiments employing genetic deletion of TRPA1 or specific pharmacologic inhibitors. Either method of abrogating TRPA1 function eliminates sensitivity to a variety of reactive chemicals, including ones previously thought to act by inducing non-specific tissue damage. Among these are allyl isothiocyante (AITC, commonly called mustard oil) [23, 30, 31, 38], cinnamaldehyde [24], formalin [23, 39], hydrogen peroxide [40], isocyanates and tear gases [41, 42], the effects of which we will now explore in more detail.

Perhaps the most studied of these agonists is AITC. As was predicted from early work, genetic deletion of TRPA1 eliminated AITC-induced Ca^{2+} responses in isolated sensory neurons (dorsal root or trigeminal) [30, 31]. Consequently, AITC-induced thermal hyperalgesia and mechanical allodynia [31] were significantly reduced in animals lacking functional TRPA1. Addressing the same question pharmacologically, HC-030031, a specific TRPA1 antagonist, inhibited AITC-induced flinching in the rat [23, 38]. As a result of these studies and others, AITC is now thought of as a selective TRPA1 agonist, at least at low concentrations. Similar inhibition of pain behaviours was observed in a cinnamaldehyde-induced flinching model with another TRPA1 antagonist, AP-18 [24].

Formaldehyde is a protein- and DNA-crosslinking agent that is the active ingredient in formalin. Injecting formalin into the paw of an animal, most often a rodent, results in immediate pain behaviours that persist for about five minutes. That phase is followed by approximately 5-10 minutes during which the animals display relatively little pain behaviour. Subsequently, a second period of pain behaviours occurs that is thought to be caused by continued afferent input and central sensitization. This formalin model has been used since the 1970s in the study of pain, but until recently the mechanism by which formalin causes pain was not well understood. Recent work has shown that animals lacking functional TRPA1, either because of treatment with HC-030031 or through genetic deletion, fail to show pain behaviours in response to formalin injection [23]. Furthermore, in a saphenous skin nerve preparation either pharmacological block or genetic deletion eliminates formalin-induced neuronal firing without affecting capsaicin-induced firing. Thus formalin, too, specifically agonizes TRPA1 when applied to isolated cells at concentrations up to 0.01% [23, 43]. As with AITC however, higher concentrations of formalin will cause tissue damage that is independent of TRPA1.

Acrolein is one of the key noxious products of combustion, and thus an important component of inhaled smoke from cigarettes and other sources. It is a reactive α,β unsaturated aldehyde that induces cough, shortness of breath and mucus production through the activation of c-fibers that innervate the lung. Genetic deletion of TRPA1 in mice eliminates acrolein-induced calcium influx in cultured neurons. Additional work in guinea pigs has demonstrated that treatment with HC-030031 prevents cigarette smoke-induced extravasation in the trachea [44]. The dependence of acrolein responses on TRPA1 highlights the function of TRPA1 in the respiratory system and underscores its importance outside of the pain arena.

Other noxious chemicals requiring TRPA1 to exert their effects include chlorine [40], 2-pentanal [31, 45], cinnamaldehyde [24, 33], allicin [34, 35], tear gases [41, 42], methyl isocyante, and hexamethylene diisocyanate (HDI) [42]. It is likely that TRPA1 antagonists are relevant to the inhibition of pain caused by any of these reactive chemicals.

However, in addition to the huge number of exogenous reactive chemicals that activate TRPA1, the channel also responds to reactive chemicals produced by the body. These include 4-hydroxynonenal (4-HNE) [39, 46, 47], 4-hydroxy oxononenal (4-ONE) [46], hydrogen peroxide [40], reactive prostaglandins [36, 48-50], and nitric oxide [51]. 4-HNE is a product of liposome peroxidation that is produced during inflammation or oxidative stress and which causes pain-related behaviours upon injection. The 4-HNE-induced pain response is dependent on TRPA1, as shown by the fact that genetic deletion of TRPA1 reduces the sensitivity of animals to 4-HNE [47], and confirmed by the dramatic increase in TRPA1 channel activity exhibited following 4-HNE treatment [39, 47]. Similarly, A- and J-series prostaglandins that are metabolites of PGE2 and PGD2, respectively, activate TRPA1 in heterologous expression systems and induce calcium influx in the subset of sensory neurons that respond to other TRPA1 activators [48-50, 52]. These responses were blocked by the non-specific TRP channel antagonist ruthenium red as well as by HC-030031 [48]. As would be expected for a TRPA1 agonist, injection of 15-delta PGJ2 evokes pain when injected *in vivo* [53]. It is these endogenous agonists of TRPA1 that most likely define the role of the channel in pathological conditions.

In addition to the ligands that activate TRPA1 through covalent modification, there are a number of TRPA1 agonists that specifically bind and activate the channel in a cysteine-independent fashion. One of the most important of these is calcium, which dramatically increases TRPA1 current [54-56]. As a result, TRPA1 activity may be coupled with a variety of other channels and signalling pathways that modulate intracellular calcium levels. Intracellular alkalinization also potentiates TRPA1 activity and augments the ability of the channel to sense tissue damage [57, 58]. In addition, recent work has shown that intracellular zinc agonizes TRPA1 with an EC50 of 2 μM [59]. Interestingly, zinc is also an irritant and inhaled zinc can cause zinc fume fever in welders.

Exogenous non-covalent agonists include menthol, which exerts a species-dependent effect on the channel [22], icilin [8] and 2-APB [36]. More clinically relevant is the observation that a variety of anaesthetic compounds activate TRPA1. These include propofol and etomidate, which cause burning pain upon injection [60]. Volatile anaesthetics such as isofluorane [60] and local anaesthetics including lidocaine [61] also activate TRPA1 in cultured cells.

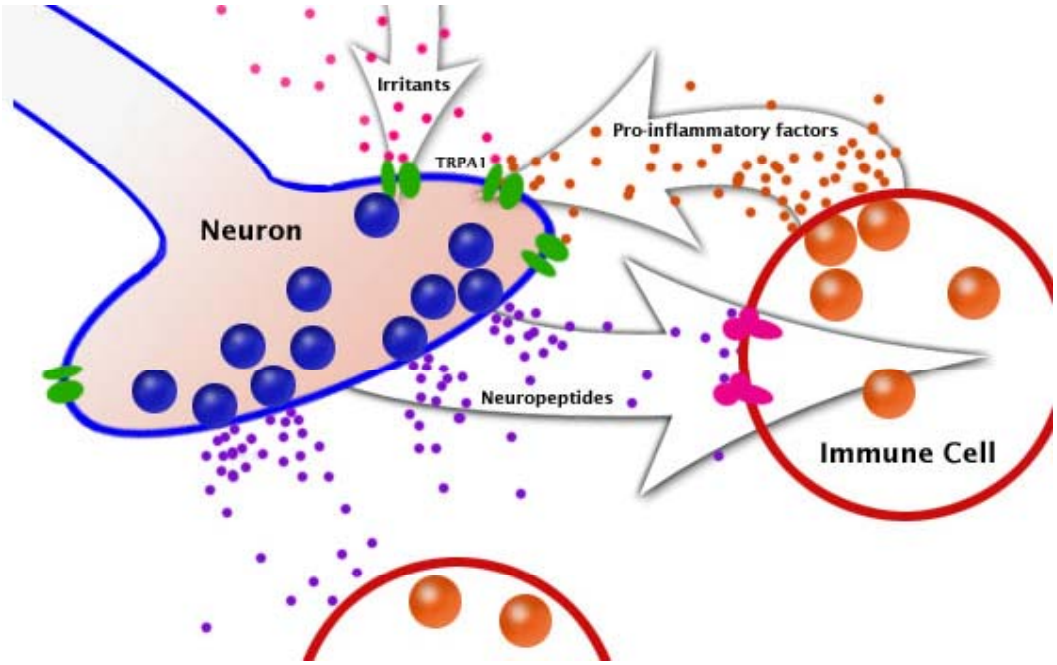


Fig. (1). Irritant mediated activation of TRPA1 leads to neuronal firing and the subsequent release of neurotransmitters and neuropeptides. These and other factors recruit immune cells to the site of neuronal activity. These immune cells release additional TRPA1 agonists and re-excite the neuron, leading to a feed forward loop.

The ability of TRPA1 to sense both damaging and irritating chemicals and its promiscuity have led to the proposal that TRPA1 functions *in vivo* as a general "irritancy receptor".

TRPA1 IN INFLAMMATION AND INFLAMMATORY PAIN

In addition to their well-established role in the detection of noxious stimuli, there is much data that suggest sensory neurons also play an important role in the generation and maintenance of inflammatory pain. Because of the robust expression of TRPA1 in sensory neurons and the channel's ability to respond to so many endogenously produced chemicals, TRPA1 is uniquely positioned to influence inflammation and inflammatory pain. Indeed, a reduction in the amount of functional TRPA1 inhibits several types of inflammatory pain. In addition, studies examining the role of TRPA1 in asthma suggest that inhibiting TRPA1 reduces not only inflammatory pain, but also inflammation *per se* [62]. These findings have led us and others to propose that TRPA1 is a key mediator of neurogenic inflammation (See Fig. **1**).

Numerous reports provide data that are consistent with this hypothesis. TRPA1 activation induces edema and secondary hyperalgesia. This is clearly shown in a range of studies exploring the effects of AITC on various tissues. Applied to the mouse ear, it results in a significant swelling that persists for several hours [63]. Injection into the paw leads to a TRPA1-dependent secondary thermal and mechanical hypersensitivity [30, 31]. In addition, injection of AITC into the masseter muscle induces nocifensive responses and changes in mechanical sensitivity that can be prevented by pre-treatment with either AP-18 or HC-030031 [64]. Similarly, intracolonic injection induces pain behaviours that are diminished by anti-sense nucleotides directed against TRPA1 [17] and *Trpa1*$^{-/-}$ mice have reduced visceral pain after intracolonic injection [65]. Taken together, these data demonstrate that activation of TRPA1 can cause cutaneous, visceral and muscular pain and, therefore, that TRPA1 antagonists have the potential to be efficacious against these types of pain.

Inflammatory pain may be particularly well-suited for treatment by TRPA1 antagonists. We propose that TRPA1 promotes inflammatory responses through its involvement in both neural (direct) and immune cell (indirect) activation. TRPA1 stimulation leads to increased firing of sensory neurons. Such neuronal activity induces the release of a variety of neuropeptides and neurotransmitters, such as NK-A, substance P, and CGRP. These agents induce vasodilation and, in conjunction with other factors, recruit immune cells to the site of activity. The recruited immune cells will, in turn, secrete numerous signalling molecules, including the TRPA1 agonists hypochlorite (neutrophils), hydrogen peroxide (granulocytes), and prostaglandins (mast cells, macrophages, dendritic cells) that will reactivate the neuron [48, 66, 67]. Simultaneously, the tissue damage that often accompanies TRPA1 activation leads to the production of reactive oxygen species that subsequently induce liposome peroxidation and the generation of additional TRPA1 activators including 4-ONE and 4-HNE [39, 47]. Consequently, TRPA1 is positioned as a key mediator of the feed forward loop that allows local inflammation to persist.

Consistent with a potential role for TRPA1 in inflammation, *in vivo* experiments have shown that inhibition or deletion of TRPA1 is analgesic in a number of animal models of inflammatory pain. For example, experiments relying on two independently generated TRPA1 knockout lines demonstrate that functional TRPA1 is required for bradykinin-induced thermal hyperalgesia [31] and mechanical allodynia [30]. Sensory neurons isolated from Trpa1$^{-/-}$ mice exhibit vastly attenuated calcium influx in response to bradykinin. Thus, although bradykinin functions

through its own GPCR and does not directly act on TRPA1, it requires functional TRPA1 for its effects. These results suggest that TRPA1 may be important for the maintenance of inflammatory pain that did not originate with TRPA1 activation.

Experiments with pharmacological antagonists of TRPA1 also support the hypothesis that TRPA1 plays a broad role in inflammatory pain. Injection of complete Freund's adjuvant (CFA) into rodent paws results in an inflammatory response and an increased sensitivity to thermal and mechanical pain. The TRPA1 antagonists AP-18 [24] and HC-030031 [38] both significantly reduce CFA-induced mechanical hypersensitivity. Since *Trpa1*$^{-/-}$ mice do not show such a reduction in hypersensitivity following AP-18 treatment, we may conclude that this is an "on-target effect". This effect does not represent a general anti-inflammatory effect, as AP-18 has no effect on CFA-induced heat hyperalgesia [24].

Treatment with TRPA1 antagonists results in larger pain deficits than genetic deletion. TRPA1 knockouts showed no defect in mechanical hypersensitivity after inflammation, whereas HC-030031 or AP-18 treated animals had reduced CFA-induced mechanical hypersensitivity [24, 38]. The discrepancy between these experiments and those relying on acute pharmacological inhibition highlights the challenges in using transgenic animals to probe the *in vivo* function of TRP channels. Specific pharmacological agents will be a necessary partner of genetically modified animals in the elucidation of the *in vivo* function of TRP channels, particularly in the pain area.

NEUROPATHIC AND DIABETIC PAIN

The role of TRPA1 in neuropathic pain remains relatively unexplored. Deletion of TRPA1 in the mouse did not impact the development of mechanical allodynia after spared nerve injury. In contrast, HC-030031 reversed mechanical allodynia approximately six weeks after spinal nerve ligation surgery in the rat, again illustrating the conflict between studies relying on genetic deletion *vs* pharmacological inhibition of the channel [38]. In the same model, cold hypersensitivity was reduced one week post spinal nerve ligation by administration of anti-sense nucleotides directed against TRPA1. Mechanical hypersensitivity was unaffected. Most recently, in a study examining the role of TRPA1 in diabetic neuropathy, Chembridge-5861528 (an antagonist structurally similar to HC-030031) significantly reduced mechanical hypersensitivity in the streptazotocin model of diabetes mellitus. Interestingly, prolonged pre-treatment with Chembridge-5861528 also prevented the development of mechanical hypersensitivity in this model, even after the acute effects of the drug had worn off, suggesting a role for TRPA1 in the disease process [25]. Additional studies will be required to elucidate both the degree to which TRPA1 is involved in neuropathic pain and the mechanism of its involvement.

TRPA1 IN COLD PAIN

One of the most controversial areas in TRPA1 biology has been its role in cold sensing. It was originally proposed that TRPA1 was the noxious cold receptor, responding to temperatures below 15°C [8]. Subsequent *in vitro* work showed that there was little overlap between cold-sensitive and AITC-responsive trigeminal neurons, challenging the role for TRPA1 in cold detection in a native system [10]. Genetic deletion of TRPA1 failed to resolve this controversy. Work from the Julius and Basbaum groups in mice that lacked functional TRPA1 suggested that the percentage of cold-responsive neurons was similar to that in wild type mice, arguing against a role for TRPA1 in the detection of noxious cold [31]. However, *Trpa1*$^{-/-}$ animals from the Corey and Woolf labs exhibited a deficit in cold responsiveness that was more apparent in female animals [30]. Still more recent studies show that cooling activates TRPA1 in a recombinant expression system and a subset of cold-responsive neurons seemed to be absent from acutely isolated trigeminal neurons taken from mice lacking functional TRPA1. In addition, in accompanying behavioural studies, TRPA1-deficient mice demonstrated altered responses to cold, including a dramatic decrease in the number of responses on a 0° C cold plate [68].

Regardless of whether TRPA1 is important for the detection of acute noxious cold, it is clear that in some pathological conditions, cold hypersensitivity can be reduced by antagonizing TRPA1. For example, in the CFA model of inflammatory pain, treatment with AP-18 dramatically decreased cold responses [24]. Cold hypersensitivity can also be inhibited by lowering functional TRPA1 levels with antisense oligonucleotides [15, 69]. More recently, it was shown that cloquinol induced cold allodynia depends on the presence of functional TRPA1 in mice [70].

Additional work will be required to determine the role of TRPA1 in cold sensation in both normal and pathological conditions. In addition, it will be important to define the relationship between TRPA1 and TRPM8, a known cold sensor [71-73].

CONCLUSIONS

TRPA1 plays a variety of physiological roles as a sensor of irritating chemicals and cold, and a participant in both inflammatory pain and airway hyperresponsiveness. In addition to its obvious allure as a target for inflammatory pain, it may also be therapeutically useful for targeting some types of neuropathic pain and even asthma. The wide availability of selective TRPA1 blockers may result in the discovery of additional therapeutic applications for new, improved antagonists. It is likely that the excitement surrounding TRPA1 will soon lead to the discovery of such agents, and the true evaluation of its therapeutic potential in the clinic.

REFERENCES

[1] Caterina, M.J.; Schumacher, M.A.; Tominaga, M.; Rosen, T.A.; Levine, J.D.; Julius, D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature,* **1997**, *389*(6653), 816-824.

[2] Gavva, N.R.; Bannon, A.W.; Surapaneni, S.; Hovland, D.N. Jr.; Lehto, S.G.; Gore, A.; Juan, T.; Deng, H.; Han, B.; Klionsky, L.; Kuang, R.; Le, A.; Tamir, R.; Wang, J.; Youngblood, B.; Zhu, D.; Norman, M.H.; Magal, E.; Treanor, J.J.; Louis, J. C. The vanilloid receptor TRPV1 is tonically activated *in vivo* and involved in body temperature regulation. *J. Neurosci.,* **2007**, *27*(13), 3366-3374.

[3] Gavva, N.R.; Treanor, J.J.; Garami, A.; Fang, L.; Surapaneni, S.; Akrami, A.; Alvarez, F.; Bak, A.; Darling, M.; Gore, A.; Jang, G.R.; Kesslak, J.P.; Ni, L.; Norman, M.H.; Palluconi, G.; Rose, M.J.; Salfi, M.; Tan, E.; Romanovsky, A.A.; Banfield, C.; Davar, G. Pharmacological blockade of the vanilloid receptor TRPV1

elicits marked hyperthermia in humans. *Pain,* **2008**, *136*(1-2), 202-210.

[4] Minke, B. Drosophila mutant with a transducer defect. *Biophys. Struct. Mech.,* **1977**, *3*(1), 59-64.

[5] Clapham, D.E. TRP channels as cellular sensors. *Nature,* **2003**, *426*(6966), 517-524.

[6] Jaquemar, D.; Schenker, T.; Trueb, B. An ankyrin-like protein with transmembrane domains is specifically lost after oncogenic transformation of human fibroblasts. *J. Biol. Chem.,* **1999**, *274*(11), 7325-7333.

[7] Montell, C. Drosophila TRP channels. *Pflugers Arch,* **2005**, *451*(1), 19-28.

[8] Story, G.M.; Peier, A.M.; Reeve, A.J.; Eid, S.R.; Mosbacher, J.; Hricik, T.R.; Earley, T.J.; Hergarden, A.C.; Andersson, D.A.; Hwang, S.W.; McIntyre, P.; Jegla, T.; Bevan, S.; Patapoutian, A. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell,* **2003**, *112*(6), 819-829.

[9] Nagata, K.; Duggan, A.; Kumar, G.; Garcia-Anoveros, J. Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. *J. Neurosci.,* **2005**, *25*(16), 4052-4061.

[10] Jordt, S.E.; Bautista, D.M.; Chuang, H.H.; McKemy, D.D.; Zygmunt, P.M.; Hogestatt, E.D.; Meng, I.D.; Julius, D. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature,* **2004**, *427*(6971), 260-265.

[11] Yu, S.; Ouyang, A. TRPA1 in bradykinin-induced mechanical hypersensitivity of vagal C fibers in guinea pig esophagus. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **2009**, *296*(2), G255-265.

[12] Hjerling-Leffler, J.; Alqatari, M.; Ernfors, P.; Koltzenburg, M. Emergence of functional sensory subtypes as defined by transient receptor potential channel expression. *J. Neurosci.,* **2007**, *27*(10), 2435-2443.

[13] Kwan, K.Y.; Glazer, J.M.; Corey, D.P.; Rice, F.L.; Stucky, C.L. TRPA1 modulates mechanotransduction in cutaneous sensory neurons. *J. Neurosci.,* **2009**, *29*(15), 4808-4819.

[14] Biro, T.; Kovacs, L. An "ice-cold" TR(i)P to skin biology: the role of TRPA1 in human epidermal keratinocytes. *J. Invest. Dermatol.,* **2009**, *129*(9), 2096-2099.

[15] Obata, K.; Katsura, H.; Mizushima, T.; Yamanaka, H.; Kobayashi, K.; Dai, Y.; Fukuoka, T.; Tokunaga, A.; Tominaga, M.; Noguchi, K. TRPA1 induced in sensory neurons contributes to cold hyperalgesia after inflammation and nerve injury. *J. Clin. Invest.,* **2005**, *115*(9), 2393-2401.

[16] Frederick, J.; Buck, M.E.; Matson, D.J.; Cortright, D.N. Increased TRPA1, TRPM8, and TRPV2 expression in dorsal root ganglia by nerve injury. *Biochem. Biophys. Res. Commun.,* **2007**, *358*(4), 1058-1064.

[17] Yang, J.; Li, Y.; Zuo, X.; Zhen, Y.; Yu, Y.; Gao, L. Transient receptor potential ankyrin-1 participates in visceral hyperalgesia following experimental colitis. *Neurosci. Lett.,* **2008**, *440*(3), 237-241.

[18] Caspani, O.; Zurborg, S.; Labuz, D.; Heppenstall, P.A. The contribution of TRPM8 and TRPA1 channels to cold allodynia and neuropathic pain. *PLoS One,* **2009**, *4*(10), e7383.

[19] Staaf, S.; Oerther, S.; Lucas, G.; Mattsson, J.P.; Ernfors, P. Differential regulation of TRP channels in a rat model of neuropathic pain. *Pain,* **2009**, *144*(1-2), 187-199.

[20] Schmidt, M.; Dubin, A.E.; Petrus, M.J.; Earley, T J.; Patapoutian, A. Nociceptive Signals Induce Trafficking of TRPA1 to the Plasma Membrane. *Neuron,* **2009**, *64*(64), 498-509.

[21] Xu, H.; Blair, N.T.; Clapham, D.E. Camphor activates and strongly desensitizes the transient receptor potential vanilloid subtype 1 channel in a vanilloid-independent mechanism. *J. Neurosci.,* **2005**, *25*(39), 8924-8937.

[22] Karashima, Y.; Damann, N.; Prenen, J.; Talavera, K.; Segal, A.; Voets, T.; Nilius, B. Bimodal action of menthol on the transient receptor potential channel TRPA1. *J. Neurosci.,* **2007**, *27*(37), 9874-9884.

[23] McNamara, C.R.; Mandel-Brehm, J.; Bautista, D.M.; Siemens, J.; Deranian, K.L.; Zhao, M.; Hayward, N.J.; Chong, J.A.; Julius, D.; Moran, M.M.; Fanger, C.M. TRPA1 mediates formalin-induced pain. *Proc. Natl. Acad. Sci. USA,* **2007**, *104*(33), 13525-13530.

[24] Petrus, M.; Peier, A.M.; Bandell, M.; Hwang, S.W.; Huynh, T.; Olney, N.; Jegla, T.; Patapoutian, A. A role of TRPA1 in mechanical hyperalgesia is revealed by pharmacological inhibition. *Mol. Pain,* **2007**, *3*, 40.

[25] Wei, H.; Hamalainen, M.M.; Saarnilehto, M.; Koivisto, A.; Pertovaara, A. Attenuation of mechanical hypersensitivity by an antagonist of the TRPA1 ion channel in diabetic animals. *Anesthesiology,* **2009**, *111*(1), 147-154.

[26] Perner, R.J.; Kort, M.E.; Didomenico, S., Jr.; Chen, J.; Vasudevan, A. TRPA1 Antagonists. PCT/US2009/030005, 16 July **2009**.

[27] Klionsky, L.; Tamir, R.; Gao, B.; Wang, W.; Immke, D.C.; Nishimura, N.; Gavva, N.R. Species-specific pharmacology of Trichloro (sulfanyl) ethyl benzamides as transient receptor potential ankyrin 1 (TRPA1) antagonists. *Mol. Pain,* **2007**, *3*, 39.

[28] Chen, J.; Zhang, X.F.; Kort, M.E.; Huth, J.R.; Sun, C.; Miesbauer, L.J.; Cassar, S.C.; Neelands, T.; Scott, V.E.; Moreland, R.B.; Reilly, R.M.; Hajduk, P.J.; Kym, P.R.; Hutchins, C.W.; Faltynek, C.R. Molecular determinants of species-specific activation or blockade of TRPA1 channels. *J. Neurosci.,* **2008**, *28*(19), 5063-5071.

[29] Corey, D.P.; Garcia-Anoveros, J.; Holt, J.R.; Kwan, K.Y.; Lin, S.Y.; Vollrath, M.A.; Amalfitano, A.; Cheung, E.L.; Derfler, B.H.; Duggan, A.; Geleoc, G.S.; Gray, P.A.; Hoffman, M.P.; Rehm, H.L.; Tamasauskas, D.; Zhang, D.S. TRPA1 is a candidate for the mechanosensitive transduction channel of vertebrate hair cells. *Nature,* **2004**, *432*(7018), 723-730.

[30] Kwan, K.Y.; Allchorne, A.J.; Vollrath, M.A.; Christensen, A.P.; Zhang, D.S.; Woolf, C.J.; Corey, D.P. TRPA1 Contributes to Cold, Mechanical, and Chemical Nociception but Is Not Essential for Hair-Cell Transduction. *Neuron,* **2006**, *50*, 277-289.

[31] Bautista, D.M.; Jordt, S.E.; Nikai, T.; Tsuruda, P.R.; Read, A.J.; Poblete, J.; Yamoah, E.N.; Basbaum, A.I.; Julius, D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell,* **2006**, *124*, 1269-1282.

[32] Asai, Y.; Holt, J.R.; Geleoc, G.S. A quantitative analysis of the spatiotemporal pattern of transient receptor potential gene expression in the developing mouse cochlea. *J. Assoc. Res. Otolaryngol.,* **2010**, *11*(1), 27-37.

[33] Bandell, M.; Story, G.M.; Hwang, S.W.; Viswanath, V.; Eid, S.R.; Petrus, M.J.; Earley, T.J.; Patapoutian, A. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron,* **2004**, *41*(6), 849-857.

[34] Bautista, D.M.; Movahed, P.; Hinman, A.; Axelsson, H.E.; Sterner, O.; Hogestatt, E.D.; Julius, D.; Jordt, S.E.; Zygmunt, P.M. Pungent products from garlic activate the sensory ion channel TRPA1. *Proc. Natl. Acad. Sci. USA,* **2005**, *102*, 12248-12252.

[35] Macpherson, L.J.; Geierstanger, B.H.; Viswanath, V.; Bandell, M.; Eid, S.R.; Hwang, S.; Patapoutian, A. The pungency of garlic: activation of TRPA1 and TRPV1 in response to allicin. *Curr. Biol.,* **2005**, *15*, 929-934.

[36] Hinman, A.; Chuang, H.H.; Bautista, D.M.; Julius, D. TRP channel activation by reversible covalent modification. *Proc. Natl. Acad. Sci. USA,* **2006**, *103*(51), 19564-19568.

[37] Macpherson, L.J.; Dubin, A.E.; Evans, M.J.; Marr, F.; Schultz, P.G.; Cravatt, B.F.; Patapoutian, A. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature,* **2007**, *445*(7127), 541-545.

[38] Eid, S.R.; Crown, E.D.; Moore, E.L.; Liang, H.A.; Choong, K.C.; Dima, S.; Henze, D.A.; Kane, S.A.; Urban, M.O. HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. *Mol. Pain,* **2008**, *4*, 48.

[39] Macpherson, L.J.; Xiao, B.; Kwan, K.Y.; Petrus, M.J.; Dubin, A.E.; Hwang, S.; Cravatt, B.; Corey, D.P.; Patapoutian, A. An ion channel essential for sensing chemical damage. *J. Neurosci.,* **2007**, *27*(42), 11412-11415.

[40] Bessac, B.F.; Sivula, M.; von Hehn, C.A.; Escalera, J.; Cohn, L.; Jordt, S.E. TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J. Clin. Invest.,* **2008**, *118*(5), 899-910.

[41] Brone, B.; Peeters, P.J.; Marrannes, R.; Mercken, M.; Nuydens, R.; Meert, T.; Gijsen, H.J. Tear gasses CN, CR, and CS are potent activators of the human TRPA1 receptor. *Toxicol. Appl. Pharmacol.,* **2008**, *231*(2), 150-156.

[42] Bessac, B.F.; Sivula, M.; von Hehn, C.A.; Caceres, A.I.; Escalera, J.; Jordt, S.E. Transient receptor potential ankyrin 1 antagonists block the noxious effects of toxic industrial isocyanates and tear gases. *FASEB J.,* **2009**, *23*(4), 1102-1114.

[43] Kerstein, P.C.; del Camino, D.; Moran, M.M.; Stucky, C.L. Pharmacological blockade of TRPA1 inhibits mechanical firing in nociceptors. *Mol. Pain,* **2009**, *5*, 19.

[44] Andre, E.; Campi, B.; Materazzi, S.; Trevisani, M.; Amadesi, S.; Massi, D.; Creminon, C.; Vaksman, N.; Nassini, R.; Civelli, M.; Baraldi, P.G.; Poole, D.P.; Bunnett, N.W.; Geppetti, P.; Patacchini, R. Cigarette smoke-induced neurogenic inflammation is mediated by alpha,beta-unsaturated aldehydes and the TRPA1 receptor in rodents. *J. Clin. Invest.,* **2008**, *118*(7), 2574-2582.

[45] Inoue, T.; Bryant, B.P. Multiple Cation Channels Mediate Increases in Intracellular Calcium Induced by the Volatile Irritant, Trans-2-Pentenal in Rat Trigeminal Neurons. *Cell Mol. Neurobiol.,* **2010**, *30*(1), 35-41.

[46] Taylor-Clark, T.E.; McAlexander, M.A.; Nassenstein, C.; Sheardown, S.A.; Wilson, S.; Thornton, J.; Carr, M.J.; Undem, B.J. Relative contributions of TRPA1 and TRPV1 channels in the activation of vagal bronchopulmonary C-fibres by the endogenous autacoid 4-oxononenal. *J. Physiol.,* **2008**, *586*(14), 3447-3459.

[47] Trevisani, M.; Siemens, J.; Materazzi, S.; Bautista, D.M.; Nassini, R.; Campi, B.; Imamachi, N.; Andre, E.; Patacchini, R.; Cottrell, G.S.; Gatti, R.; Basbaum, A.I.; Bunnett, N.W.; Julius, D.; Geppetti, P. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through activation of the irritant receptor TRPA1. *Proc. Natl. Acad. Sci. USA,* **2007**, *104*(33), 13519-13524.

[48] Taylor-Clark, T.E.; Undem, B.J.; Macglashan, D.W. Jr.; Ghatta, S.; Carr, M.J.; McAlexander, M.A. Prostaglandin-induced activation of nociceptive neurons via direct interaction with transient receptor potential A1 (TRPA1). *Mol. Pharmacol.,* **2008**, *73*(2), 274-281.

[49] Takahashi, N.; Mizuno, Y.; Kozai, D.; Yamamoto, S.; Kiyonaka, S.; Shibata, T.; Uchida, K.; Mori, Y. Molecular characterization of TRPA1 channel activation by cysteine-reactive inflammatory mediators. *Channels (Austin,)* **2008**, *2*(4).

[50] Maher, M.; Ao, H.; Banke, T.; Nasser, N.; Wu, N.T.; Breitenbucher, J.G.; Chaplan, S.R.; Wickenden, A.D. Activation of TRPA1 by farnesyl thiosalicylic acid. *Mol. Pharmacol.,* **2008**, *73*(4), 1225-1234.

[51] Miyamoto, T.; Dubin, A.E.; Petrus, M.J.; Patapoutian, A. TRPV1 and TRPA1 mediate peripheral nitric oxide-induced nociception in mice. *PLoS One,* **2009**, *4*(10), e7596.

[52] Materazzi, S.; Nassini, R.; Andre, E.; Campi, B.; Amadesi, S.; Trevisani, M.; Bunnett, N.W.; Patacchini, R.; Geppetti, P. Cox-dependent fatty acid metabolites cause pain through activation of the irritant receptor TRPA1. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(33), 12045-12050.

[53] Cruz-Orengo, L.; Dhaka, A.; Heuermann, R.J.; Young, T.J.; Montana, M.C.; Cavanaugh, E.J.; Kim, D.; Story, G.M. Cutaneous nociception evoked by 15-delta PGJ2 via activation of ion channel TRPA1. *Mol. Pain,* **2008**, *4*, 30.

[54] Zurborg, S.; Yurgionas, B.; Jira, J.A.; Caspani, O.; Heppenstall, P.A. Direct activation of the ion channel TRPA1 by Ca2+. *Nat. Neurosci.,* **2007**, *10*(3), 277-279.

[55] Doerner, J.F.; Gisselmann, G.; Hatt, H.; Wetzel, C.H. Transient receptor potential channel A1 is directly gated by calcium ions. *J. Biol. Chem.,* **2007**, *282*(18), 13180-13189.

[56] Wang, Y.Y.; Chang, R.B.; Waters, H.N.; McKemy, D.D.; Liman, E.R. The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. *J. Biol. Chem.,* **2008**, *283*(47), 32691-32703.

[57] Fujita, F.; Uchida, K.; Moriyama, T.; Shima, A.; Shibasaki, K.; Inada, H.; Sokabe, T.; Tominaga, M. Intracellular alkalization causes pain sensation through activation of TRPA1 in mice. *J. Clin. Invest.,* **2008**, *118*(21), 4049-4057.

[58] Dhaka, A.; Uzzell, V.; Dubin, A.E.; Mathur, J.; Petrus, M.; Bandell, M.; Patapoutian, A. TRPV1 is activated by both acidic and basic pH. *J. Neurosci.,* **2009**, *29*(1), 153-158.

[59] Hu, H.; Bandell, M.; Petrus, M.J.; Zhu, M.X.; Patapoutian, A. Zinc activates damage-sensing TRPA1 ion channels. *Nat. Chem. Biol.,* **2009**, *5*(3), 183-190.

[60] Matta, J.A.; Cornett, P.M.; Miyares, R.L.; Abe, K.; Sahibzada, N.; Ahern, G.P. General anesthetics activate a nociceptive ion channel to enhance pain and inflammation. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(25), 8784-8789.

[61] Leffler, A.; Fischer, M.J.; Rehner, D.; Kienel, S.; Kistner, K.; Sauer, S.K.; Gavva, N.R.; Reeh, P.W.; Nau, C. The vanilloid receptor TRPV1 is activated and sensitized by local anesthetics in rodent sensory neurons. *J. Clin. Invest.,* **2008**, *118*(2), 763-776.

[62] Caceres, A.I.; Brackmann, M.; Elia, M.D.; Bessac, B.F.; del Camino, D.; D'Amours, M.; Witek, J.S.; Fanger, C.M.; Chong, J.A.; Hayward, N.J.; Homer, R.J.; Cohn, L.; Huang, X.; Moran, M.M.; Jordt, S.E. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(22), 9099-9104.

[63] Helyes, Z.; Pinter, E.; Szolcsanyi, J.; Horvath, J. Anti-inflammatory and antinociceptive effect of different somatostatin-analogs. *Neurobiology (Bp),* **1996**, *4*(1-2), 115-117.

[64] Ro, J.Y.; Lee, J.S.; Zhang, Y. Activation of TRPV1 and TRPA1 leads to muscle nociception and mechanical hyperalgesia. *Pain,* **2009**, *144*(3), 270-277.

[65] Cattaruzza, F.; Spreadbury, I.; Miranda-Morales, M.; Grady, E.F.; Vanner, S.J.; Bunnett, N.W. Transient Receptor Potential Ankyrin-1 has a Major Role in Mediating Visceral Pain in Mice. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **2009** [Epub ahead of print].

[66] Bessac, B.F.; Jordt, S.E. Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. *Physiology (Bethesda),* **2008**, *23*, 360-370.

[67] Bessac, B.F.; Sivula, M.; von Hehn, C.A.; Escalera, J.; Cohn, L.; Jordt, S.E. TRPA1 is a major oxidant sensor in murine airway sensory neurons. *J. Clin. Invest.,* **2008**, *118*(5), 1899-1910.

[68] Karashima, Y.; Talavera, K.; Everaerts, W.; Janssens, A.; Kwan, K.Y.; Vennekens, R.; Nilius, B.; Voets, T. TRPA1 acts as a cold sensor *in vitro* and *in vivo*. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(4), 1273-1278.

[69] Katsura, H.; Obata, K.; Mizushima, T.; Yamanaka, H.; Kobayashi, K.; Dai, Y.; Fukuoka, 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*(1), 112-123.

[70] Andersson, D.A.; Gentry, C.; Moss, S.; Bevan, S. Clioquinol and pyrithione activate TRPA1 by increasing intracellular Zn2+. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(20), 8374-8379.

[71] Bautista, D.M.; Siemens, J.; Glazer, J.M.; Tsuruda, P.R.; Basbaum, A.I.; Stucky, C.L.; Jordt, S.E.; Julius, D. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature,* **2007**, *448*(7150), 204-208.

[72] Dhaka, A.; Murray, A.N.; Mathur, J.; Earley, T.J.; Petrus, M.J.; Patapoutian, A. TRPM8 Is Required for Cold Sensation in Mice. *Neuron,* **2007**, *54*(3), 371-378.

[73] Colburn, R.W.; Lubin, M.L.; Stone, D.J. Jr.; Wang, Y.; Lawrence, D.; D'Andrea, M.R.; Brandt, M.R.; Liu, Y.; Flores, C.M.; Qin, N. Attenuated cold sensitivity in TRPM8 null mice. *Neuron,* **2007**, *54*(3), 379-386.

Received: December 1, 2009 Revised: January 16, 2010 Accepted: February 22, 2010