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Transient Receptor Potential Channels in Pain and Itch

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ABSTRACT

Pain and itch (pruritus) are distinct, complex sensory sensations that indicate actual or potential tissue damage. While both involve neural network activation, their physiological mechanisms differ: pain is a necessary signal of harm that triggers protective responses, whereas itch primarily provokes the urge to scratch. In pruritus, the general discourse deals with histaminergic and nonhistaminergic itch. Painful or itching stimulation applied to peripheral receptors evokes a cascade of nociceptive signaling in ascending somatosensory pathways, culminating in a distributed, multidimensional brain representation that is required to localize, interpret, modulate, and appropriately respond to the stimulus.

The Transient Receptor Potential (TRP) channel superfamily is distinct in that channels are nonselective for cations (for sodium and calcium) and are involved in a diverse array of biological functions, making them attractive targets for synthesizing novel therapeutics. They regulate many functions, such as sensory perception, pain and itch, immunity, digestion, cancer, development, urological, cardiovascular, and nervous systems. Moreover, these channels are widely distributed in various tissue membranes, and TRP channel mutations are observed in several diseases, such as hereditary diseases and many other TRP channelopathies. Among them are the TRPA1 channel, members of the vanilloid subfamily (TRPV1, TRPV3, and TRPV4), and members of the melastatin subfamily (TRPM2, TRPM3, and TRPM8).

TRP ankyrin 1 (TRPA1) is a calcium-permeable channel that is co-expressed on a subpopulation of TRPV1-expressing nociceptive nerve fibers' terminals and distal endings of mechanosensitive C-fibers, contributing to the transduction of noxious signals. TRPA1 is considered to detect non-histaminergic itch and hypersensitivity to noxious cold. Various irritant chemicals (e.g., pungent compounds) and endogenous products of tissue injury activate TRPA1.

TRP vanilloid 1 (TRPV1) is also a nonselective ion channel with high calcium permeability that is activated by capsaicin, heat (>43°C), and pH (below < 6). It is a prominent nociceptor in C-fiber sensory neurons involved in pain and itch pathways. TRPV1 is considered to detect non-histami-

nergic itch. It has become the first family member with a postulated and subsequently verified link to pain and itch, suggesting this protein receptor as a hub for algesic and pruritogenic signals. Both channels are attractive therapeutic targets in pain and itch.

KEYWORDS: algesia, allodynia, hyperalgesia, nociception, pruriception

Transient Receptor Potential Channels in Pain and Itch

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INTRODUCTION

Pain and itch (pruritus) are unpleasant sensations that indicate actual or potential tissue damage. Pain is a necessary alert signal that sets off protective responses, while itch mainly provokes the desire to scratch. Both are multifaceted or multidimensional experiences. However, pain and itch usually perform an important biological function for the body in normal states. For pruritus, the general discourse mainly deals with histaminergic and nonhistaminergic itch [1-4].

In the normal state, painful or itching stimulation applied to peripheral receptors evokes a cascade of nociceptive signaling in ascending somatosensory pathways that project to a distributed multidimensional brain representation required to localize, interpret, modulate, and appropriately respond to the stimulus [5].

It is well known that most cellular functions are regulated by the influx of different ions across the cell membrane through selective or nonselective channels. These channel gene families demarcate their important role in mammalian physiology. The transient receptor potential (TRP) channel superfamily is multimodal, nonselective for cations (calcium and sodium), and involved in a diverse array of biological functions, making them attractive targets for therapeutics [4,6-8].

They are multifunctional signaling molecules with important roles in health and disease. More than two decades of intensive preclinical and clinical research support the involvement of TRP channels in pain, itch, and neurogenic inflammation. In this regard, expression of TRP channels in cell types mediating innate (such as macrophages, dendritic cells, keratinocytes, mast cells, and natural killer cells) and adaptive (lymphocytes) immune response suggests involvement in the regulation of inflammation [9].

Furthermore, TRP ion channels are molecular sensors of chemical, thermal, and mechanical noxious stimuli that evoke pain and itch sensations. Among them are the TRPA1 channel, members of the vanilloid subfamily (TRPV1, TRPV3, and TRPV4), and finally members of the melastatin subfamily (TRPM2, TRPM3, and TRPM8) [10].

TRP channels regulate many functions, such as sensory perception, pain and itch, immunity,

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digestion, cancer, and development, and are involved in urological, cardiovascular, and nervous systems [6-7]. Moreover, these channels are widely distributed in various tissue membranes, and mutations in TRP channels are observed in several diseases, among them hereditary diseases and many other TRP channelopathies [11].

Given the importance of TRPA1 and TRPV1 in mediating itch signaling, investigation of these ion channels has been of considerable interest for their potential roles in contributing to chronic pruritus. Beyond their known expression in the nerve endings of primary afferent neurons, TRP channels have been found in keratinocytes, the epidermis, and mast cells, and are upregulated in affected skin in several dermatological pathologies associated with chronic itch, including atopic dermatitis, psoriasis, and prurigo nodularis [1].

ANKYRIN SUBFAMILY: TRPA1

The TRPA subfamily has only one member (TRPA1) due to numerous ankyrin repeats for the N-terminus. It is a calcium-permeable nonselective cation channel that contributes to the transduction of noxious and itch signals. On the one hand, TRPA1 is considered a chemical nocisensor as it is sensitive to various irritant chemicals and endogenous products of tissue injury. Among them are pungent compounds in spices, such as cinnamon, mustard oil, and wasabi, as well as chloroquine, acrolein, formalin, cannabinoids, and various reactive chemicals, oxygen, nitrogen, and carbonyl species. This channel is activated by mechanical stimuli and signals associated with tissue damage, pain, and itch [6].

On the other hand, TRPA1 was proposed as a noxious cold receptor in mice and insects. Although TRPA1 channels from insects to birds (endotherms) show heat-dependent activation, indicating the importance of TRPA1 in detecting ambient warm to hot temperatures, in mammals, TRPA1 temperature sensitivity remains controversial. Human TRPA1 has bimodal thermosensitivity, and mouse TRPA1 is involved in noxious heat sensitivity, but additional systematic analyses are needed to determine the general mechanism of mammalian TRPA1 thermosensitivity [12].

Further, the diversity of functions underlines its expression in cell types such as sensory vagal neurons, jugular veins, nodose ganglia, epithelial cells, and various other cells, making this channel relevant to a wide range of diseases and an attractive therapeutic target [6, 13].

Several natural compounds (TRPA1 agonists), such as curcumin, resveratrol, and chalcones, have been shown to activate and desensitize TRPA1, suggesting that the development of synthetic TRPA1 agonists could be an attractive path toward safe and efficacious pain therapy [14].

Regarding TRPA1 antagonists, several pharmaceutical companies are actively pursuing novel small-molecule antagonists and optimizing existing scaffolds. They will eventually provide clinically meaningful pain and itch relief. Preclinical research suggests that a centrally acting TRPA1 antagonist is likely to provide superior efficacy over the peripherally restricted mode of action. Development of centrally acting small-molecule TRPA1 antagonists has been challenging, especially regarding drug-like properties and medicinal chemistry optimization [14].

Finally, several rare gain-of-function variants of TRPA1 have been shown to cause pain in humans. Furthermore, several inflammatory pain mediators associated with various chronic pain conditions have been shown to increase TRPA1 sensitivity. Therefore, TRPA1 could be considered a promising target for drug therapy in humans.

Regarding itching, TRPA1 is critical for acute non-histaminergic itch and chronic itch in dry skin, and is partially involved in acute histaminergic itch. TRPA1 represents a promising target for the development of antipruritic drugs too [1].

VANILLOID SUBFAMILY

This group contains seven members (TRPV1-7) and is named for the sensitivity of the founding member, TRPV1, to the vanilloid capsaicin. These protein channels are polymodal, being activated by capsaicin, pH, and osmolarity changes, mechanical stimuli, high temperatures, lipids, and voltages. TRPVs can be further categorized by their thermal sensitivity: TRPV1, TRPV2, TRPV3, and TRPV4 are gated by warmth and painful heat and are termed thermo-TRP channels, along with TRPM3 and TRPM8. TRPV5, TRPV6, and TRPV7 are not activated by temperature and thus comprise a second subset of this subfamily. They have been associated with the fine regulation of calcium absorption in epithelia [6,7].

The temperature-driven activation of TRPV subfamily cation channels contributes to pruritus, flares, skin barrier dysfunction, atopic dermatitis development, and asthma attacks. The blocking of TRPV channels may attenuate temperature-mediated itch, skin barrier dysfunction, and the exacerbation of atopic dermatitis [1].

TRPV1

As mentioned, thermo-TRPs are crucial for physiological functions as they constantly monitor internal body temperature and warn about ambient hazards associated with heat and cold. They also act as polymodal sensors, integrating signals from mechanical and chemical stimuli in addition to temperature. For example, TRPV1 exhibits remarkable sensitivity to capsaicin, the spicy molecule of hot chili peppers, resulting in a sensation of heat and pain.

Since the TRPV1 ion channel was cloned in David Julius' laboratory in 1997 [15], there have been extensive efforts to reveal structural and functional details of this protein, which culminated in the Nobel Prize (2021) in Physiology and Medicine for him. Thus, nowadays it is one of the best characterized members of the TRP superfamily of ion channels. Its expression was highly enriched in dorsal root and trigeminal sensory ganglia, and in particular in small to medium diameter nociceptive neurons, offering a compelling potential basis for capsaicin-evoked pain [16].

TRPV1 responds to temperatures above 43°C and activates at neutral pH (≤ 6) at room temperature, and channel conformation changes. The opened channel typically allows influx of sodium and calcium ions into the cell, which results in neuronal depolarization. Consecutive action potential transmission causes release of neuropeptides from nociceptors, such as calcitonin gene-related peptide or substance P, ultimately sending harmful signals, such as pain, itching, burning, and stinging sensations to the spinal cord and brain, as well as activating neurogenic inflammation [17, 18].

The structure of TRPV1 ankyrin repeat domain was initially solved by X-ray crystallography. Later, cryo-EM was exploited to visualize conformational transition in TRPV1 that uncovered a two-step mechanism of proton modulation, substates reflecting phosphatidyl inositol lipid and vanilloid agonist binding stoichiometry and competition, and adaptive behavior of the selectivity filter, which accommodates permeation of small and large organic cations. Knockout (KO) *Trpv1* mice display a distinct thermoregulatory phenotype, and the development of inflammation-induced and injury-induced hyperalgesia (increased pain sensitivity) is impaired [19].

Drugs that target TRPV1 to modulate pain through an agonist strategy utilize overactivation that results in a desensitized and inactivated channel receptor state. When a high dose of agonist agent (capsaicin or resiniferatoxin-RFT) binds directly to the channel, a conformational change occurs that reduces calcium-dependent depolarization. A pain signal transmitted to the spinal cord decreases, and hence, the release of inflammatory neuropeptides diminishes, re-

sulting in hypo-algesia propagation. Thus, applied agonists will cause reduced pain, itching, and burning sensations.

Regarding TRPV1, competitive antagonists directly block the active vanilloid binding site while non-competitive agents indirectly bind nearby to allosterically modulate the channel and prevent the required conformational change from occurring. However, they may bind, and the ending result is the same, as channel activation is inhibited, so there is decreased calcium and sodium influx. This prevention of signal transduction reduces nociceptive signaling, inhibits thermal hyperalgesia, and opposes inflammatory pain [17].

However, harmful side effects of TRPV1-targeted antagonist drugs include the risk of thermos hypoesthesia or temperature dysregulation. Impaired temperature sensitivity can range from feeling chills to hyperthermia, and places patients at risk for burn injuries. Recent studies have provided insight that TRPV1 nociception and thermoregulation pathways have biased allosteric mechanisms, indicating that even more specific channel targeting may reduce unwanted side effects [17].

More than twenty-five years after its cloning [15, 16], TRPV1 has become the first subfamily member linked to thermal pain and itch. Histamine activates primary sensory neurons via the histamine type 1 receptor (H1R) linked to TRPV1, and histamine-evoked scratching is attenuated in knockout mice lacking TRPV1 [1, 2].

We have recently found that an intraplantar injection of histamine in mice resulted in significant thermal hyperalgesia and mechanical allodynia ipsilaterally that persisted for 1 h. Pretreatment with the TRPV1 antagonist AMG-517, but not the TRPA1 antagonist HC-030031, significantly attenuated the magnitude and time course of thermal hyperalgesia and mechanical allodynia elicited by histamine, indicating that these effects are mediated by TRPV1 [20, 21].

In addition, non-histaminergic pruritus, such as that in cholestasis, has also been related to TRPV1 sensitization by pruritogens. TRPV1 also indirectly contributes to non-histaminergic itch via protease-activated receptor 2 (PAR2) and PAR4, which are involved in chronic neurogenic inflammation. The latter sensitizes TRPV1 channels and induces itch [1, 22].

TRPV3

The third vanilloid member was cloned from mouse skin keratinocytes and various human tissues and was immediately confirmed to be sensitive to innocuous thermal stimulation in the warm range (34–38° C). TRPV3 is expressed in mammalian skin, tongue, dorsal root ganglion (DRG), trigeminal ganglion (TG), spinal cord, and brain. This calcium-permeable protein demonstrates increasing current responses to repeated applications of heat or agonists, termed sensitization [19, 23-25].

From a structural point of view, firstly, TRPV3 ankyrin repeat domain was determined by X-ray crystallography. Subsequently, numerous TRPV3 structures solved in a diverse range of conformations provided valuable information for understanding the molecular mechanisms underlying thermo-TRP temperature sensations [19]. Recently, it has been established that cryo-EM structures illuminate the opening and inactivation of wild-type human TRPV3 in response to binding of two types of agonists: either the natural cannabinoid tetrahydrocannabivarin (THCV) or synthetic agonist 2-aminoethoxydiphenylborane (2-APB). THCV binds to the vanilloid site, while 2-APB binds to the S1-S4 base site. Despite binding to distally located sites, both agonists induce similar pore opening and cause dissociation of a lipid that occupies the vanilloid site in their absence. These data uncover different but converging allosteric pathways through which small-molecule agonists activate TRPV3 and provide a framework for drug design and under-

standing the role of lipids in ion channel function [25].

TRPV3 is involved in multiple physiological and pathological functions of the skin, such as atopic dermatitis and Olmsted syndrome. It is expressed in diverse human tissues, among them the skin, testes, DRG, spinal cord, and brain. Plant-derived camphor activates TRPV3 and sensitizes responses of the channel to warmth. Warm temperature stimulates TRPV3 in keratinocytes, releasing various inflammatory factors that activate pruriceptors in sensory neurons to transmit itch signals [26, 27].

TRPV3 can also be activated by chemicals such as eugenol, thymol, and carvacrol, major components of oregano, savory, clove, and thyme. In mice, TRPV3 was not detected in DRG neurons, but it was detected in keratinocytes and is essential in causing allergic and pruritic dermatitis in rodents. In contrast, in primates, TRPV3 expression is also observed in DRG, TG sensory neurons, hypothalamus, and several nonneuronal tissues [28].

A recent study provided a clear link between TRPV3 and atopic dermatitis, showing that interleukin-31 (IL-31) induces B-type natriuretic peptide (BNP) synthesis and release from sensory neurons. BNP subsequently binds to the natriuretic peptide receptor (NPR1) on keratinocytes to upregulate TRPV3 transcripts. It could increase the surface expression of TRPV3, resulting in heightened TRPV3 activity and increased serpin E1 release. In turn, serpin E1 activates sensory fibers in the skin and promotes itch transduction [29].

An increasing number of studies have shown that keratinocyte-expressed TRPV3 is involved in chronic pruritus and itch transmission [30]. In particular, Han et al. [31] reported that citrusinine-II, a plant-derived natural acridone alkaloid from *A. monophyla*, is a potent and selective antagonist of TRPV3 that has a strong antipruritic effect in both in vivo and in vitro experiments. These data indicate the potential of citrusinine-II-targeted therapy for itch [31].

In another study, it was found that keratinocytes isolated from patients with atopic dermatitis exhibited enhanced heat sensitivity and hyperactivity of TRPV3 [32]. TRPV3 was upregulated in the skin of the MC903-induced chronic atopic dermatitis mouse model. Heat stimulation to MC903-treated mice increased scratching behavior and produced higher levels of thymic stromal lymphopoietin (TLSP), nerve growth factor (NGF), prostaglandin E2 (PGE2), and IL-33 from the epidermis, which were attenuated by the pharmacologic inhibition of TRPV3 [32].

Taken together, recent studies in rodents evaluated the relation of TRPV3 to itch in atopic dermatitis and psoriasis. However, less is known concerning the clinical relevance of TRPV3 in human studies. Further research is needed to reveal the roles of TRPV3 in human skin dysesthesia and abnormalities in detail [1].

TRPV4

TRPV4 was discovered as a multimodal ion channel activated by diverse physical stimuli, including warm temperature, hypotonic cell swelling, membrane stretch, and low pH. TRPV4 is also sensitive to various chemical stimuli, including synthetic agonists like GSK1016790A and phorbol esters. Several endogenous lipid molecules, such as arachidonic acid (AA) and its metabolites, as well as endocannabinoids, activate or regulate the TRPV4 channel. Certain synthesized chemical antagonists, HC-067047 and GSK2798745, inhibit TRPV4 activity. One of the major regulatory pathways of this channel is through protein phosphorylation catalyzed by various protein (e.g., PKC, PKA), and other (e.g., SGK1, and Src) kinases [33]

The physiological roles of TRPV4 are tissue-specific, and the mechanisms behind this specificity remain mostly unclarified. It is noteworthy that mutations in the TRPV4 channel have been associated with a broad spectrum of congenital diseases, with most of these mutations mainly

resulting in gain-of-function. Mutations have been identified in human patients showing a variety of phenotypes and symptoms, mostly related to skeletal and neuromuscular disorders. Since TRPV4 is so widely expressed throughout the body, it comes as no surprise that the literature is growing in evidence linking this protein to malfunction in systems other than the skeletal and neuromuscular [34].

This channel is activated by heat, with the temperature threshold around 24°C. Experiments on rats treated with selective TRPV4 agonists and antagonists revealed that this channel is involved in the recruitment of behavioral and autonomic warmth-defensive responses to regulate core body temperature. The disorder region of the TRPV4 N-terminus and its ankyrin repeat domain portion were extensively studied in isolation using mutagenesis and different methods, such as X-ray crystallography, mass spectrometry, fluorescence spectroscopy, and others. Subsequently, high-resolution cryo-EM structures were solved for human TRPV4 in complex with GTPase RhoA, as well as human and mouse TRPV4 in the apo state and in complex with agonists, competitive antagonists, and nonselective ion channel blocker ruthenium red [25].

An unexpected role for TRPV4 in itch came with the discovery that scratching behavior elicited by histamine [35, 36] and serotonin [37] was reduced in KO mice lacking TRPV4, and that serotonin-evoked scratching, and the activation of DRG neurons were reduced by a TRPV4 antagonist in wild-type mice [37] (for a review, see [38]).

Scratching elicited by the intradermal injection of endothelin-1 was also attenuated in

TRPV4 KO mice [35]. Scratching elicited by cinnamaldehyde (CA) was also reduced in TRPV4 KO mice [39]. Signs of chronic itch in dry skin and contact dermatitis models were also reduced in KO mice lacking TRPV4 [38, 40].

Recent studies suggest that chemicals from traditional Chinese medicines such as vitexin (apigenin-8-C-glucoside) [41] or cimifugin (from the herb *Saposhnikovia divaricata*) [42] may target TRPV4 to relieve acute and chronic itch. Thus, TRPV4 appears to be a good target for the development of drugs to alleviate itch [1].

MELASTATIN SUBFAMILY

TRPM channels are a genetically and functionally diverse subfamily comprising eight members (TRPM1–8). They share or are similar in some characteristics, but differ in others. For example, TRPM2, TRPM6, and TRPM7 are unique, containing enzymatically active protein domains in their C-termini. TRPM1 (the first identified) is considered a marker for metastasized melanoma. TRPM3 is proposed to be thermosensitive to temperature in the warm range. TRPM4 and TRPM5 are the only members of the TRP channel superfamily selective for just monovalent ions do not permeable to calcium. TRPM8 was first identified in transformed prostate cells as an androgen-responsive channel, but it also serves as a sensor for cold temperatures in the peripheral nervous system [6, 43, 44]. Accumulating evidence has recently established TRPM channels as crucial regulators of energy metabolism [45].

TRPM2

TRPM2 was first cloned from human fetal brain, and later demonstrated that it can be activated by exposure to high temperature (> 35°C), apparently through direct heat-evoked channel gating. The structures of TRPM2 were solved by cryo-EM for a zebrafish *(Danio rerio)* ortholog, both in the closed apo state and in the open state activated by the agonist ADP ribose and calcium cations. Subsequent studies of sea anemone *(Nematostella vectensis),* zebrafish, and human TRPM2 unveiled additional conformations and binding sites, thereby providing insights into

pharmacology and structural differences between the TRPM2 orthologs [46].

It has recently been proposed that the warmth sensitivity of TRPM2 is uniquely dependent on calcium ion permeability, and it is regulated by using a positive feedback mechanism. The altered sensation of innocuous heat in KO mice supports the hypothesis that TRPM2 expressed in somatosensory neurons mediates an innocuous “warm” signal. In addition, hypothalamic TRPM2 channels have been shown to function as temperature sensors, which limit fever responses and detect increased temperatures to prevent overheating [19].

Further, aberrant expression of TRPM2 is associated with various pathological conditions. Overexpression of TRPM2 promotes cell survival in multiple malignancies, including neuroblastoma, lung, prostate, stomach, and pancreatic cancers. TRPM2 also mediates different neurological disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), and epilepsy, and contributes to ischemia/reperfusion injury [47].

TRPM3

TRPM3 is an ion channel inhibited by GPCRs through direct interaction with G protein (Gβγ) released upon their activation. This GPCR-TRPM3 signaling pathway contributes to the analgesic effect of morphine. The structure of mouse TRPM3 has been solved recently in the apo form, in complex with phosphatidyl-inositol-4,5-bisphosphate (PIP2) lipid bound to the interface between the S1–S4 bundle and pre-S1 helix, and in complex with G-protein (Gβ1γ2) [48]. This channel is activated by heat in the range 31-45° C. TRPM3 plays a critical role in nociception, and *Trpm3* KO mice exhibit reduced noxious heat sensitivity at cellular and behavioral levels [19].

TRPM8

The menthol-sensitive TRPM8 channel is activated by cooling stimuli and is involved in cold allodynia and aberrant cold sensitivity. Initially, it was shown to be overexpressed in different types of cancer cells and was first cloned from human prostate carcinoma cells without characterization of ion channel functions. Later authors found that TRPM8 represents an ion channel that is activated by thermal stimuli in the cool to cold range between 8-28° C, as well as by cooling compounds menthol and icilin. Increased TRPM8 activity in DRG and the spinal cord

raises calcium influx and activates MAPK pathways, which boost neuronal excitability and central sensitization, allowing TRPM8 to influence spinal and supraspinal pain circuits. This amplifies nociceptive signaling, neuronal sensitization, and cold hypersensitivity, which are transmitted toward the CNS by Aβ and C fibers [19,49,50].

In collaboration with American colleagues, we found that menthol dose-dependently increased the latency for noxious heat-evoked withdrawal of the treated hindpaw with a weak mirror-image effect, indicating antinociception. Menthol at the highest concentration (40%) reduced mechanical withdrawal thresholds, with no effect at lower concentrations. At the time, menthol had a biphasic effect on the cold avoidance reflex. At high concentrations (10% and 40%), menthol reduced avoidance of colder temperatures (15° C and 20° C) compared to 30° C, while at lower concentrations (0.01–1%), menthol enhanced cold avoidance. In a −5° C cold plate test, 40% menthol significantly increased the nocifensive response latency (cold hypo-algesia), while lower concentrations were not different from vehicle controls. These results are generally consistent with neurophysiological and human psychophysical data and support TRPM8 as a potential peripheral target of pain modulation [51].

While skin cooling has been used for centuries to relieve itch, it was only recently shown that skin cooling and menthol can alleviate both histaminergic and nonhistaminergic itch-related

behavior in mice in a TRPM8-dependent manner [52]. Cold fibers are thought to activate inhibitory spinal interneurons that suppress spinal itch transmission. Indeed, mice lacking a particular class of itch-inhibitory spinal interneurons exhibited a reduction in the antipruritic effect of menthol [1].

CONCLUSIONS

Ubiquitous expression of TRP channels underscores their relevance across virtually every facet of human physiology and pathology. They remain viable targets for the development of novel therapeutics to treat acute and chronic pain, itch, inflammation, and various diseases. For example, TRP channel antagonists are obvious tools to dampen channel function when the underlying disease involves hyperactivity. Indeed, many such molecules are either in development or have been employed in various phases of clinical trials. These studies are also merged with known binding and channel regulation of several natural products that are ligands for these channels. While antagonists are the obvious route to treatment, developing novel channel agonists is a viable approach for some conditions. In particular, the TRPM8 and TRPV1 channel agonists, menthol and capsaicin, respectively, have been used to treat holistically a plethora of conditions, including pain, pruritus, headaches, and others. Thus, the role of TRP channels in the specific disease is critical for therapies.

REFERENCES

1. *Tsagareli MG, Follansbee T, Iodi Carstens, Carstens E. (2023) Targeting transient receptor potential (TRP) channels, mas-related G-Protein-coupled receptors (Mrgprs), and protease-activated receptors (PARs) to relieve itch. Pharmaceuticals; 16: 1707, doi: 10.3390/ ph16121707.*
2. *Tsagareli MG, Carstens EE. (2023) Chapter one – allodynia and hyperalgesia in pruritus: involvement of TRP channels. In: Tsagareli MG, and Follansbee T (eds). Histaminergic and Non-Histaminergic Itch. Nova Science: New York, pp. 1–23.*
3. *Yuan T, Li J, Shen L, Zhang W, Wang T, Xu Y, Zhu J, Huang Y, Ma C. (2024) Assessment of itch and pain in animal models and human subjects. In: Ma C, and Huang Y. (eds.) Translational Research in Pain and Itch. Chap. 1, 2nd ed., Springer, pp. 1-22, doi: 10.1007/ 978-981-99-8921-8_1.*
4. *Tsagareli MG, Nozadze I, Gurtskaia G, Tsagareli M, Carstens Iodi M, Gedevanishvili G., Carstens E. (2025). Nociceptive modulation by inactivation of TRPA1 and TRPV1 channels. Med Res Arch, [online] 13(12), doi: 10.18103/mra.v13i12.7150.*
5. *Motzkin JC, Basbaum AI, Crowther AJ (2024) Neuroanatomy of the nociceptive system: From nociceptors to brain networks. Int Rev Neurobiol; 179:1-39, doi: 10.1016/bs.irn.2024. 10.008.*
6. *McKemy DD. (2024) What makes TRP channels attractive therapeutic targets? In: Szallasi A. (ed). TRP Channels as Therapeutic Targets. Chap. 1, 2nd ed., Elsevier, pp. 1-7, doi: 10.1016/ B978-0-443-18653-0.00018-6.*
7. *Rangel-Yescas GE, Islas LD. (2024) Biology of TRP channels. In: Szallasi A. (ed). TRP Channels as Therapeutic Targets. Chapt. 2, 2nd ed., Elsevier, pp. 9-34, doi: 10.1016/B978-0-443-18653-0.00020-4.*
8. *Tsagareli M.G., Nozadze I. (2020) An overview on transient receptor potential channels superfamily. Behavioural Pharmacol; 31(5):413-434, doi: 10.1097/FBP.0000000000000524.*
9. *Koivisto A-P, Szallasi A. (2024) Targeting TRP channels for pain, itch and neurogenic inflammation. Int. J.Mol. Sci; 25: 320, doi: 10.3390/ijms25010320.*
10. *Moore C, Gupta R, Jordt SE, Chen Y, Liedtke WB. (2018) Regulation of pain and itch by TRP channels. Neurosci Bull; 34(1):120-142, doi: 10.1007/s12264-017-0200-8.*
11. *Kiselyov K. (2024) TRP channelopathies: TRP channel dysfunction in hereditary diseases. In:*

Szallasi A. (ed). TRP Channels as Therapeutic Targets. Chap. 5, 2nd ed., Elsevier, pp. 71-34, doi: 10.1016/B978-0-443-18653-0.00017-4.

12. *Tominaga M, Iwata M. (2025) TRPA1 and thermosensitivity. J Physiol Sci; 75(1): 100010, doi: 10.1016/ j.jphyss.2025.100010.*
13. *Talavera K, Startek JB, Alvarez-Collazo J, Boonen B, Alpizar YA, Sanchez A, Naert R, Nilius B. (2020) Mammalian transient receptor potential TRPA1 channels: From structure to disease. Physiol Rev; 100 (2):725-803, doi: 10.1152/physrev.00005.2019.*
14. *Koivisto A, Jalava N, Rantanen M, Chapman H, Holm P, Pertovaara A. (2024) TRPA1 antagonists for pain relief. In: Szallasi A. (ed). TRP Channels as Therapeutic Targets. Chap. 12, 2nd ed. Elsevier, pp. 185-202, doi: 10.1016/B978-0-443-18653-0.00032-0.*
15. *Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature; 389(6653):816–824, doi: 10.1038/ 39807.*
16. *Caterina M.J. (2021) How do you feel? A warm and touching 2021 Nobel tribute. J Clin Invest; 131(24): e156587, doi: 10.1172/JCI156587.*
17. *Tsai EH, Gan TJ. (2026) TRPV1 receptor modulators: Emerging therapies for neuropathic, osteoarthritic, and perioperative pain. CNS Drugs; 40(4):523-534, doi: 10.1007/s40263-026-01272-1.*
18. *Fernández-Carvajal A, Fernández-Ballester G, Ferrer-Montiel A. (2022) TRPV1 in chronic pruritus and pain: Soft modulation as a therapeutic strategy. Front Mol Neurosci; 15: 930964, doi: 10.3389/fnmol.2022.930964.*
19. *Nadezhdin KD, Sobolevsky AI. (2024) Thermo-TRPs: Temperature-sensitive TRP channels. In: Szallasi A. (ed). TRP Channels as Therapeutic Targets. Chap. 4, 2nd ed. Elsevier, pp. 53-70, doi: 10.1016/B978-0-443-18653-0.00032-0.*
20. *Tsagareli, M.G.; Carstens, E.E. Allodynia and hyperalgesia in pruritus: Involvement of TRP channels. In: Tsagareli, MG, Follansbee T. (eds). Histaminergic and Non-Histaminergic Itch; Nova Science: New York, USA, 2023; Chapter 1; pp. 1–23.*
21. *Tsagareli MG, Nozadze I, Tsiklauri N, Carstens MI, Gurtskaia G, Carstens E. (2020) Thermal hyperalgesia and mechanical allodynia elicited by histamine and non-histaminergic itch mediators: Respective involvement of TRPV1 and TRPA1. Neuroscience; 449:35-45, doi: 10.1016/j.neuroscience.2020.09.048.*
22. *Shirolkar P, Mishra SK. (2022) Role of TRP ion channels in pruritus. Neurosci Lett; 768: 136379, doi: 10.1016/j.neulet.2021.136379.*
23. *Peier AM, Reeve AJ, Andersson DA, Moqrich A, Earley TJ, Hergarden AC, Story GM, Colley S, Hogenesch JB, McIntyre P, Bevan S, Patapoutian A. (2002) A heat-sensitive TRP channel expressed in keratinocytes. Science; 296(5575): 2046-2049, doi: 10.1126/science.1073140.*
24. *Xu H, Ramsey IS, Kotecha SA, Moran MM, Chong JA, Lawson D, Ge P, Lilly J, Silos-Santiago I, Xie Y, DiStefano PS, Curtis R, Clapham DE. (2002) TRPV3 is a calcium-permeable temperature-sensitive cation channel. Nature; 418(6894): 181-186, doi: 10.1038/nature00882.*
25. *Nadezhdin KD, Neuberger A, Khosrof LS, Talyzina IA, Khau J, Yelshanskaya MV, Sobolevsky AI. (2024) TRPV3 activation by different agonists is accompanied by lipid dissociation from the vanilloid site. Sci Adv; 10(18): eadn2453, doi: 10.1126/sciadv.adn2453.*
26. *Guo Y, Song Y, Liu W, Wang T, Ma X, Yu Z. (2023) Novel insights into role of keratinocytes-expressed TRPV3 in the Skin. Biomolecules; 13(3):513, doi: 10.3390/biom13030513.*
27. *Sanjel B, Shim W-S. (2023) Molecules that channel stimulus into pruritus. In: Tsagareli MG, Follansbee T. (eds.). Histaminergic and Non-Histaminergic Itch. Nova Science: New York, USA, Chap. 4, pp. 59–92.*
28. *Steinhoff M, Bíró TA. (2009) TR(i)P to pruritus research: Role of TRPV3 in inflammation and itch. J Invest Dermatol; 129:531–535, doi: 10.1038/jid.2008.440.*
29. *Larkin C, Chen W, Szabó IL, Shan C, Dajnoki Z, Szegedi A, Buhl T, Fan Y, O'Neill S, Walls D*

(2021) Novel insights into the TRPV3-mediated itch in atopic dermatitis. J Allergy Clin Immunol; 147:1110–1114.e5, doi: 10.1016/j.jaci.2020.09.028.

30. *Um JY, Kim HB, Kim JC, Park JS, Lee SY, Chung BY, Park CW, Kim HO. (2022) TRPV3 and itch: The role of TRPV3 in chronic pruritus according to clinical and experimental evidence. Int J Mol Sci; 23(23):14962, doi: 10.3390/ijms232314962.*
31. *Han Y, Luo A, Kamau PM, Takomthong P, Hu J, Boonyarat C, Luo L, Lai R. (2021) A plant-derived TRPV3 inhibitor suppresses pain and itch. Br J Pharmacol; 178:1669–1683, doi: 10. 1111/ bph.15390.*
32. *Seo SH, Kim S, Kim SE, Chung S, Lee SE. (2020) Enhanced thermal sensitivity of TRPV3 in keratinocytes underlies heat-induced pruritogen release and pruritus in atopic dermatitis. J Invest Dermatol; 140:2199–2209.e6, doi: 10.1016/j.jid.2020.02.028.*
33. *Parthasarathy A, Zhang DX. (2026) An update on regulation of the polymodal TRPV4 channel by protein phosphorylation. Channels; 20(1):2611698, doi: 10.1080/19336950.2025. 2611698.*
34. *Hernández-Vega AM, García-Villegas R, Rosenbaum T. (2024) Roles for TRPV4 in disease: A discussion of possible mechanisms. Cell Calcium; 124:102972, doi: 10.1016/j.ceca.2024. 102972.*
35. *Chen Y, Fang Q, Wang Z, Zhang JY, MacLeod A, Hall RP, Liedtke WB. (2016) Transient receptor potential vanilloid 4 ion channel functions as a pruriceptor in epidermal keratinocytes to evoke histaminergic itch. J Biol Chem; 291:10252–10262, doi: 10.1074/jbc. M116.716464*
36. *Kim S, Barry DM, Liu XY, Yin S, Munanairi A, Meng QT, Cheng W, Mo P, Wan L, Liu SB. (2016) Facilitation of TRPV4 by TRPV1 is required for itch transmission in some sensory neuro populations. Sci. Signal; 9: ra71, doi: 10.1126/scisignal.aaf1047.*
37. *Akiyama T,, Ivanov M, Nagamine M, Davoodi A, Carstens MI, Ikoma A, Cevikbas F, Kempkes C, Buddenkotte J, Steinhoff M. (2016) Involvement of TRPV4 in serotonin-evoked scratching. J Invest Dermatol; 136, 154–160, doi: 10.1038/JID.2015.388.*
38. *Zhang Q, Henry G, Chen Y. (2021) Emerging role of transient receptor potential vanilloid 4 (TRPV4) ion channel in acute and chronic itch. Int J Mol Sci; 22: 7591, doi: 10.3390/ ijms22147591.*
39. *Domocos D, Follansbee T, Nguyen A, Nguyen T, Carstens MI, Carstens E. (2020) Cinnamaldehyde elicits itch behavior via TRPV1 and TRPV4 but not TRPA1. Itch; 5: e36, doi: 10.1097/ itx.0000000000000036.*
40. *Luo J, Feng J, Yu G, Yang P, Mack MR, Du J, Yu W, Qian A, Zhang Y, Liu S. (2018) Transient receptor potential vanilloid 4-expressing macrophages and keratinocytes contribute differentially to allergic and nonallergic chronic itch. J Allergy Clin Immunol; 141: 608–619.e7, doi: 10.1016/j. jaci.2017.05.051.*
41. *Qin Z, Xiang L, Zheng S, Zhao Y, Qin Y, Zhang L, Zhou L. (2023) Vitexin inhibits pain and itch behavior via modulating TRPV4 activity in mice. Biomed Pharmacother; 165: 115101, doi: 10.1016/j. biopha.2023.115101.*
42. *Yan J, Ye F, Ju Y, Wang D, Chen J, Zhang X, Yin Z, Wang C, Yang Y, Zhu C. (2021) Cimifugin relieves pruritus in psoriasis by inhibiting TRPV4. Cell Calcium; 97: 102429,*
43. *doi: 10.1016/j.ceca.2021.102429.*
44. *Vydra Bousova K, Zouharova M, Jiraskova K, Vetyskova V. (2023) Interaction of calmodulin with TRPM: An initiator of channel modulation. Int J Mol Sci; 24(20):15162, doi: 10.3390/ Ijms242015162.*
45. *Huffer K, Denley MCS, Oskoui EV, Swartz KJ. (2024) Conservation of the cooling agent binding pocket within the TRPM subfamily. Elife; 13:RP99643, doi: 10.7554/eLife.99643.*
46. *Han M, Xu G. (2026) The TRPM family: key players and mechanisms in energy metabolism. Cell Calcium; 135:103135, doi: 10.1016/j.ceca.2026.103135.*
47. *Yu X, Xie Y, Zhang X, Ma C, Liu L, Zhen W, Xu L, Zhang J, Liang Y, Zhao L, Gao X, Yu P, Luo J, Jiang LH, Nie Y, Yang F, Guo J, Yang W. (2021) Structural and functional basis of the selectivity filter as a gate in human TRPM2 channel. Cell Rep; 37(7):110025, doi: 10.1016/ j.celrep.2021.110025.*
48. *Sultana S, Xie Y, Kamal MS, Li W. (2026) Pathophysiological significance of the TRPM2 ion chan-*

nel as a potential target in cancer, neurological disorders, and ischemia/reperfusion injury. Pharmacol Res; 225:108132, doi: 10.1016/j.phrs.2026.

49. *Zhao C, MacKinnon R. (2023) Structural and functional analyses of a GPCR-inhibited ion channel TRPM3. Neuron; 111(1):81-91.e7, doi: 10.1016/j.neuron.2022.10.002.*
50. *Dhande M, Chouhan D, Verma N, Deb PK, Verma N, Tiwari V. (2026) Neurobiology of TRPM channels in pain processing: Current advances and future directions. Life Sci; 386: 124169, doi: 10.1016/j.lfs.2025.124169.*
51. *Gandini MA, Zamponi GW. (2024) Navigating the controversies: Role of TRPM channels in pain states. Int J Mol Sci; 25(19):10284, doi: 10.3390/ijms251910284.*
52. *Klein AH, Sawyer CM, Carstens MI, Tsagareli MG, Tsiklauri N, Carstens E. (2010) Topical application of L-menthol induces heat analgesia, mechanical allodynia, and a biphasic effect on cold sensitivity in rats. Behav Brain Res; 212(2):179-186, doi: 10.1016/j.bbr.2010.04.015.*
53. *Mahmoud O, Soares GB, Yosipovitch G. (2023) Transient receptor potential channels and itch. Int J Mol Sci; 24: 420, doi: 10.3390/ijms24010420.*