ANNUAL REVIEWS

Annual Review of Pharmacology and Toxicology

TRP Channel Cooperation for Nociception: Therapeutic Opportunities

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Annu. Rev. Pharmacol. Toxicol. 2021. 61:655–77

First published as a Review in Advance on September 25, 2020

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

https://doi.org/10.1146/annurev-pharmtox-010919-023238

Keywords

TRP channel, TRPV1, TRPA1, TRPM3, nociception, drug development

Abstract

Chronic pain treatment remains a sore challenge, and in our aging society, the number of patients reporting inadequate pain relief continues to grow. Current treatment options all have their drawbacks, including limited efficacy and the propensity of abuse and addiction; the latter is exemplified by the ongoing opioid crisis. Extensive research in the last few decades has focused on mechanisms underlying chronic pain states, thereby producing attractive opportunities for novel, effective and safe pharmaceutical interventions. Members of the transient receptor potential (TRP) ion channel family represent innovative targets to tackle pain sensation at the root. Three TRP channels, TRPV1, TRPM3, and TRPA1, are of particular interest, as they were identified as sensors of chemical- and heat-induced pain in nociceptor neurons. This review summarizes the knowledge regarding TRP channel–based pain therapies, including the bumpy road of the clinical development of TRPV1 antagonists, the current status of TRPA1 antagonists, and the future potential of targeting TRPM3.

1. INTRODUCTION

Although generally described as an unpleasant sensation, pain is vital to detect noxious stimuli in the environment and to protect our body from the harm they may cause. By contrast, chronic pain no longer fulfills a physiological goal; it represents an incapacitating pathology that burdens millions of patients worldwide (1, 2). The condition may arise from a degenerative process or an incurred lesion, but determining the culprit is not always obvious. Yet the prevalence of chronic pain is only expected to increase in our aging population. Effective treatment of this healthcare problem is therefore a top priority, but it unfortunately continues to present a major challenge. Currently marketed analgesics such as nonsteroidal anti-inflammatory drugs and paracetamol can be very effective to treat common aches but often provide inadequate pain relief in chronic pain patients. On the other hand, opioids are the most potent analgesics available, yet their use is burdened by unwanted side effects, the development of tolerance, and an increasing addiction problem (3–5). Other classes of analgesic drugs such as gabapentinoids, tricyclic antidepressants, and serotonin/norepinephrine reuptake inhibitors are effective in only a limited number of chronic pain patients. Consequently, there is a high demand for novel analgesics with an improved efficacy and safety profile.

Promising pain research in the last few decades has shed light on the physiology of pain and the pathological events underlying chronic pain conditions, thereby creating opportunities for novel pharmaceutical interventions. One attractive approach is to target the very beginning of the pain pathway (**Figure 1**), focusing on nociceptive receptors. In particular, several members of the transient receptor potential (TRP) superfamily are notorious for their role in nociception, for example, the burning sensation associated with chili peppers or the pungent taste of wasabi.

2. TRP ION CHANNELS: A PROMISCUOUS FAMILY

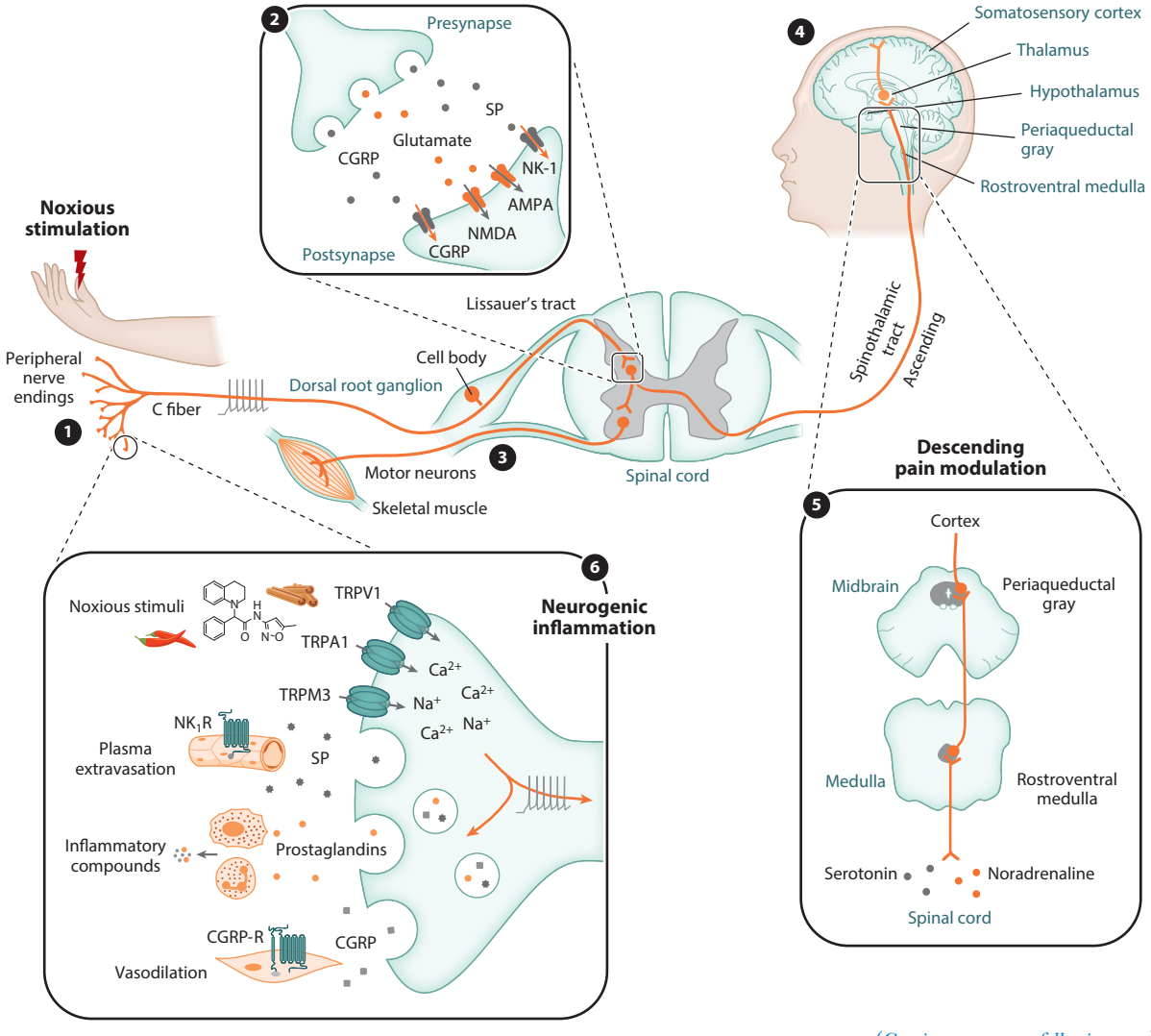
The discovery of TRP channels traces back to 1969, when a *Drosophila melanogaster* mutant was isolated that exhibited a transient receptor potential in its photoreceptors upon continuous illumination (6, 7). The *Drosophila trp* gene was cloned in 1989 (8), and further research led to the identification of a large TRP family consisting of more than 50 members. In mammals, 28 TRP channels have been characterized, grouped into six subfamilies: transient receptor potential ankyrin (TRPA), TRP canonical, transient receptor potential melastatin (TRPM), TRP mucolipin, TRP polycystin, and transient receptor potential vanilloid (TRPV) (9). These ion channels generally possess six transmembrane domains, but the overall structure differs a lot between subfamilies. Furthermore, there is wide heterogeneity in gating mechanisms, including activation by various endo- or exogenous ligands as well as voltage- and temperature-gated channels. As such, the TRP family contributes to a variety of physiological functions, including vision, hearing, taste perception, thermosensation, and responding to different environmental stimuli (9). Nonetheless, this ion channel family is probably most (in)famous for its role in nociception. In fact, TRP channels constitute the largest group of nociceptive ion channels involved in pain sensation in mammals.

The growing interest in TRP channels as transducers of painful stimuli started about 20 years ago, following the discovery and subsequent cloning of TRPV1. Initially referred to as vanilloid receptor subtype 1 (or VR1), the ion channel was described as a nonselective cation channel activated by the hot chili pepper component capsaicin, by thermal stimuli within the noxious range, and by protons (10). Being an integrator of diverse noxious stimuli, TRPV1 is predominantly expressed in a subset of primary sensory neurons, particularly in thinly myelinated Aδ fibers and in unmyelinated C fibers, both peptidergic and nonpeptidergic (see the sidebar titled Peptidergic Versus Nonpeptidergic Afferents). In addition, the capsaicin receptor has been identified in the

spinal cord and brain as well as in nonneuronal tissues like the bladder and skin, although the function of the ion channel in these tissues remains poorly understood (11).

3. TRPV1 AND THE HEAT-ACTIVATED TRP TRIO

The generation of TRPV1-deficient mice further highlighted the channel's involvement in acute and inflammatory pain. Indeed, these animals lacked a pain response to capsaicin and also had a significantly reduced (but still robust) avoidance response to noxious heat. More intriguingly, TRPV1-deficient mice failed to develop heat hyperalgesia following tissue inflammation induced by the injection of carrageenan or Freund's Complete Adjuvant (12, 13). Based on these promising preclinical results, TRPV1 soon aroused interest as a novel target for inflammatory pain conditions, and to date, TRPV1 remains the most thoroughly studied member of the TRP family.



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Pain and neurogenic inflammation following the activation of nociceptive transient receptor potential (TRP) channels. (①) In the event of painful stimulation, nociceptive ion channels, including transient receptor potential vanilloid 1 (TRPV1) (for instance, activated by capsaicin, the pungent component of chili peppers), transient receptor potential ankyrin 1 (TRPA1) (with cinnamaldehyde, responsible for the flavor and odor of cinnamon, as the known channel activator), and transient receptor potential melastatin 3 (TRPM3) (with CIM0216 as the potent synthetic agonist), can be activated. (②) The nociceptive signal travels up to the spinal cord via myelinated Aδ fibers or unmyelinated C fibers, terminating in the upper laminae of the dorsal horn. Within the spinal cord, second-order neurons are activated through the release of neurotransmitters, including glutamate, substance P (SP), and calcitonin gene–related peptide (CGRP). While CGRP acts on the CGRP receptor complex, SP activates the neurokinin-1 receptor (NK-1). In the case of glutamate, the two major receptors in the spinal cord are the α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA) receptor and the *N*-methyl-D-aspartate (NMDA) receptor. (③) To ensure a rapid withdrawal reflex, the nociceptive information is communicated to motor neurons. (④) A sensation of pain arises when the spinothalamic tract passes the information to the brain, where the thalamus relays the nociceptive signal to the cortical areas. (⑤) Descending systems originate in these higher brain regions that can attenuate or facilitate the nociceptive input in the dorsal horn. Both the periaqueductal gray and rostroventral medulla are key structures for the descending modulation of pain. Activation of TRP channels within the antinociceptive pathway is one of the strategies to obtain analgesia. (⑥) Besides currents conducted toward the central nervous system, the activation of calcium-permeable TRP channels in the terminals of peptidergic C fibers can promote the local release of inflammatory mediators, including SP, CGRP, and prostaglandins. Via the NK-1 receptor expressed on endothelial cells, SP increases vascular permeability, leading to plasma extravasation and thereby to swelling of the area. CGRP is a potent vasodilator, exerting its effect by interaction with the CGRP receptor on vascular smooth muscle cells. This vasodilatation causes the typical symptoms of warmth and redness. Furthermore, SP, CGRP, and prostaglandins act on neighboring mast cells and immune cells, resulting in the release of inflammatory and proalgesic mediators. This inflammatory soup in turn activates other sensory nerve endings, enlarging the response. Together these symptoms characterize neurogenic inflammation, a vicious cycle of ongoing nociceptor activation and inflammation manifesting itself as hypersensitivity (140, 141).

Moreover, its discovery as a heat sensor opened the door toward the identification of a subset of thermosensory TRP channels, the so-called thermoTRPs (14), which include channels that are activated by heating and/or cooling over a broad thermal range between 5 and 55°C. Whereas the role of the menthol-sensitive channel TRPM8 in conveying cool temperatures is well established and generally accepted, the exact contribution of, for instance, TRPA1 to the detection of noxious cold or TRPV3, TRPV4, and TRPM2 to warmth sensation remains to be fully uncovered (15).

Recent research revealed that noxious heat sensing in mice depends on a set of three TRP channels, including not only TRPV1 but also TRPA1 and TRPM3. The functionality of at least one of these three TRPs was shown to be sufficient yet crucial to maintain acute heat sensitivity,

PEPTIDERGIC VERSUS NONPEPTIDERGIC AFFERENTS

Interestingly, transient receptor potential vanilloid 1 (TRPV1) expression has been reported in two distinct populations of C fibers. One specific class, termed peptidergic afferents, can induce the release of neuropeptides, including calcitonin gene–related peptide (CGRP) and substance P (SP). However, not all nociceptors express SP and CGRP. As they develop, some nociceptive C fibers switch off the tropomyosin receptor kinase A (TrkA) with high affinity for the neurotrophin nerve growth factor (NGF). Instead, they begin to express the glial cell line–derived neurotrophic factor (GDNF) receptor Ret. These neurons are known as nonpeptidergic nociceptors. Peptidergic nociceptors preserve TrkA expression, and only these neurons express SP and CGRP (11, 137). Interestingly, elevated levels of both NGF and GDNF have been described in circumstances of inflammation, in which these factors are known to upregulate the expression of TRPV1 and as such facilitate thermal hyperalgesia (138, 139). Conversely, the neuropeptides released from peptidergic afferents following TRP activation are known to evoke neurogenic inflammation (**Figure 1**) and as such contribute to the typical hypersensitivity and pain symptoms following tissue injury (140).

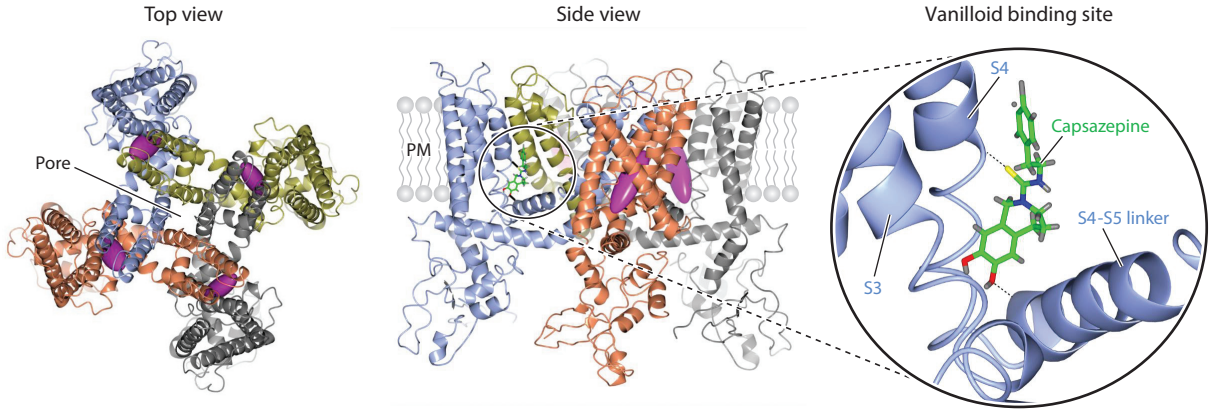


Figure 2

Structure of transient receptor potential vanilloid 1 (TRPV1). Ribbon diagrams show the structure of TRPV1 as seen from the extracellular side (*left*), with indication of the central pore, and from the side (*middle*), with indication of the plasma membrane (PM). Differently colored protein regions indicate the four identical subunits of the tetrameric channel. The location of the vanilloid binding site is shown by the magenta ellipsoids. On the right, the details are given for one vanilloid binding site, the location of which is indicated in the side view (*black circle*). This binding site is occupied by a molecule of capsazepine, a competitive vanilloid antagonist. Structures were rendered using CCP4MG version 2.10.11 (**http://www.ccp4.ac.uk/MG/**) based on atomic coordinates in the Protein Data Bank (**https://www.rcsb.org/structure/5IS0**) (142).

as triple-knockout mice, deficient for all three heat-activated channels, lacked the protective withdrawal reaction when exposed to noxious heat, making them vulnerable to burn injuries (16). Notably, all three channels, which show a partly overlapping expression profile in nociceptor neurons, have been implicated in pathological pain signaling and hypersensitivity and are therefore appealing targets for analgesic drug development. Here we provide an overview of lessons learned from drug development projects targeting these three TRP channels and provide an outlook on how these may one day lead to novel analgesic drugs to treat various types of pathological pain.

3.1. Targeting TRPV1: The Hot-Headed Family Member

Although the molecular basis—the interaction with the TRPV1 vanilloid binding site (**Figure 2**)—took longer to decipher, chili peppers have been known to provoke pungency and pain since time immemorial. In addition to their widespread use to spice up food, the principle of capsaicin-induced acute pain is utilized nowadays in, for example, capsaicin-containing pepper sprays for policing or self-defense. On the other hand, TRPV1 also shows analgesic potential. Different approaches (**Figure 3**) have been tested to combat pain, starting with agonists and antagonists of TRPV1 (17).

3.1.1. Inhibiting pain by overwhelming TRPV1. The desensitizing and analgesic effects of capsaicin (**Supplemental Table 1**) are nothing new. To illustrate, alcohol or gum enriched with capsaicin is an old trick to relieve toothache, where the analgesic effect arises from a lasting refractory state following the excitation of sensory neurons by capsaicin. During this refractory or desensitized period, sensory neurons no longer respond to subsequent capsaicin stimulation or to other stimuli such as noxious heat. Of course, desensitization can also be exploited therapeutically. Indeed, a wide range of capsaicin-containing formulations, including creams and occlusive patches, are on the market for a variety of pain syndromes, ranging from minor muscle or joint

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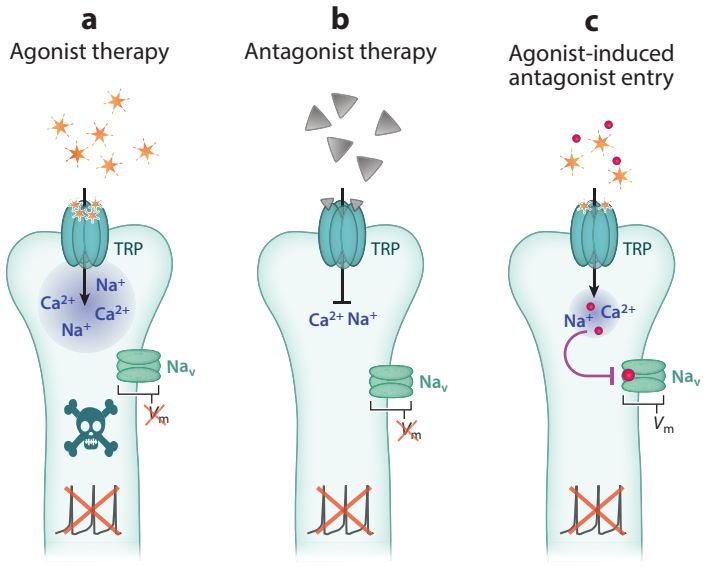

Figure 3

Therapeutic transient receptor potential (TRP) strategies. (*a*) In agonist therapy, opening the TRP channel (TRPV1, TRPA1, or TRPM3) by application of an ultrapotent agonist induces a massive influx of sodium and calcium ions in the peripheral nerve endings. The extracellular calcium entering through TRP channels overwhelms the intracellular calcium-buffering capacity and thereby activates calcium-dependent proteases and cytoskeleton breakdown. As a result, local nociceptor function is impaired for an extended period. An example of this strategy includes pain management with a capsaicin 8% patch to rapidly deliver the TRPV1 agonist into the skin. (*b*) In antagonist therapy, selective TRP inhibitors induce a full block of the TRP channel and prevent depolarization of the membrane potential by blocking the influx of cations like sodium and calcium. As a consequence, voltage-dependent sodium channels (Na_V) are not activated. (*c*) In agonist-induced antagonist entry, when combining a TRP channel agonist with local anesthetics like inhibitors of Na_V, the membrane-impermeable sodium channel blocker enters the nerve endings via the TRP pore. For example, anesthetic drugs are chaperoned through the pore of the TRPV1 channel and block Na_V channels in all nerve endings expressing TRPV1.

aches to chronic painful conditions such as diabetic neuropathy, postherpetic neuralgia, and arthritis (18, 19). However, the clinical value of these capsaicin-containing formulations is limited by the delicate balance between their irritating and desensitizing effects. Indeed, when the concentration acquired at the sensory nerve endings is not sufficiently high, capsaicin application will merely result in a stinging sensation without the anticipated desensitization. On the other hand, one should be cautious not to produce neurotoxic effects at the high therapeutic doses. Accordingly, systemic administration of capsaicin is not attainable in clinical practice, despite its powerful therapeutic potential (18, 20).

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In an attempt to optimize the desensitization window, resiniferatoxin (RTX) (**Supplemental Table 1**) is currently under clinical investigation. RTX is another pungent plant product that is derived from *Euphorbia resinifera*, the dried latex of which has a long history of medical use (21). RTX is considered to be the ultrapotent analog of capsaicin and is currently the most powerful TRPV1 agonist known. As such, stimulation of the TRP channel by RTX induces an exceedingly prolonged calcium influx, desensitizing the sensory neurons that express TRPV1 and thereby surpassing the pharmacological mechanism of action associated with capsaicin (20, 22). At present,

RTX is undergoing clinical trials, in which it is being administrated via intrathecal or epidural injection, to test its lasting pain relief potential in patients with advanced cancer. Preliminary results suggest that RTX can indeed evoke cell death of TRPV1-positive nociceptor neurons in humans, causing prolonged pain relief (23). In addition, intra-articular injection of the plant derivative is in development for moderate to severe knee pain due to osteoarthritis, for which Sorrento Therapeutics recently progressed to phase III trials (**https://clinicaltrials.gov/ct2/show/NCT04044742**). On the other hand, RTX was found to be unsuccessful in treating interstitial cystitis (24).

Notably, in addition to overwhelming the ion channel, the activation of TRPV1 in the antinociceptive descending pain pathway might be an alternative strategy to combat pain. In this respect, endocannabinoids, including anandamide, were identified as channel agonists, and more recently, AM404, a metabolite of paracetamol, was also shown to be a potent activator of TRPV1 in the brain (25–29). Yet besides activating and desensitizing TRPV1 using channel agonists, TRPV1 antagonists also possess the therapeutic potential to revolutionize pain management. To support early clinical development of these TRPV1-targeting drugs, capsaicin once again proved to be very useful. Capsaicin is well known to provoke cutaneous neurogenic inflammation following topical application or intradermal injection (**Figure 1**). The resulting flare, induced by an increase in dermal blood flow, can serve as a basis to evaluate TRPV1 target engagement in both animals and humans (**Figure 4**).

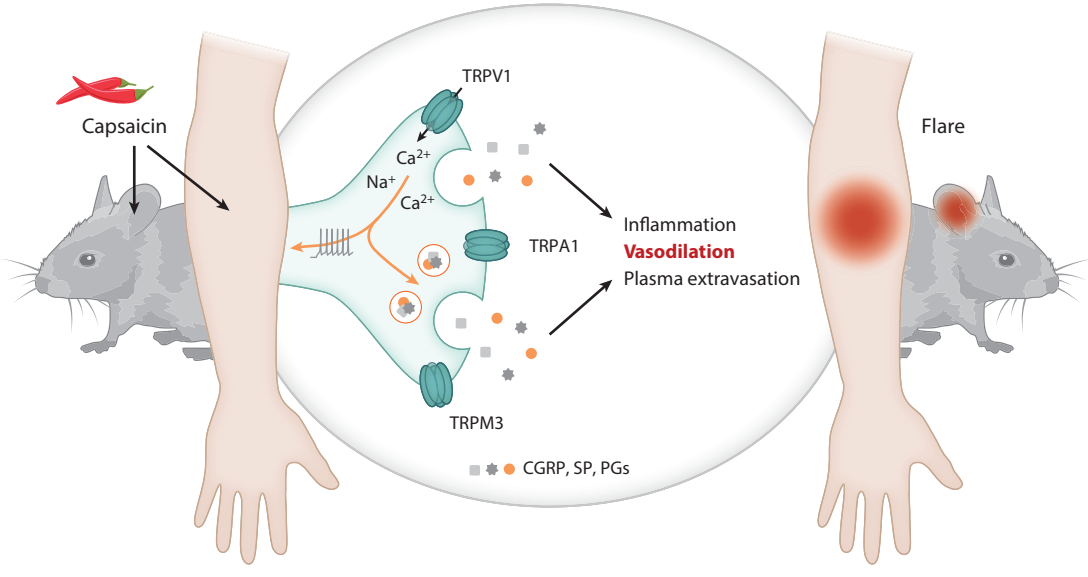

Figure 4

Capsaicin target engagement biomarker. Following its administration on the ears of anesthetized mice or on the volar surface of subjects' forearms, capsaicin will activate calcium-permeable transient receptor potential vanilloid 1 (TRPV1) channels expressed on the peripheral nerve endings of sensory neurons innervating the skin. As a result, several mediators, including calcitonin gene–related peptide (CGRP), substance P (SP), and prostaglandins (PGs), are released from peptidergic dermal afferents, in turn evoking inflammation, vasodilatation, and plasma extravasation, generally known as neurogenic inflammation. The vasodilatory component can be observed as a flare response with the naked eye and also measured quantitatively as an increase in dermal blood flow. This offers a noninvasive method to evaluate TRPV1 target engagement both preclinically and in early clinical drug development (110, 143, 144). In addition to TRPV1, comparable models can be developed for other transient receptor potential channels, for example, the cinnamaldehyde target engagement biomarker for transient receptor potential ankyrin 1 (TRPA1) (109, 145). Also transient receptor potential melastatin 3 (TRPM3) lends itself to a similar assay to test the channel's activity in rodents and humans.

3.1.2. First-generation TRPV1 antagonists: too hot to handle. Following the discovery of capsazepine (**Supplemental Table 1**), which is the oldest TRPV1 antagonist and which competes with capsaicin and RTX for the vanilloid binding site (**Figure 2**), TRPV1 research boomed (30, 31). Given that TRPV1-deficient mice demonstrate reduced heat sensitivity and do not develop inflammatory heat hyperalgesia, it was a straightforward step for the pharmaceutical industry to develop TRPV1 antagonists for pain relief. However, initial endeavors were far from successful due to a lack of efficacy and unwanted side effects.

SB-705498 (**Supplemental Table 1**) from GlaxoSmithKline (GSK) was the first TRPV1 antagonist to enter clinical development in 2005. The compound was well tolerated at single oral doses up to 400 mg and reduced both the flare evoked by topical application of capsaicin and the dermal inflammation induced by ultraviolet B irradiation (32). Based on the evident target engagement, GSK initiated phase II clinical studies. SB-705498 failed to demonstrate a positive effect on the intensity of dental pain after a third molar tooth extraction, which is a well-characterized model of acute inflammatory pain (33). In addition, the compound proved to be inferior to placebo against migraine headache and photo- and phonophobia (34). Following these unsuccessful results, GSK started aiming for respiratory and skin disorders as an alternative indication. Intranasal doses of the TRPV1 antagonist were evaluated in the management of rhinitis, but only modest symptom attenuation could be obtained (35). Likewise, in the case of chronic cough, the compound failed to reduce cough counts, despite its confirmed engagement with TRPV1 (36). Finally, drug development attempts were terminated after an SB-705498-containing cream was found unsuccessful in atopic dermatitis (37). In contrast to other first-generation TRPV1 antagonists (see below), effects on core body temperature have not been reported for SB-705498. This may reflect the cautious developmental approach of GSK, which may not have resulted in a similar degree of TRPV1 antagonism as achieved with other compounds (36, 38).

Amgen also invested substantially in small-molecule TRPV1 antagonists. Of the compounds evaluated in vitro, the polymodal antagonist AMG-517 (**Supplemental Table 1**), a potent blocker of TRPV1 when activated not only by capsaicin but also by other stimuli such as heat or low pH, was selected for clinical development (39, 40). However, the expectation of developing a selective new analgesic drug was soon tempered due to problematic hyperthermic side effects. In a single-dose phase I study, AMG-517 evoked a marked but reversible increase in body temperature. The maximum temperature recorded in humans was 39.9°C (40). A similar hyperthermic reaction was also observed in animals treated with AMG-517, where the effect was attenuated upon repeated dosing, suggesting that it may be circumvented in a clinical setting as well (41). Consequently, Amgen proceeded to a multiple-dose phase I trial, which demonstrated that humans also habituate to the hyperthermic effect induced by 10 mg of AMG-517. Furthermore, the temperature elevation was found to be plasma concentration dependent, with some individual susceptibility (40). Still, it was unknown whether the concentration of AMG-517 required to obtain analgesia in humans was below or above the threshold triggering hyperthermia. To investigate this hypothesis, a phase II study was initiated in patients with acute dental pain after molar extraction. These subjects also experienced marked hyperthermia, but unlike in the phase I trial, temperature elevations persisted for days, and individual susceptibility was no longer a unique case. About one-third of the participants experienced elevated body temperatures of 39 to 40.2°C, with the highest temperature registered in a subject who received the lowest dose: only 2 mg of AMG-517 (40). The different hyperthermic responses in these phase I and phase II studies were unexpected, but they were most likely an on-target effect of TRPV1 blockade, since TRPV1-knockout mice do not develop this drug-induced hyperthermia (42). Consequently, clinical studies involving AMG-517 were discontinued before uncovering its analgesic potential (40).

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Recently, Abbott (divided into Abbott and Abbvie since 2013) published clinical results on its polymodal antagonist ABT-102 (**Supplemental Table 1**). The analgesic capacities were assessed in a phase I crossover trial involving etoricoxib, tramadol, and low doses of ABT-102. Painful stimulation was evoked using a CO_2 laser on both normal and inflamed skin. In this experimental pain study, a 6-mg dose of ABT-102 was found to be effective in reducing pain and even superior to the active controls (43). Unfortunately, this compound also displayed temperature-related side effects, prompting its withdrawal from clinical development. In rats, ABT-102 evoked hyperthermia, an adverse on-target effect that attenuated within 2 days of recurrent dosing (44). Similarly, ABT-102 induced a dose-dependent increase in core body temperature in humans, although participants' temperatures never exceeded 39°C. By the seventh dosing day, the body temperature was no longer significantly different from that of placebo subjects (45, 46). Accordingly, the TRPV1 antagonist would have presented worthy therapeutic potential in the context of chronic pain if it were not for another temperature-related side effect. Indeed, ABT-102 was also found to affect the vital ability to sense noxious heat in subjects. With the first dose of the antagonist, subjects developed elevated cutaneous and oral heat pain thresholds as well as increased withdrawal latencies from a 49°C water bath. Unlike the hyperthermic effects, these deficits maintained after repeated dosing. Thermosensation only recovered when the antagonist was nearly washed out. As a result, development of ABT-102 was terminated due to a substantial clinical risk of burns (46).

Similarly, AstraZeneca also attempted to develop a polymodal TRPV1 antagonist. AZD1386 (**Supplemental Table 1**) entered clinical development in 2008 with a single-dose study to investigate its effect on capsaicin- and heat-induced pain (47). Relying on its encouraging antinociceptive effect and evident target engagement, AstraZeneca continued to invest in its antagonist. A slight hyperthermic effect was noted but was not considered clinically significant, as the largest rise in body temperature was 1.2°C, and the highest temperature recorded was 38°C (48, 49). Furthermore, the hyperthermia attenuated after multiple dosing, unlike the impact on skin heat perception, with the heat pain threshold increasing 4.8°C on average compared to placebo (47, 49). Based on these overall mild temperature-related side effects, AstraZeneca proceeded to evaluate the analgesic activity of its antagonist. In patients experiencing pain following third molar extraction, the primary end point focused on pain intensity over an 8-h period but did not reach statistical significance. Instead, AZD1386 was shown to induce a very rapid but temporary analgesic effect. Within 15 min after receiving an oral solution containing AZD1386, subjects rated their pain intensity considerably lower than placebo. Surprisingly, the reported analgesia did not last longer than 1 h, unlike the elevated heat pain threshold, which persisted up to 5 h after dosing. Furthermore, the analgesic effect was obtained at relatively low plasma concentrations, which only peaked after 1 h, when the analgesia had already started to decrease (48). As such, the potential of AZD1386 for chronic pain conditions remained undecided, and unfortunately, the drug candidate had to be withdrawn from further development, as several patients in an osteoarthritic pain study exhibited elevated hepatic enzymes. Note that this study also hinted at some analgesic effect, as a reduction in pain intensity using the numerical rating scale could be demonstrated. However, no clinically relevant effect on the Western Ontario and McMaster osteoarthritis index (WOMAC) could be obtained (50).

In addition, Johnson & Johnson Pharmaceutical Research & Development developed a TRPV1 antagonist aimed at treating osteoarthritic pain. Mavatrep, otherwise known as JNJ-39439335 (**Supplemental Table 1**), originated from a benzo[*d*]imidazole platform and is a potent competitive antagonist of capsaicin-, pH-, and heat-evoked currents mediated by TRPV1 (51). After confirming dose-dependent target engagement (52), the analgesic potential was assessed in patients with chronic osteoarthritic pain of the knee. Mavatrep diminished pain intensity following a stair climbing effort and improved osteoarthritic pain and stiffness using the

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WOMAC questionnaire, although the latter could not be confirmed in a multiple-dose study (53, 54). Unfortunately, this compound also suffered from temperature-related side effects. As only the hyperthermic effect, and not the thermohypoesthesia, minimized upon repeated dosing, the company terminated clinical development (52).

Many other pharmaceutical companies experienced similar obstacles in the quest for TRPV1 antagonists. MK-2295/NGD8243 (**Supplemental Table 1**), a development collaboration between Merck and Neurogen, demonstrated significantly impaired sensitivity to noxious heat, with subjects requiring more than 1 min extra to pull their hand out of a 49°C water bath. Moreover, up to 40% of subjects did not recognize 70°C water as noxious (55, 56). On the other hand, V116517 (**Supplemental Table 1**) from Purdue Pharma displayed a clean safety profile in humans, although a dose-dependent increase in body temperature was described in rats (57, 58). Nonetheless, no additional clinical studies have been registered for this compound. Likewise, clinical development of GRC-6211 (**Supplemental Table 1**), a potent and competitive TRPV1 antagonist created by Eli Lilly and Glenmark Pharmaceuticals, was suspended without providing details (59–61). Japan Tobacco halted development of JTS-653 (**Supplemental Table 1**), one of the most potent polymodal TRPV1 antagonists (62), and DWP05195 (structure not disclosed) from Daewoong Pharmaceutical did not go beyond phase II trials (63). At the moment, only tivanisiran (formerly SYL1001), a small interfering RNA targeting TRPV1, is still under development for dry eye disease, where the nociceptive channel is thought to be involved in ocular pain and inflammation (64). So far, topical administration of tivanisiran has been shown effective without tolerability issues (65, 66).

3.1.3. Second-generation TRPV1 antagonists: a more cooled down approach. In an attempt to achieve TRPV1-mediated analgesia without the accompanying hyperthermic effects, modality-selective antagonists were developed. Amgen was one of the pioneers that experimented with different TRPV1 modulators. Interestingly, AMG-8562 (**Supplemental Table 1**), an antagonist that blocks capsaicin- but not heat-induced TRPV1 stimulation and potentiates activation by noxious pH, did not elicit hyperthermia in rats. Conversely, AMG-7905 (**Supplemental Table 1**), a compound that antagonizes activation by capsaicin but potentiates heat and proton stimulation, produced striking hypothermia. Accordingly, AMG-8562 was considered the long-awaited holy grail in TRPV1 drug development. The thermoneutral molecule was further evaluated in different experimental pain models, where it produced analgesic effects similar to those described for first-generation polymodal TRPV1 antagonists. However, AMG-8562 presented a different pharmacological profile on human TRPV1, as the compound blocked activation by protons instead of the potentiation demonstrated in rats. Hence, it was expected that AMG-8562 would still evoke hyperthermia in humans. As a result, the compound was never pursued clinically. Nonetheless, Amgen demonstrated that it is possible to separate the hyperthermic effects associated with TRPV1 modulation from the antihyperalgesic effects, paving the way for second-generation TRPV1 antagonists (67).

Other pharmaceutical companies soon jumped on board to develop TRPV1 antagonists without the undesirable effects on body temperature and noxious heat threshold. NEO6860 (structure not disclosed), developed by the NEOMED Institute, is one such new-generation antagonist that blocks TRPV1 activation by capsaicin but not by heat or protons. A first-in-human study in 2015 discovered no relevant effect on core body temperature nor on heat pain threshold at doses ascending from 50 to 1,200 mg. The only temperature-related adverse event was a slight perception of feeling hot. Due to the well-known involvement of TRPV1 in heat sensation, this was believed to be an on-target effect. Other evidence of target engagement was provided by a reduction of the pain and flare induced by intradermal capsaicin injection (68, 69). To evaluate the analgesic

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properties of its modality-selective antagonist, NEOMED set up a proof-of-concept study in patients with osteoarthritic knee pain. NEO6860 provided pain relief based on the numerical rating scale pre- and postexercise; the patient's global impression of change; and, to a smaller degree, the WOMAC (70). However, further research is necessary to fully comprehend the therapeutic potential of this compound.

PharmEste also developed a TRPV1 antagonist with an apparently clean safety profile. Remarkably, PHE377, alternatively named V-377 (**Supplemental Table 1**), was reported to antagonize both capsaicin- and proton-induced activation of TRPV1 (71). As such, there was skepticism when the first-in-human trial was initiated. According to the company website, different phase I clinical trials confirmed the favorable safety profile of PHE377. However, PharmEste did not support this claim with data, and for reasons unknown, PHE377's clinical development was terminated.

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3.1.4. The future of TRPV1 antagonists: extinguished? In conclusion, despite significant investments, there is currently no TRPV1 antagonist registered for clinical use. The efforts to develop small molecules targeting TRPV1 have produced multiple potent and selective inhibitors, but none of these compounds came close to market authorization. Several reasons contributed to this unsatisfactory return on investment. Most importantly, acute pharmacological inhibition of TRPV1 has been shown to evoke hyperthermia, independent of the chemical structure of the antagonist. This on-target side effect appears to be related to the blockade of constitutively active TRPV1 channels on thermal afferents in the abdomen. These neurons show basal activity under physiological circumstances, which leads to a tonic suppression of autonomic cold defense mechanisms. Acute inhibition of TRPV1 reduces the basal activity of these thermal afferents, which in turn reduces the tonic suppression, ultimately causing hyperthermia. Although concerning, the evoked temperature elevation is not permanent, as compensatory mechanisms can develop over time (72). Indeed, the hyperthermic effect of some TRPV1 antagonists attenuated upon repeated administration in humans, while the analgesic effect remained. Still, in an attempt to overcome this side effect, TRPV1 antagonists with different pharmacological profiles were investigated. The hyperthermic effect correlated with the proton mode of TRPV1 activation: Antagonists with a high potency to block stimulation by protons induced hyperthermia, whereas antagonists that potentiated the TRPV1-mediated proton response even caused hypothermia (72, 73). Interestingly, Garami et al. (74) recently suggested that in humans, but not in rats, the heat activation mode of TRPV1 also plays a role in temperature regulation, while the capsaicin mode was shown to be irrelevant. As such, second-generation, modality-selective TRPV1 antagonists still hold some promise, but the clinical potential of this generation has not yet been thoroughly explored.

In addition to the numerous attempts to block TRPV1 activity, interest in selective channel agonists to target local anesthetics toward sensory neurons was rekindled. Most local anesthetics, including lidocaine, exert their effect by interacting with sodium channels embedded in the cell membrane, thereby reducing the neuronal excitability and consequently generating anesthesia. However, as these drugs do not specifically target sensory neurons, common side effects include low blood pressure and local paralysis. In an attempt to stay away from sympathetic and motor nerve fibers, sodium channel blockers in combination with TRPV1 agonists were shown to produce pain-specific local anesthesia by chaperoning the anesthetic through the pore of the TRPV1 channel (75) (**Figure 3**). In mice, this approach was shown to induce a long-lasting anesthetic effect without the burning sensation generally known to accompany capsaicin injection (76). However, to the best of our knowledge, the clinical potential of this combination was never investigated in humans.

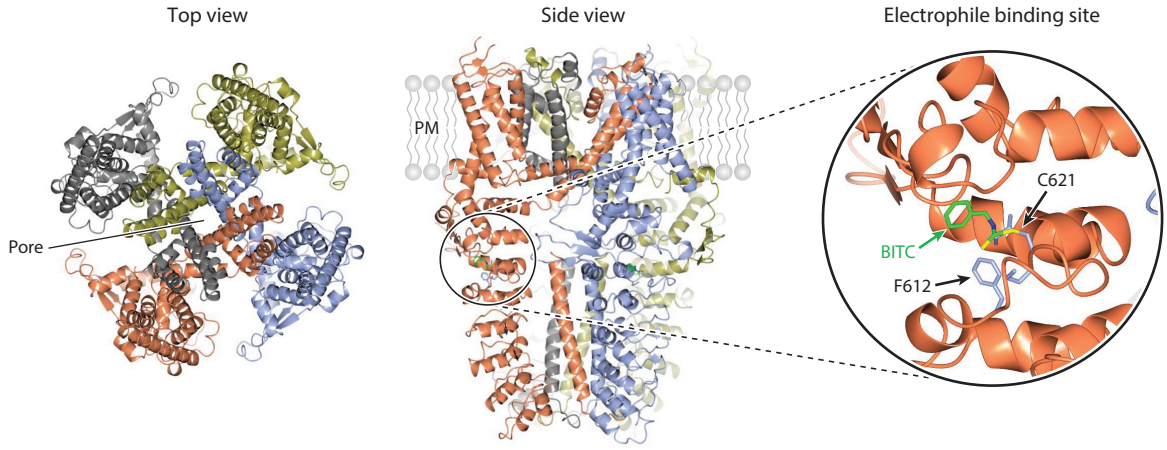

Figure 5

Structure of transient receptor potential ankyrin 1 (TRPA1). Ribbon diagrams show the structure of TRPA1, as seen from the extracellular side (*left*), with indication of the central pore, and from the side (*middle*), with indication of the plasma membrane (PM). Differently colored protein regions indicate the four identical subunits of the tetrameric channel. The details of one electrophile binding site indicated in the side view (*black circle*) are shown (*right*). This covalent binding site is occupied by a molecule of benzyl isothiocyanate (BITC), one of the many electrophilic agonists of TRPA1. BITC is covalently bound to cysteine residue C621; the reactivity of this cysteine is strongly enhanced by the neighboring phenylalanine residue F612 through a thiol-π interaction. Structures were rendered using CCP4MG version 2.10.11 (**http://www.ccp4.ac.uk/MG/**), based on atomic coordinates in the Protein Data Bank (**https://www.rcsb.org/structure/6PQP**) (146).

3.2. TRPA1: The Only Child of the Family

TRPA1 caught the eye as an alternative drug target to TRPV1. This single mammalian member of the TRPA subfamily is highly coexpressed with TRPV1 in nociceptor neurons and was initially described as a noxious cold sensor, although its role in cold sensing in mice and humans remains a matter of debate (77–80). In contrast, the role of TRPA1 in nociceptive responses to chemical compounds is well established. TRPA1 can be activated by a broad variety of irritants, which include numerous natural pungents like cinnamaldehyde (**Supplemental Table 2**), responsible for the flavor and odor of cinnamon; allicin, present in garlic; and allyl isothiocyanate (**Supplemental Table 2**), the pungent ingredient in wasabi and mustard oil. Additionally, acrolein, a toxicant in cigarette smoke and tear gasses, is also a known channel agonist. All of these compounds activate TRPA1 through the covalent modification of reactive cysteine and lysine residues in the channel's N terminus (**Figure 5**). Nonelectrophilic agonists have also been described, including menthol, nicotine (81), and cannabidiol. Moreover, multiple endogenous compounds released under conditions of oxidative stress and inflammation have been described to activate TRPA1 (82–84), evidently pointing toward a role of the channel in nociception and pathological pain. This has been confirmed in several animal models that showed effects of genetic ablation or pharmacological inhibition of TRPA1 in inflammatory pain as well as in diabetic and chemotherapy-induced painful neuropathy (85–91).

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Notably, familial episodic pain syndrome (FEPS) provides genetic evidence that variations in the *TRPA1* gene are capable of altering pain sensitivity in humans. FEPS results from a point mutation in the channel gene, which is associated with an enhanced response to both chemical and thermal TRPA1 activation. Individuals suffering from the syndrome describe debilitating episodes of upper body pain, which are often elicited by fasting, cold, and physical stress (92). Based on all

these findings, TRPA1 is considered to be a potential analgesic and anti-inflammatory target and as such has been pursued by several pharmaceutical companies.

3.2.1. TRPA1 antagonists: thinner on the ground. In contrast to TRPV1, where agonist-induced desensitization is an established means of analgesia (see above), the use of TRPA1 agonists to induce pain relief has been poorly explored. However, a recent study related the antinociceptive effect of acetaminophen to activation of TRPA1 in the spinal cord. It was proposed that the sodium and calcium influx following stimulation of spinal TRPA1 would generate excitatory postsynaptic currents and that the subsequent inhibition of voltage-gated sodium and calcium channels on the central terminals in the dorsal horn would reduce neuronal excitability, providing a mechanism for the antinociceptive effect of the common analgesic, at least preclinically (93). Nonetheless, pharmaceutical companies have mainly invested in channel antagonists, as was the case with TRPV1.

Over a decade ago, Hydra Biosciences took the first step toward developing therapeutic TRPA1 antagonists with HC-030031 (**Supplemental Table 2**), a xanthine derivative (94). The compound was shown to diminish mechanical hypersensitivity in rodent models of inflammatory and neuropathic pain (95) and was found to be effective in reducing inflammation and hyperactivity of the airways in wild-type mice exposed to airway allergens (96). HC-030031 soon became a stalwart friend in preclinical TRPA1 research and put the channel in the spotlight as a drug target for diabetic neuropathy, chemotherapy-induced neuropathic pain, inflammatory bowel disease, and respiratory disorders (97). Yet, from a pharmacokinetic perspective, the caffeine (**Supplemental Table 2**) derivative showed less promise, with a half-life of around 30 min in rats, high clearance, and only micromolar potency (98). Nevertheless, it inspired pharmaceutical companies to develop more potent TRPA1 antagonists with enhanced pharmacokinetic properties.

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In 2012, Glenmark Pharmaceuticals selected GRC-17536 (**Supplemental Table 2**) from a series of caffeine-based TRPA1 antagonists to initiate phase I development. The drug was well tolerated and demonstrated a decent pharmacokinetic profile (99). A subsequent proof-of-concept study in patients with painful diabetic peripheral neuropathy delivered a promising, statistically significant, and clinically relevant response in the subgroup without denervation, without affecting the central nervous system or inducing other drug-related adverse effects (100). Glenmark also evaluated the compound in patients with refractory chronic cough, but no significant improvement in cough frequency could be established (**https://www.clinicaltrialsregister.eu/ctr-search/trial/2013-002728-17/results**). GRC-17536 is now a candidate for out-licensing, but full clinical development is supposedly hampered by the drug's poor pharmaceutical characteristics, as the structure is rather bulky, lipophilic, and poorly soluble (101).

Although Glenmark was the first company to take a TRPA1 antagonist to clinical development, Hydra Biosciences also continued to work on better channel blockers. In 2012, the company announced a phase I clinical trial for CB-625 (**Supplemental Table 2**), a joint collaboration effort with Cubist Pharmaceuticals, but pharmacokinetic concerns supposedly halted further development of this nanomolar potent TRPA1 modulator (101, 102).

In general, the first class of xanthine-based antagonists suffered from pharmaceutical and pharmacokinetic flaws despite strong inhibitory potencies. As such, several companies shifted their focus toward other classes of small-molecule TRPA1 antagonists. In recent years, companies such as Abbott, Amgen, AstraZeneca, Janssen, Merck, Pfizer, and Roche have all described channel modulators with diverse medicinal chemistry. Some of these compounds were shown to reduce nocifensive behavior in rats exposed to cinnamaldehyde or mustard oil, but for the most part, not much is known about these development attempts (101). Among other concerns, inconsistent effects across species proved to be a hurdle in the progress of these drug candidates (103, 104). For example, Amgen disclosed a series of species-specific TRPA1 modulators, including AMG9090

(**Supplemental Table 2**), an antagonist of hTRPA1 and a partial agonist of rTRPA1 (105). Interestingly, an antagonist developed by Novartis (**Supplemental Table 2**) was shown to affect the cold perception of naïve mice at −5°C without altering body temperature (106). On the other hand, none of the subjects included in a human TRPA1-specific pain study reported a cold sensation after intradermal injections of JT010 and A-967079 (**Supplemental Table 2**), a selective agonist and antagonist of TRPA1, respectively (107).

In 2015, the Orion Corporation initiated a phase I study for ODM-108, a negative allosteric TRPA1 modulator. The chemical structure of this antagonist has not been disclosed so far, but it likely resembles the alkyne-containing compounds (**Supplemental Table 2**) patented in the same year (108). Unfortunately, the structural modifications were no golden ticket to success, as Orion terminated the study early due to complex pharmacokinetic results. However, no safety concerns were reported (**https://clinicaltrials.gov/ct2/show/NCT02432664**).

Also, Hydra Biosciences tried a different approach. In addition to its HC-030031 analog CB-625, the company developed HX-100 (structure not disclosed), a structurally different TRPA1 antagonist. Based on promising preclinical safety and efficacy results, Hydra Biosciences teamed up with Boehringer Ingelheim to advance HX-100 into clinical trials for painful diabetic neuropathy and allergic asthma. The phase I clinical trial followed a standard single- and multiple-ascending-dose design and was completed in 2016. Hydra Biosciences was expected to quickly proceed to phase II development, but dose-related skin and musculoskeletal adverse events in healthy volunteers put an end to its development. Recently, Eli Lilly acquired Hydra's preclinical program of TRPA1 antagonists and committed to develop new therapeutic options for chronic pain patients. Currently, the company is evaluating the safety, tolerability, and pharmacokinetic effects of LY3526318, previously known as HX-260 (**Supplemental Table 2**). In addition, target engagement will be assessed using cinnamaldehyde-induced dermal blood flow changes in healthy females (**https://clinicaltrials.gov/ct2/show/NCT03977974**, **https://clinicaltrials.gov/ct2/show/NCT04183283**) (109, 110).

3.2.2. The future of TRPA1 antagonists: desolate? Taking into account that TRPA1-focused drug development started only a decade ago, it is not surprising that antagonists are largely outnumbered by TRPV1-targeted drug candidates. However, the numerous patent applications filed by different pharmaceutical companies demonstrate a general interest in selective TRPA1 channel antagonists (for a review, see 111). The therapeutic potential of the ion channel is further supported by promising clinical results, yet development efforts are tormented by poor pharmaceutical and pharmacokinetic properties, and at the moment, the growing interest in TRPA1 appears to have stumbled over the hurdles along the way. Besides the pharmacokinetic concerns, TRPA1 antagonists might exert an effect on body temperature and/or thermosensation. These temperature-related side effects remain a subject of discussion, as several preclinical studies in mice delivered contradictory results on the importance of TRPA1 in thermoregulation (16, 112, 113). However, unlike for TRPV1, an effect on human thermosensation or thermoregulation has not yet been described for TRPA1. The bumpy road traveled with TRPV1 antagonists makes investors rather reticent when it comes to TRP drug development. In addition, the striking differences between human and rodent TRPA1 are another complication damping industry's enthusiasm. As the sequence homology of human and rodent TRPA1 is less than 80%, the relevance of these animal experiments may be questioned (114). In this respect, experiments in primates might be more instructive, as it is known that monkeys and humans share similar TRPA1 pharmacology (103). Finally, TRPA1 can be found in a large variety of tissues, although the channel is primarily expressed on nociceptive neurons. This opens the door to several on-target yet undesirable side effects. Nonetheless, no major adverse events were described for the few TRPA1 antagonists

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that advanced to clinical development, making us wonder whether TRPA1 has been undeservedly left behind. In this respect, characterizing the TRPA1 binding sites for channel antagonists could breathe new life into development efforts.

Still, if medicinal chemistry efforts fail to produce successful compounds, TRPA1 agonists might be able to ease the pain. In addition, monoclonal antibodies against TRPA1 also provide therapeutic possibilities. The true potential of this approach has been questioned due to the limited extracellular pore loop of TRP channels. Yet in 2014, Amgen described two monoclonal antibodies, 2B10 and 2D1, acting as antagonists of multiple modes of TRPA1 activation (115). Unfortunately, the feasibility of using these antagonist antibodies as therapeutics remains an important, underexplored opportunity in TRP-targeted drug development.

To conclude, the last has not been said when it comes to TRPA1-targeted drug development, and as our knowledge on TRPA1 modulation increases, this only child might pave the road to effective TRP-based analgesics.

3.3. TRPM3: The New Kid on the Block

Recently, the spotlight moved to TRPM3. Initially described as a volume-regulated, calcium-permeable channel in the human kidney, this member of the melastatin subfamily is now recognized as a polymodal nociceptor that is sensitive to a variety of physical and chemical stimuli, most notably the endogenous neurosteroid pregnenolone sulfate (**Supplemental Table 3**) and the 1,4-dihydropyridine nifedipine (**Supplemental Table 3**) (116, 117). TRPM3 messenger RNA was detected at levels similar to those of TRPV1 and TRPA1 in mouse sensory neurons, and the three channels show a largely overlapping expression profile (118, 119). Likewise, activation of TRPM3 can provoke pain, as exemplified by the pain evoked by injection of pregnenolone sulfate into the hind paw of wild-type but not TRPM3-knockout mice (119). Later, the synthetic small molecule CIM0216 (**Supplemental Table 3**) was described as a more potent chemical ligand (120). Remarkably, CIM0216 was shown to open an alternative permeation pathway in addition to the central calcium-permeable pore. Furthermore, combined stimulation with pregnenolone sulfate and the antifungal drug clotrimazole (**Supplemental Table 3**), but not with nifedipine and clotrimazole, causes the alternative permeation pathway to open (121). Activation of this additional permeation pathway is thus strongly stimulus dependent but can greatly enhance action potential firing and thereby intensify TRPM3-mediated pain (121).

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In addition to its role as a nociceptive chemosensor, TRPM3 is activated by heat. Like TRPV1-knockout animals, elimination of TRPM3 abolishes inflammatory heat hyperalgesia. Preclinical studies in rodents are highly promising and indeed suggest that pharmacological inhibition of TRPM3 can alleviate pain and hyperalgesia (122). Interestingly, recent evidence also described a role for G protein–coupled receptors, including μ-opioid receptors, in the modulation of TRPM3 but not TRPV1 and TRPA1. Upon activation of peripheral μ-opioid receptors, the $G_{\beta\gamma}$ subunit can dissociate and directly bind to TRPM3, thereby inhibiting the channel's activity (123–128). On top of that, the nonsteroidal anti-inflammatory drug diclofenac (**Supplemental Table 3**) was shown to antagonize the melastatin 3 family member in addition to its effect on other TRPs (129, 130). In humans, a de novo mutation in TRPM3's S4–S5 linker region (V990M) was recently described in subjects with developmental and epileptic encephalopathies. In addition to epilepsy and intellectual disability, some probands reported altered heat and pain thresholds (131). In vitro characterization of the V990M mutant further revealed that it causes a strong gain of channel function, including increased sensitivity to pregnenolone sulfate and heat (132, 133).

Altogether, inhibition of TRPM3 has drawn attention as a viable strategy to combat pain. Of course, if we have learned anything from the past TRP drug development attempts, it is the

importance of a favorable safety profile in addition to the analgesic potential. So far, TRPM3 is presumed not to contribute to body temperature regulation, as neither channel agonists nor antagonists were shown to disturb core temperature in mice (134, 135). Nonetheless, potentially dangerous impairments in the acute response to noxious heat remain a concern, as TRPM3 antagonists were shown to attenuate heat sensation (134, 135). Yet only minor deficits are expected as long as the capsaicin receptor remains functional. In line with the clinical results obtained with TRPA1, the functionality of TRPV1 is thought to be sufficient to maintain the initial response to acute heat, as 43°C is hypothesized to be the turning point between innocuous warmth and noxious heat (136). Besides these possible temperature-related issues, the broad expression pattern of TRPM3 raises a reasonable concern for other on-target side effects. Still, the generation of knockout mice revealed no major deficits, and administration of TRPM3 antagonists like primidone (**Supplemental Table 3**) and the flavone isosakuranetin (**Supplemental Table 3**) was reassuringly devoid of serious side effects (134, 135). Nonetheless, the biggest impediment in the development of TRPM3-targeted drugs is the availability of suitable drug candidates.

Supplemental Material >

4. CONCLUSION AND FUTURE PERSPECTIVES

Pain management continues to present an area of substantial unmet medical need. With the discovery of nociceptive TRP channels, one hoped to develop a new class of potent, safe analgesics. Yet for the time being, the ugly side of TRP channels has been most prominent. Activation or inhibition of a TRP channel may be beneficial for pain relief while at the same time inducing unacceptable adverse effects. Indeed, clinical development of TRPV1 antagonists was largely halted because the drugs caused hyperthermia and put patients at risk for scalding injuries by elevating the heat pain threshold. On the other hand, TRPA1-targeted drug development suffered from pharmaceutical and pharmacokinetic complications. Nonetheless, companies that find a way to successfully exploit the fair face of TRP channels might hold a golden ticket for pain relief. In this respect, TRPM3 is hitting the headlines as the latest TRP member to tackle. Bearing in mind the lessons learned from past endeavors, this ion channel might indeed be just the cannon we need to combat persistent pain.

DISCLOSURE STATEMENT

J.V. and T.V. are coinventors on the patent *Novel Treatment of Pain* (WO/2012/149614). J.d.H. (principal investigator) and D.B. (clinical project manager) conducted the TRPA1 target engagement study of LY3526318 in healthy female subjects (**https://clinicaltrials.gov/ct2/show/NCT04183283**).

ACKNOWLEDGMENTS

Scientific illustration toolkits from Motifolio were used to generate some of the figures in this article.

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