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Pain Transduction: A Pharmacologic Perspective

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Abstract

Pain represents a necessary physiological function yet remains a significant pathological process in humans across the world. The transduction of a nociceptive stimulus refers to the processes that turn a noxious stimulus into a transmissible neurological signal. This involves a number of ion channels that facilitate the conversion of nociceptive stimulus into an electrical signal. Because an understanding of nociceptive physiology complements a discussion of analgesic pharmacology, the relationships between the two are presented together. In this review article, a critical evaluation is provided on the findings relating to both the physiology and pharmacology of relevant acid-sensing ion channels (ASIC), and transient receptor potential (TRP) cation channels, and voltage-gated sodium (Nav) channels.

Keywords

Analgesia; Anesthesia; Pain; Nociceptive transduction; Voltage-gated sodium channels; Acid-sensing ion channels

1. Introduction

Pain is a complex sensation that manifests in many different ways. Nociceptive stimuli are generally categorized into the following types: inflammatory, neuropathic, and nociceptive. Briefly, inflammatory pain corresponds to the release of inflammatory mediators such as interleukins, prostaglandins, and cytokines. These mediators sensitize nociceptive neurons. Examples of inflammatory-related pain include appendicitis and rheumatoid arthritis. Neuropathic pain refers to pain that results from damage to the nervous system structures that relay nociceptive information to the central nervous system. The pain associated with diabetic neuropathy and spinal cord injury is an example of neuropathic pain. Nociceptive pain refers to discomfort that results from an encounter with a noxious stimulus. Nociceptive pain includes thermal pain, chemical pain, and mechanical pain.

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Declaration of Interest

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Nociceptive transduction exists as an essential component of the nociceptive pathway, which consists of nociceptive transduction, transmission, modulation, and perception (Figure 1). Nociceptive transduction refers to the process by which external stimuli are converted to electrical signals that can be perceived as pain. Transduction is an indispensable part of the process for detecting external stimuli: without transduction, the human body would lose the ability to taste, touch, hear, see, or feel pain. Transduction occurs when, in response to a physical stimulus, somatosensory processes facilitate the opening of ion-gated channels [1, 2]. This transforms a physical stimulus into an electrochemical signal that can be delivered to and perceived by higher-order nervous centers [1, 2].

By evaluating high impact articles on nociceptive transduction from PubMed, we have critically reviewed the research on the pharmacological interventions that alter nociceptive transduction pathways. This review investigates the literature on transduction by examining the following topics: clinically significant pathways of nociceptive transduction, physiological function of nociceptive transducing channels, pharmacological interventions that affect transducing channels, and outstanding questions in nociceptive transduction research.

2. Clinically significant pathways of nociceptive transduction

There are a variety of transduction pathways that underlie the body's capability to transduce nociceptive stimuli. Table 1 lists the clinically significant channels in the process of nociceptive transduction and the related pharmacologic agents. As the majority of clinical research on nociceptive transduction has centered around relatively few transduction channels, we have focused our review to discuss the following physiological and pharmacological pathways: acid sensing ion channels (ASIC), and various transient receptor potential (TRP) cation channels (TRPV1, TRPA1, TRPV3, and TRPM8), voltage-gated sodium channels.

Nociceptive transduction, an area of active research, is the process by which the human body transforms a hot, cold, mechanical, or chemical stimulus into a perceivable signal. Nociceptive transduction primarily involves free nerve endings (C and A δ fibers) that spread between epidermal cells, somatosensory organs, and involves both the peripheral and central nervous systems. To gain an appreciation and understanding of the pharmacology related to these highly sensitive systems, one must explore the pertinent physiology. Briefly, these sensory systems facilitate the opening of ion channels in response to physical stimuli. The opening of ion channels leads to changes in membrane potential, the opening of additional channels, and the eventual depolarization of the afferent nerve, generating a nociceptive signal. Figure 2 illustrates this process.

3. Acid sensing ion channel (ASIC) physiology

Acid sensing ion channels (ASICs) are a group of non-voltage sensitive, proton-induced sodium channels found throughout the body [3]. ASICs function to detect changes in pH and have been associated with various disease processes in the central and peripheral nervous system including epilepsy, depression, migraines, and neuropathic pain. Some studies have

demonstrated the expression of ASICs on somatosensory organs and free nerve endings [4]. Interestingly, only two ASIC channels (ASIC3 and ASIC1b) function in acidic nociception (e.g. inflammation, ischemia) [5]. The importance of ASICs in nociception and other processes is increasingly apparent; however the exact mechanisms, physiology, and roles of this family have yet to be elucidated [3]. Regardless, a number of agents have been shown to modulate ASIC nociceptive transduction activity and may become useful, pain-relieving agents.

4. Acid sensing ion channel (ASIC) pharmacology

Amiloride, traditionally used as a potassium-sparing diuretic, has been identified as a direct inhibitor of ASICs [6]. One particular study, for example, showed that amiloride down-regulated the expression of ASIC-related proteins in human nucleus pulposus cells [7]. This study observed a similar outcome with NSAIDs, specifically ibuprofen [7]. Researchers demonstrated the clinical pain-relieving efficacy by amiloride where the subcutaneous injections of amiloride significantly attenuated the human pain response in those receiving painful proton iontophoresis [8]. We suggest that this avenue, along with related medications, be explored further.

Aminoglycosides, a class of antibiotic used to treat Gram-negative infections, have been shown to decrease ASIC current in the dorsal root ganglia of rats [9]. However, no research group has attempted to demonstrate any clinical utility for aminoglycosides in the treatment of inflammatory pain.

Voilley et al [10] showed that non-steroidal anti-inflammatory drugs (NSAIDs), through a cyclooxygenase inhibition-independent pathway, inhibit ASIC expression and activity. However, only certain NSAIDs (aspirin, flurbiprofen, ibuprofen, diclofenac) showed ASIC blocking activity [10]. These differences were attributed to the specific molecular structure of individual NSAIDs [10]. Jones et al [8] also showed that, like amiloride, topical application of NSAIDs prior to proton iontophoresis significantly attenuated the pain response.

As discussed previously, local anesthetics exert their primary action by inhibiting voltage-gated sodium channels. Because only uncharged forms of these weak bases can diffuse through the cellular phospholipid bilayer, the penetration of these agents into neurons is pH-dependent. In acidic inflammatory conditions, the charged form of local anesthetics predominates, essentially preventing their primary action and limiting their clinical utility. However, one such study demonstrated that, even in acidic conditions, local anesthetics modulate ASIC activity [11]. With further research, this modulation may represent an alternative mechanism and potential for extending the clinical use of local anesthetics.

5. Transient receptor potential cation channel (TRP) physiology

TRP channels are a broad family of channels that are involved in a host of physiological processes and are particularly important in the process of nociceptive transduction. This review examines the following nociceptive transduction-relevant TRP channels: TRPV1, TRPA1, TRPV3, and TRPM8.

TRPV1 channels, discovered barely two decades ago, permit the passage of calcium ions and are understood to play a key role in nociceptive transduction [12]. The activity of TRPV1 is potentiated by heat, acidity, and molecules such as capsaicin [13]. Like TRPV1 channels, TRPA1 channels are involved in nociceptive transduction and are sensitive to thermal, mechanical, and chemical stimuli [14]. TRPA1 channels are expressed on both neurons and non-neuronal cells (e.g., endothelial cells, fibroblasts, keratinocytes); however, the purpose of non-neuronal TRPA1 channels is currently unknown [15]. The role of TRPA1 in nociceptive transduction is illustrated by the finding that a gain-of-function mutation in TRPA1 channels results in a 5-fold increase of inward current at resting potential [16]. Specifically, the increased current was shown to be responsible for the pathogenesis of Familial Episodic Pain Syndrome, a debilitating syndrome characterized by severe episodes of pain that is often localized to the upper body [16]. TRPV3 channels, like TRPV1 channels, respond to heat and are thought to participate in the transduction of warm sensation and heat pain. While TRPV1 channels activate at temperatures $\geq 42^{\circ}\text{C}$, TRPV3 channels activate at 33°C [17, 18]. As temperatures reach noxious levels, TRPV3 and TRPV1 both demonstrate increased activity. Interestingly, although TRPV1 and TRPV3 channels share significant homology, TRPV3 channels do not respond to capsaicin or acidic pH [17]. Cutaneous TRPM8 channels are the principle receptors involved in environmental cold sensation and cold pain transduction [13, 19].

6. Transient receptor potential cation channel (TRP) pharmacology

6.1 TRPV1

Capsaicin is a highly selective and potent agonist of the TRPV1 receptor [20]. While it activates nociceptive channels, capsaicin has been used as an analgesic. Topical capsaicin is thought to act by attenuating a hypersensitivity response in the skin that reduces nociception through TRPV1 desensitization, a process known as defunctionalization, which includes a temporary loss of membrane potential, a loss of membrane transport capabilities, and a retraction of epidermal and dermal nerve fiber endings [20, 21]. Capsaicin-mediated defunctionalization of TRPV1 has been shown to be effective in the treatment of neuropathic pain [22]. Routine use of capsaicin analgesics is hampered by burning and painful sensations upon first use. However, current research shows promising advances in this area through the application of high doses of capsaicin [21], and the development of resiniferatoxin, an ultrapotent capsaicin analog that causes cytotoxicity and ablation of pain transmitting C-fibers [23].

Hyaluronic acid (HA), a component of the extracellular matrix (ECM), is known to increase the elasticity and viscosity of the ECM; however, recently, HA was shown to have pain-relieving properties. This effect was observed following injection of HA preparations into the knees of osteoarthritic patients [24]. Similarly, long-term (24 weeks) pain relief was demonstrated following injection of HA into the knee [25]. Until recently, the mechanism of pain relief for HA preparations was unknown; however, Caires et al [26] showed that HA may provide pain relief via attenuation of TRPV1 channels.

Direct antagonism of TRPV1 channels is not currently used to treat pain in a clinical setting. This is partially due to poor efficacy during clinical trials and the risk of dangerous body

temperature elevations seen in early generations of TRPV1 antagonists [27, 28]. Despite these setbacks, research related to TRPV1 antagonism shows promising results. For example, one researcher evaluated the mechanism and efficacy of two TRPV1 antagonists, JNJ-38893777 and JNJ017203212, in the treatment of migraine headaches [29]. Their work demonstrated a significant reduction in calcitonin-related gene peptide (CGRP) release from the trigeminal system following administration [29]. Notably, these antagonists decreased CGRP more significantly than sumatriptan, a widely used migraine treatment [29]. The authors, however, acknowledged the complex pathophysiology of migraine headaches and recommend additional clinical studies with regard for the association of TRPV1 to migraine pathogenesis and the clinical utility of these agents. Additional agents exhibit direct antagonism of TRPV1, but lack sufficient research to demonstrate clinical utility. Capsazepine, a TRPV1 antagonist, is commonly used in TRP channel research but has not been used in human treatment [30]. One such study treated mice with a selective TRPV1 antagonist, A-889425, and identified a corresponding blockade of pain-relaying A-delta nerve fibers, indicating a potential avenue for novel nociceptive modulation [31]. Another group discovered a highly selective and potent antagonist, KYS-05090, to TRPV1 and proposed potential clinical utility [32]. Finally, antibodies directed against TRPV1 channels have been developed [33]. To date, however, no research has attempted to evaluate the potential anti-nociceptive properties of these antibodies. Nguelefack et al [34] evaluated the analgesic mechanism of orally administered methanol/methylene chloride in a mouse model of induced pain. Although a full mechanism was not elucidated, analgesia was partially attributed to antagonism of TRPV1.

TRPV1 sensitivity and activity is promoted by prostaglandin E2 (PGE2) and bradykinin (BK) [35]. In vitro studies suggest increased transcription and expression of TRPV1 receptors when cultured cells are exposed to high concentrations of PGE2 and BK [36]. However, a recent in vivo study demonstrated that, due to an unknown mechanism, increased transcription did not necessarily result in increased TRPV1 surface expression [36]. Given these results, it seems unlikely that the mechanism by which PGE2 and BK affect TRPV1 has been fully elucidated. Theoretically, however, there is potential for modulating the nociceptive activity of TRPV1 channels via NSAID inhibition of prostaglandin production.

TRPV1 channels are widely considered to be among the most important channels in nociceptive transduction. As such, significant research has probed potential analgesic therapies via modulation of TRP channels. Many potential therapies have been proposed; however, few have been tested in human models.

6.2 TRPA1

TRPA1 channels are activated by a host of endogenous mediators and substances including allyl isothiocyanate (the spicy substance in wasabi and horseradish), acrolein, cinnamaldehyde, formalin, iodoacetamide, methanethiosulphonate, mustard oil, and pentenal [14, 15, 37, 38]. In addition, activation of TRPA1 channels by chemotherapeutic agents such as bortezomib (a proteasome inhibitor) and oxaliplatin (a platinum-containing

alkylating agent) has been shown to contribute to pain induction and allodynia in cancer patients [38, 39].

Interestingly, agonism of TRPA1 does not exclusively cause pain. In fact, like TRPV1, high doses of some activating agents desensitize TRPA1, leading to analgesia. Carnosol, for example, is a selective TRPA1 agonist found in traditional herbal Chinese medicines and has been shown to have anti-nociceptive effects [40]. Kojima et al [41] observed substantial expression of TRPA1 channels on enterochromaffin cells of the gastrointestinal tract and evaluated the effects of a selective TRPA1 agonist, ASP-7663, on constipation-induced abdominal pain. Their findings indicated that the analgesic effect of the TRPA1 agonist was due to direct desensitization of TRPA1 channels [41]. Materazzi et al [42] studied the effects of parthenolide, a constituent of feverfew, on migraine headaches. They found that parthenolide, a partial agonist of TRPA1 channels, desensitized the trigeminal system by inhibiting CGRP release [42].

Zhang et al [43] injected allyl isothiocyanate into the dorsal root ganglia of rats and found that nitro-oleic acid blocked the nociceptive response. This work indicates that nitro-oleic acid might prove useful in reducing or avoiding certain types of neurogenic pain.

Historically, pyrazolone and derivatives of pyrazolone have been used in the treatment of conditions such as migraine headaches. These drugs were widely used, despite an unknown mechanism of action, until their hematologic side effects (e.g., agranulocytosis) became apparent. Recently, however, the mechanism of pyrazolones was elucidated [38]. Using rat and mouse dorsal root ganglia neurons stimulated by allyl isothiocyanate (a known TRPA1 agonist), it was shown that treatment with pyrazolones selectively reduced calcium influx via TRPA1 channels [38]. Additionally, the group showed that pyrazolones could attenuate bortezomib-induced pain hypersensitivity with decreased hematologic side effects. If side effects can be appropriately managed, pyrazolone derivatives may have a promising future in clinical analgesia.

Nagatomo et al [44] found that caffeine suppresses the activity of TRPA1 channels in humans. This observation may support the findings of a meta-analysis that showed that the addition of caffeine to common analgesic medications produced a small but statistically significant increase in the analgesic's pain-relieving properties [45].

TRPA1 channels represent an interesting and novel mechanism for producing clinical analgesia. Their significance is not lost in the scientific community: currently, a number of TRPA1 antagonists are in clinical trials [40].

6.3 TRPV3

Bang et al [46] showed that resolvin D1, an endogenous lipid metabolite, diminished the activation of TRPV3 channels in the presence of TRPV3 agonists. Resolvins have been shown to decrease post-operative inflammation and pain, making them an attractive potential pain relief agent [46]. Further work by Bang et al [47] demonstrated that isopentenyl pyrophosphate also inhibited the activation of TRPV1 and TRPV3 channels. However, Huang et al [48], using TRPV3 knockout mice, demonstrated that TRPV3 channels

contribute only minimally to detecting warm sensation and noxious heat. Given this finding, it is likely that achieving analgesia through antagonism of TRPV channels will be best achieved by targeting other TRP channels as opposed to specific antagonist of TRPV3.

6.4 TRPM8

Cutaneous TRPM8 channels are the principle receptors involved in environmental cold sensation and cold nociceptive transduction [49]. Cooling agents, such as menthol, activate these receptors [49]. Although it would seem that the analgesic effects attributed to cold-packs or menthol would be TRPM8 mediated, menthol has been found to be non-selective for TRPM8 channels [50]. In fact, Gaudioso et al [51] linked the analgesic properties of menthol to blockage of voltage-gated sodium channels. Regardless, there is evidence to suggest that manipulation of the TRPM8 channel has potential for providing therapeutic benefit. Lashinger et al [52] showed that N-(3-aminopropyl)-2-[(3-methylphenyl)methyl]oxy)-N-(2-thienylmethyl)benzamide hydrochloride (AMTB), a TRPM8 antagonist, attenuated the volume-induced painful micturition response in rats and was useful in limiting nociception in painful-bladder syndrome. Similarly, Andrews et al [53] introduced the TRPM8 antagonist PF-05105679, which may provide relief from cold-related pain. Lippoldt et al [54] recently identified specific TRPM8 neurons as a potential therapeutic target for those suffering from cold allodynia. Agents affecting TRPM8, while not currently in clinical use, have the potential to serve as treatment agents in certain clinical cohorts.

7. Voltage-gated sodium channel physiology

Voltage-gated sodium (Nav) channels, while not classically associated with nociceptive transduction, are heavily involved in the transition from transduction to transmission and the generation of action potentials. Specifically, nociceptive transduction is mediated by the transducer potential generated by TRP, ASIC, and other channels, which is sufficient to depolarize Nav channels resulting in the formation of an action potential. Because nociceptive transmission refers primarily to the upstream and more central effects of the action potential, it is relevant to discuss Nav channel physiology and pharmacology.

Voltage-gated sodium (Nav) channels play a pivotal role in many physiological processes including the transduction of nociceptive stimuli. As such, they represent an attractive target for pain-attenuating pharmacologic agents. Nine Nav isoforms (Nav 1.1–1.9) have been demonstrated in mammals, all of which share significant homology. Of these isoforms, Nav 1.3, 1.7, 1.8, and 1.9 are the most important channels in nociceptive transduction [55, 56]. Although it is widely known that the soma of dorsal root ganglion neurons express these channels, only limited research explores the peripheral expression of sodium channels in pain-transducing free nerve endings. Limitations in this research may be tied to the extraordinarily small diameter of free nerve endings and the associated difficulty in generating appropriate research models. Regardless, it has been shown that Nav 1.6, 1.7, 1.8, and 1.9 are present in many epidermal free nerve endings [57]. Other Nav channels are found on skeletal muscle, cardiac muscle, the cerebral cortex, and cerebellum [56]. Due to the widespread distribution of Nav channels and significant channel homology, anesthetics

that antagonize Nav channels must be administered locally to avoid undesired, systemic side effects.

The physiology of Nav channels is best understood in terms of the physical structure and function of the channel itself. Sodium channels cycle through closed, open, and inactivated states [58]. Nav channels are composed of one alpha subunit and a variable number of beta subunits. The alpha subunit spans the plasma membrane, forming a pore through which ions flow [56]. Beta subunits are important in determining tissue localization, voltage thresholds, and rates of ion flow [56]. The alpha subunit is composed of four domains that each contain six transmembrane segments (S1–S6). At resting membrane potential, Nav channels are found in a closed state, which prevents ion influx. With membrane depolarization, S4 voltage sensors cause movement of the activation gate, allowing extracellular sodium ions to enter through the alpha subunit [58]. The brief period of rapid ion influx causes a significant increase in membrane potential, eventually reaching the threshold required for action potential generation. As the membrane potential becomes more positive a loop linking domains III and IV known as the “fast inactivation gate”, also positively charged, occludes the Nav channel and stops further ion influx [56, 58, 59]. During the period of inactivation, the Nav channels are largely refractory to further stimuli until the membrane is fully repolarized.

8. Voltage-gated sodium channel pharmacology

A significant number of agents inhibit nociceptive transduction by acting on Nav channels. This review discusses local anesthetics, selective Nav inhibitors, tetrodotoxin, and other miscellaneous sodium channel blocking agents.

A significant and well-known class of nociceptive inhibitors is the local anesthetics (e.g., lidocaine, bupivacaine). Local anesthetics are widely used across multiple medical specialties. Because it is thought to be the binding site of local anesthetics, the S6 segment of domain IV is a particularly important subunit of the Nav channel. It was formerly thought that local anesthetics bound to the S6 subunit to occlude the inner pore to stop ion influx, however new research has shown that physical occlusion is incomplete [60, 61, 62, 63]. One study demonstrated that lidocaine binds to S6 and, due to the positively charged amine functional group, electrostatically repels sodium ion influx to inhibit nociceptive transduction [63]. Chemically, local anesthetics are weak bases and can therefore exist in charged and uncharged states depending on the pH of the environment into which they are introduced. In alkaline conditions, local anesthetics are predominantly uncharged which maximizes their permeability through the cellular lipid bilayer. Existence in an uncharged and highly permeable form is essential to the function of local anesthetics because they must bind to the cytoplasmic side of Nav channels [64]. Accordingly, local anesthetics are not generally used as an analgesic in conditions of acidic or inflammatory pain (e.g., rheumatoid arthritis) because protonation results in ionization, decreased permeability, and poor efficacy. Figure 3 illustrates the relation of Nav channel physiology to local anesthetic pharmacology. As mentioned previously, when used as an anesthetic, this class of medication can only be used locally due to the potential of detrimental systemic effects.

Specific agents have been identified that selectively inhibit certain Nav channels. Hains et al [65, 66] used a rat model of spinal cord injury to demonstrate increased pain-related behaviors due to the upregulation of Nav 1.3 in the dorsal root ganglia and thalamic nuclei. Subsequently, intrathecal administration of antisense oligodeoxynucleotides was shown to knockdown Nav 1.3 expression, attenuating spinal cord injury-induced neuropathic pain. Similarly, another study showed that Nav 1.3 expression is increased in the injured neurons of diabetics [67]. In this study, Adeno-associated virus was used as a vector for shRNA in diabetic rats to selectively decrease Nav 1.3 expression and was found to reduce evidence of tactile allodynia [67]. Nav 1.3 expression has been shown to increase in the peripheral nervous system of animals following chronic constriction injury and peripheral nerve axotomy [68, 69]. Such animals are considered "primed" for heightened pain sensation by a precipitating injury. A possible explanation for the observed decrease in pain-related behavior in the aforementioned studies is that inhibition of Nav 1.3 decreased the amplitude of Nav1.3 current to levels closer to those seen in "unprimed" animals. While selective manipulation of Nav 1.3 channel populations could potentially be a novel way to treat neuropathic pain, no chemical agents have been identified.

Efforts to produce selective inhibitors of Nav 1.7 have been the focus of significant research. Lee et al [70] developed a monoclonal antibody to the S3/S4 domain of Nav 1.7. In mouse models of nociception, they found selective inhibition of Nav 1.7 to mediate a decreased response to both neuropathic and inflammatory pain [70]. Significantly, no side effects were observed in these test animals [70]. Another study found that methyl eugenol, an herbal extract, inhibited Nav 1.7 preferentially in both the inactivated and open states [19]. This compound has not been evaluated in animal or human studies as it pertains to induction of analgesia [19]. Another group studied the effects of selective Nav 1.7 and Nav 1.8 antagonists (ProTxII and A-803467) in mice with osteoarthritis. Both were found to reduce mechanical and thermal pain sensation after spinal injection [71]. One was additionally noted to reduce pain sensation when administered systemically [71]. Other researchers developed a series of small molecules and identified a highly selective antagonist known as 12k [72]. These studies, and many like them, demonstrate the significant potential role of Nav 1.7 antagonists in producing clinically significant analgesia.

Yue et al [73] demonstrated upregulation of Nav 1.8 by histamine in dorsal root neurons via histamine receptor-2 (HR2) receptor-dependent pathways. In rats, the HR2 blockers cimetidine and famotidine were shown to downregulate Nav 1.8 and resulted in a decreased response to thermal and mechanical pain stimuli [73]. Significantly, blockade of histamine receptor-1 by pyrilamine resulted in neither Nav 1.8 downregulation nor detectable changes in pain sensation [73]. While traditionally used for gastroesophageal reflux, this research suggests that HR2 antagonists may find clinical use in treating pain. Another study developed a novel set of pyrazine-based compounds to selectively block Nav 1.8 channels [74]. They tested oral administration of the compound in a rat model of neuropathic pain and noted dose-dependent pain-relief [74]. Similar to the work of Rahman et al [71], Mert et al [75] compared the use of A-803467, a Nav 1.8 antagonist, to lidocaine in a rat model of diabetic neuropathy. They found that systemic administration of the specific antagonist relieved pain six times better than lidocaine [75]. Because Nav 1.8 channels are concentrated in small peripheral nervous system nociceptive neurons and only sparsely present in other

locations, a drug that targets Nav 1.8 specifically is likely to be free of many of the side effects incident to other sodium channel blocking drugs [56, 58].

Nav 1.9 channels most likely do not contribute to pain levels before injury has occurred [76]. However, gain-of-function mutations in Nav 1.9 have been found to contribute to painful peripheral neuropathy [77, 78]. Additionally, researchers found Nav 1.9 to be involved with both acute and chronic inflammatory pain [79]. To demonstrate this, these researchers induced monoarthritis in mice via carrageenan injections. Nav 1.9 knockdown or knockout mice were observed to exhibit reduced pain-related behaviors in response to noxious mechanical stimuli, noxious thermal stimuli, and carrageenan injection. These results suggest that Nav 1.9 inhibition may offer pain relief to those suffering from inflammatory or arthritic diseases. Like Nav 1.8, Nav 1.9 channel-specific inhibition may provide analgesia without the adverse effects associated with generalized Nav channel inhibition. Currently, there are no Nav 1.9 channel specific-inhibitors that have been tested in a human model.

Rufinamide is a sodium channel blocker that has found clinical use for treating seizures in Lennox-Gastaut syndrome. Recently, Kharatmal et al [79] demonstrated that rufinamide decreases the activity of Nav 1.8 and 1.9 in a rat model of diabetic neuropathy. Blockage of these channels was observed to contribute to a decrease in both mechanical allodynia and thermal hyperalgesia in the study animals [79].

A number of antidepressant medications have also been shown to attenuate nociceptive transduction via action on sodium channels. Investigators explored the mechanism by which selective serotonin reuptake inhibitors (SSRIs) treat neuropathic pain [80]. Specifically, they measured the binding affinities of SSRIs to Nav 1.7 and 1.8. Of the SSRIs tested, they found that paroxetine and fluoxetine bound to Nav 1.7 and 1.8 with the greatest affinity, were associated with frequency-dependent inhibition of Nav 1.7 and 1.8, quicker onset of inactivation, and slower recovery from inactivated states [80]. It follows that SSRIs may have an analgesic effect when used to treat neuropathic pain by virtue of their ability to inhibit current through these Nav channels.

Amitriptyline, a tricyclic antidepressant, has also been observed to have utility in treating migraine pain. While details surrounding the pain-relieving mechanism of amitriptyline continue to be debated, Liang J et al [81] found amitriptyline to have a demonstrable effect on sodium channels. Specifically, Liang et al [81] showed that, in rats, amitriptyline blocks Nav 1.8 and decreased nociceptive-related behaviors in rats undergoing electrical stimulation of the dura mater. In another study, the investigators found that amitriptyline also works to inhibit current flow through Nav 1.9 [82]. Thus, it is likely that amitriptyline works to alleviate migraine pain in part due to its activity at Nav channels. Amitriptyline may also prove useful in chronic pain as demonstrated by Amorim et al [83]. After inducing chronic monoarthritis in rodents, they observed a decrease in mechanical nociceptive sensation when the rodents were treated with amitriptyline [83].

Tetrodotoxin is a well-known sodium channel blocker and potent neurotoxin. This is evidenced by the fact that resistance to tetrodotoxin defines some subsets of sodium channels (Nav 1.8, 1.9). Unfortunately, Nav 1.8 and 1.9 are largely present in the peripheral

nervous system, a fact that could explain limitations in the clinical efficacy of tetrodotoxin as an analgesic [56, 58]. For example, Hagen et al [84] tested tetrodotoxin in the setting of cancer-related pain. In this study, they noted that the side effects of tetrodotoxin were tolerable to the test subjects; however, fewer than half of the subjects reported significant pain relief [84]. Also noteworthy is the observation that those that benefitted from tetrodotoxin received only transitory relief [84]. Other studies support potential clinical use for tetrodotoxin. One such study used a rat model to show that intrathecal tetrodotoxin augments the action of isoflurane, an inhalational anesthetic, and that this effect was reversed by veratridine, a drug that opens sodium channels [85].

Menthol, while classically associated with activity at TRPM8 channels, also appears to mediate anti-nociceptive effects via voltage-gated sodium channels. Specifically, Gaudioso et al [51] found menthol to alleviate pain in mice that had been treated with a sodium-channel targeting toxin. They suggested this mechanism to underlie the analgesic effects of topical menthol-containing creams and ointments [51].

Currently, nociceptive-transducing sodium channels are an important focus of research. New therapies that specifically inactivate Nav channels are an important frontier in generating novel, clinically useful analgesics.

9. Expert Commentary

Despite significant steps toward identifying new ways to treat pain, there remain many significant avenues of inquiry in nociceptive transduction. Many of these questions are relevant to the clinical utility of therapies that modulate nociceptive transduction mechanisms.

The extent of the physiology of ASICs has yet to be elucidated. Regardless, a number of agents have been shown to modulate ASIC activity and demonstrate analgesic properties. We recommend further investigations of amiloride (and other related medications) as an analgesic agent. Aminoglycoside antibiotics were shown to attenuate ASIC activity in rats. However, no research has attempted to study the activity of aminoglycosides with regards to pain management in humans. Additionally, the chemical properties of local anesthetic agents currently limit their usage in acidic, inflamed tissues due to protonation and poor permeability. However, current research indicates that local anesthetics, such as tetracaine, can demonstrate activity at ASICs especially if given at increased doses [11]. Any clinical utility related to this finding is uninvestigated. Unanswered questions from ASIC-related research could potentially yield novel methods to treat clinical pain.

The pharmacologic use of agents affecting transducing TRP channels is becoming increasingly significant. New techniques, such as intraarticular injections of hyaluronan represent recent successes. Direct antagonism of TRPV1 channels is not in clinical practice currently due to hyperthermic safety concerns. However, research is ongoing with the discovery of new agents and the development of monoclonal antibodies directed against TRPV1. Furthermore, while there is extensive knowledge of agents that activate TRPA1, thereby inducing pain, very few agents reduce pain by this route. Pyrazolone derivatives

have shown historical benefit but serious side effects (agranulocytosis) have forced pharmacologists to revisit this class of agents. Finally, pharmacologic agents acting at TRPV3 and TRPM8 are very limited although a few agents may represent an interesting and new approach to transduction therapies.

The use of local anesthetics is well established, as is their action on Nav channels. However, detrimental effects of systemic local anesthetics and other limiting factors necessitate the development of more selective agents. As discussed previously, a number of agents have been shown to selectively inhibit or downregulate expression of certain Nav channels. Many of these agents show therapeutic promise. We highly recommend well-designed studies to advance the science and use of specific sodium channel antagonists.

10. Five-year View

A host of agents that attenuate TRPV1 activity are under investigation. We recommend further work with hyaluronic acid, as the research and results are very promising. Research and advancements of direct TRPV1 antagonists have undergone significant setbacks. However, a number of agents show promise and deserve thorough evaluation. Finally, it is currently unclear how PGE2 and BK impact TRPV1 expression and function. Without a clear understanding of this interaction, prior assumptions of the actions of NSAIDs on TRPV1 channels should be questioned.

Another important emerging avenue in pain transduction research appears to be the modulation of neuronal exocytosis by botulinum toxin. For example, one recent study administered intradermal doses of botulinum neurotoxin to healthy volunteers and noted an increase in mechanical pain thresholds [86]. Remarkably, the subjects did not experience a loss of non-nociceptive sensation, demonstrating that botulinum toxin was specific for nociceptive processes [86]. Significantly, this study observed that, because action at TRPA1 channels cannot explain the observed changes in mechanotransduction, that the activity of botulinum toxin is not specific to TRPA1 channels [86]. A group of other studies; however, have named TRPV1-expressing neurons and adenosine to be the site of action of botulinum toxin [87, 88, 89]. While more work is needed to clarify the exact mechanisms, we anticipate that further research in the areas of neuronal exocytosis and their relationship to nociception will be an area of active research in the coming years.

We recommend a renewed interest in pyrazolone derivatives with respect to TRPA1 antagonism. The utility of these agents are clear, but side effect profile is dire. We recommend research to uncover the cause of hematologic effects such that these effects can be managed and pyrazolone derivatives can find clinical use.

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** of considerable interest

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Key Issues

- Despite advances in medicine and numerous agents that counteract pain, millions of patients continue to suffer.
- Attention has been given to identify novel pharmaceutical interventions that produce analgesia by interacting with nociceptive-transducing channels. Although much has been accomplished, much work remains, and future opportunities abound.
- Nociceptive transduction, the conversion of stimuli into perceivable and transmittable signals, is the target of many therapeutic agents.
- Nociceptive transduction is also the focus of many experimental agents.
- Although, the clinical efficacy of pyrazolone derivatives by antagonizing TRPA1 is encouraging, the adverse effects of these molecules hinder their clinical utility. Thus, a critical evaluation of the underlying mechanisms of the adverse hematological effects of pyrazolone derivatives is required.
- There is an urgent need for further investigation into agents that interact with TRPV3 and TRPM8.
- The role of resolvins in nociceptive transduction warrants a careful and detailed attention.
- Evaluation of the full mechanism and clinical efficacy of mentholated products is also warranted.

Figure 1. Pain Transduction in the pain pathway

Transduction refers to the process by which a painful physical or chemical stimulus is transformed into a signal that can be carried (via transmission) to the central nervous system and perceived as pain. Pain transduction is accomplished via specialized ion channels including those noted in the figure (TRP, ASIC, and voltage-gated sodium channels). Simultaneously, the brain can alter the transmission of a pain signal via descending pain modulatory tracts. Green arrows represent activation or potentiation. Red arrows represent inactivation or attenuation.

Figure 2. Mechanism of pain transduction

Pain transduction is the conversion of a nociceptive stimulus to a transmissible neuroelectrical signal. The general mechanism of pain transduction includes the activation of various ion channels by specialized transducing channels (eg, TRP). Ion channel opening leads to ion influx, membrane depolarization, and the genesis of a nociceptive signal.

Figure 3. Voltage-gated sodium channels (Nav) cycle between closed, open, and inactivated states (A) In the closed state, an activation gate occludes the pore to prevent ion influx and propagation of a signal. (B) An appropriate stimulus can result in membrane depolarization, which is detected by the S4 voltage sensor resulting in the deflection and opening of the activation gate. In the open state, sodium ions rapidly depolarize the membrane, which may lead to an action potential and propagation of the nociceptive signal. (C) Following a period of sodium influx, the positively charged inactivation gate (derived from a loop between domains III and IV) is electrostatically driven to occlude the Nav pore and prevent further

ion passage. **(D)** Local anesthetics must be deprotonated (uncharged) to diffuse through the lipid bilayer where, upon re-protonation, it then binds at the cytoplasmic face of the Nav. Local anesthetics bind to the S6 segment of domain IV of the open-state Nav, and via a positively charged functional group, electrostatically repel sodium influx, membrane depolarization, and pain transduction. *LA⁺: protonated (charged) local anesthetic; LA: deprotonated (uncharged) local anesthetic.*

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Table 1
Nociceptive transduction channels and associated pharmacology

The key pain transducing channels are voltage-gated sodium channels, acid sensing ion channels, and transient receptor potential cation channels. This table lists the specific subtypes of the channels that are involved in pain transduction as well as the associated inhibitory, and therefore analgesic, agents.

Pain Transducing Channels	Abbreviation	Modulatory agents
Acid-Sensing Ion Channels	ASIC	<ul style="list-style-type: none"> Amiloride Aminoglycoside antibiotics NSAIDs (aspirin, flurbiprofen, ibuprofen, diclofenac) Local anesthetic agents
Transient Receptor Potential Cation Channel, Subfamily V, Member 1	TRPV1	<ul style="list-style-type: none"> Capsaicin Resiniferatoxin Hyaluronic acid JNJ-38893777, JNJ-17203212 Capsazepine A-889425 KYS-05090 Polyclonal antibodies Methanol/methylene chloride NSAIDs Isopentyl pyrophosphate
Transient Receptor Potential Cation Channel, Subfamily A, Member 1	TRPA1	<ul style="list-style-type: none"> Carnosol ASP-7663 Parthenolide Nitro-oleic acid Pyrazolone derivatives Caffeine
Transient Receptor Potential Cation Channel, Subfamily V, Member 3	TRPV3	<ul style="list-style-type: none"> Resolvin D1 Isopentyl pyrophosphate
Transient Receptor Potential Cation Channel, Subfamily M, Member 8	TRPM8	<ul style="list-style-type: none"> Menthol AMTB PF-05105679
Voltage-gated Sodium Channels	Nav 1.3, 1.7, 1.8, 1.9	<ul style="list-style-type: none"> Local anesthetic agents Nav 1.3 inhibition: gene knock-down Nav 1.7 inhibition: Monoclonal antibodies, methyl eugenol, ProTxII, 12k, SSRIs (fluoxetine, paroxetine) Nav 1.8 inhibition: A-803467, H2 blockers (cimetidine, famotidine), pyrazine-based compounds, rufinamide, SSRIs (fluoxetine, paroxetine), amitriptyline Nav 1.9 inhibition: gene knock-down, rufinamide, amitriptyline

Pain Transducing Channels	Abbreviation	Modulatory agents
		<ul style="list-style-type: none">▪ Tetrodotoxin▪ Menthol

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