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Review Article

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Targeting TRPV1 as an Alternative Approach to Narcotic Analgesics to Treat Chronic Pain Conditions

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Abstract. In spite of intense research efforts and after the dedicated Decade of Pain Control and Research, there are not many alternatives to opioid-based narcotic analgesics in the therapeutic armamentarium to treat chronic pain conditions. Chronic opioid treatment is associated with sedation, tolerance, dependence, hyperalgesia, respiratory depression, and constipation. Since the affective component is an integral part of pain perception, perhaps it is inevitable that potent analgesics possess the property of impacting pain pathways in the supraspinal structures. The question still remains to be answered is that whether a powerful analgesic can be devoid of narcotic effect and addictive potentials. Local anesthetics are powerful analgesics for acute pain by blocking voltage-gated sodium channels that are involved in generation and propagation of action potentials. Antidepressants and anticonvulsants have proven to be useful in the treatment of certain modalities of pain. In neuropathic pain conditions, the complexity arises because of the notion that neuronal circuitry is altered, as occurs in phantom pain, in that pain is perceived even in the absence of peripheral nociceptive inputs. If the locus of these changes is in the central nervous system, commonly used analgesics may not be very useful. This review focuses on the recent advances in nociceptive transmission and nociceptive transient receptor potential vanilloid 1 channel as a target for treating chronic pain conditions with its agonists/antagonists.

KEY WORDS: analgesia; hyperalgesia; morphine; narcotic analgesics; nociceptive ion channels; nociceptors; transient receptor potential (TRP) channels.

INTRODUCTION

In late 2000, the US Congress passed into law a provision that declared 2001–2010 as the Decade of Pain Control and Research. Despite extensive efforts, the mainstay of analgesics for chronic pain is still morphine and its analogs. Long-term use of morphine and its analogs is associated with the development of tolerance and induces significant adverse effects including addiction, hyperalgesia, sedation respiratory depression, and constipation leading to premature death. This decade has also witnessed several prominent members of the entertainment industry succumb to the use of powerful narcotic analgesics, especially when used in combination with other drugs. In neuropathic pain, there can be lack of sensory inputs due to peripheral nerve damage or degeneration, yet some patients experience burning pain sensation. Therefore, alternative targets have to be identified and studied. Transient receptor potential (TRP) channels have gained importance in recent years and are considered as potential targets for next generation analgesics (1–3).

The sensation of pain is an unpleasant sensory experience designed to avoid harmful environments and involves peripheral and central nervous system. Pain is a fundamental sensation that alerts us to injury and triggers various compensatory behavioral responses. Under certain circumstances, pain no longer serves as a warning system; instead, it becomes chronic and debilitating. Injuries due to physical and chemical stimuli damages the nervous tissue leading to a variety of modalities of pain, and the locus can be anywhere in the pain neuroaxis from the peripheral nerve endings to central pain centers. Spinal and supraspinal structures that comprise of spinal dorsal horn, several thalamic nuclei, and cortical areas including the areas that control emotions are involved in processing pain sensation (4,5).

There are changes that occur along the pain neuroaxis involving primary afferents, neurons in the spinal cord, thalamus, and somatosensory cortex. Recently, the involvement of non-neuronal cells such as microglia is gaining importance (6). In addition, functional imaging has provided newer insights into the involvement of specific regions in the central nervous system in processing of pain. Inputs from limbic areas such as amygdala, parietoinsular cortex and the anterior cingulate cortex, and brain stem areas such as periaqueductal gray (PAG) and rostral ventromedial medulla (RVM; components of descending pain pathway) have been implicated to play a significant role (5,7). The importance of

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the descending pathway is further highlighted by the observation that in spinalized animals, long-term potentiation (LTP) could be readily elicited in C-fiber afferent synapses at the spinal cord, whereas in animals with intact spinal cord, LTP could not be induced (8). Furthermore, use of animal models raises the question whether the areas that control cognitive and affective states are prominent in rodents as compared to primates (5).

In recent years, it is becoming apparent that distinct neurons carry a specific type of sensation from the periphery, suggesting the process of discrimination of sensation begins at the periphery. Elimination of a subset of neurons at the spinal cord level that expresses gastrin-releasing peptide receptor selectively abolished itch sensation (thought to be low-grade pain) originating from the periphery (9). Targeting TRP vanilloid 1 (TRPV1) expressed in the central terminals of the sensory neurons in the spinal cord without affecting dorsal root ganglia (DRG) or peripheral terminals led to a complete loss inflammatory thermal hypersensitivity without affecting mechanical sensitivity elicited using von Frey filaments (10). In addition, mu (μ) opioid receptors (MOR) are selectively distributed in unmyelinated peptidergic primary afferent terminals, activation of which reduces thermal hypersensitivity (11). From the distribution pattern of delta (δ) opioid receptors (DOR) in myelinated nonpeptidergic primary afferent terminals, it is expected that morphine also would affect mechanical hypersensitivity (allodynia and hyperalgesia). Morphine is a potent analgesic because it affects both thermal and mechanical hypersensitivities. These studies predict that interrupting nociceptive transmission in a given neuronal pathway at any level in the pain neuroaxis can relieve a selective modality of pain.

PHYSIOLOGY OF NOCICEPTIVE TRANSMISSION

Significant advances have been made in understanding the molecular mechanisms underlying pain perception. In 1906, Sherrington (12) called a subset of neurons that carry nociceptive information nociceptors. However, in recent years, several nociceptive ion channels that respond to specific stimuli have been cloned and characterized (13). Using heterologous expression systems, nociceptive ion channels have been shown to respond to specific physical and chemical stimuli (13). However, the nociceptors are polymodal in nature, which defies the specificity of carrying a selective modality of pain. Electrophysiological studies have found that a subset of sensory neurons is excited by noxious heat, intense pressure, or chemical agents but not by innocuous stimuli such as light touch and warmth (14). This has been well demonstrated by injecting a pungent substance such as capsaicin, an ingredient obtained from hot chili peppers that selectively activates TRPV1 receptors expressed in a subset of nociceptors. However, as a result of sensory nerve efferent function, substances such as bradykinin (BK), calcitonin gene-related peptide (CGRP), and substance P (SP) are released from the peripheral and central nerve terminals and are able to recruit fibers that carry light touch resulting in allodynia around the injected area (15,16). Based on these studies, it is becoming apparent that a subset of sensory neurons exists, whose sole function is to respond to a specific noxious stimulus. The cell bodies of the primary afferent fibers that carry specific information can be characterized based upon anatomical and functional criteria and are located in DRG and trigeminal ganglia (TG).

The DRG have the cell bodies of neurons that innervate the body except head and neck and form synapses at the dorsal horn of the spinal cord, whereas TG have the cell bodies of neurons that innervate head and neck and form synapses at the caudal spinal trigeminal nucleus (CSTN). The nerve fibers fall into four major categories depending upon their conduction velocities: myelinated Aα and Aβ conduct the fastest, and lightly myelinated Aδ and unmyelinated C-fibers conduct more slowly. Noxious thermal, mechanical, and chemical sensations are carried by the low threshold Aδ and C-fibers; non-noxious sensations such as vibration or light touch are carried by Aβ fibers. In disease conditions, Aβ fibers are involved in transmitting pain signal to non-noxious stimuli. It is hypothesized that these fibers mediate the sensation of "first" (C) and "second" (Aδ) pain, namely, the acute sharp pain and the more diffused delayed pain. At the levels of the spinal cord, there are inhibitory and excitatory neuronal circuits and a descending input from the supraspinal structures. Serotonin (5-HT) and norepinephrine (NE) play a major role in descending pathway (7). Neurons in the RVM consist of three different types of cells ("off," "on," and "neutral"). Increased activity of "off" cells results in analgesia (17). Similarly, morphine-induced analgesia is associated with inhibition of "off" cell activity and enhancement of "on" cell activity (18).

Altered sensation to thermal, mechanical, and chemical stimuli occurs as an initial step to pain perception. These sensations can be either enhanced or diminished by inputs from the periphery. It is logical to expect that enhanced nociceptive transmission from periphery manifests as pain, but the possibility must also be considered that diminished inputs from the periphery can induce plastic changes in the intermediate structures (spinal and supraspinal structures) that may be responsible for spontaneous or exaggerated firing of neurons.

MECHANISMS UNDERLYING PERIPHERAL AND CENTRAL SENSITIZATION

Peripheral Sensitization

Injury enhances the pain sensation by increasing the sensitivity of nociceptors to both thermal and mechanical stimuli (19). The process of sensitization can occur at the levels of the nociceptive ion channels, at the peripheral nerve terminals, along the axonal membrane, and at the levels of spinal dorsal horn where the central terminals of the sensory nerve form the first sensory synapse.

Peripheral sensitization is a phenomenon, which involves an increase in the responsiveness of nociceptive ion channels and a reduction in activation threshold of nociceptive neurons (19,20). Sensitization of nociceptive ion channels can result in ectopic or spontaneous discharges (21,22), alteration in ion channel expression, and increased neuronal sprouting (23). Activation of Ca^{2+} permeable nociceptive ion channels in the sensory neurons leads to the synthesis and/or release of a variety of proinflammatory agents and neuropeptides, such as adenosine triphosphate (ATP), CGRP, BK, prostaglandins

(PGs), SP, vasoactive intestinal peptide, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neuropeptide Y (NPY) (15,24). Increases in intracellular Ca^{2+} initiate several second messenger pathways, including activation of PLA_2, PLC, and Ca^{2+}-dependent kinases, which can lead to production of arachidonic acid (AA) metabolites, release Ca^{2+} from intracellular stores, and phosphorylate nociceptive ion channels, respectively. BK is thought to be synthesized and released on demand from sympathetic and sensory nerve endings (25,26). BK initiates prostanoid synthesis and mediates release of vasoactive neuropeptides (25–27).

Central Sensitization

Central sensitization occurs at spinal and supraspinal sites and involves descending projections from supraspinal sites to the spinal cord that facilitates nociceptive transmission. An increase in receptor expression and neurotransmitter/neuropeptide release can occur in the spinal dorsal horn neurons. As a result, a phenomenon known as "wind-up" occurs following repetitive C-fiber stimulation. The spinal dorsal horn neurons are in a depolarized state as a result of prolonged and amplified neuronal responses, which is dependent on *N*-methyl-D-aspartate (NMDA) receptor activation (7,19,28). In certain conditions, loss of inputs from the periphery may reorganize spinal and supraspinal circuitry, which include glutamatergic (28,29), GABAergic (16), and glycinergic (16,30) systems. Several members of the TRP family of ion channels are expressed in the spinal dorsal horn and may also participate in central sensitization (10,11,19).

Glutamate is the predominant excitatory amino acid released from the nerve terminals of nociceptors. However, some of these neurons are also peptidergic, releasing both SP and CGRP (31), and modulate synaptic transmission. Central sensitization, which is similar to LTP in the hippocampus, may result from either pathological conditions or direct spinal trauma leading to increased relay neuron responsiveness to primary inputs. The major difference is that even low frequency stimulation is capable of initiating LTP in the spinal dorsal horn neurons (16).

Glycine and GABA are inhibitory neurotransmitters; activation of their receptors causes inhibitory postsynaptic potential and decreases the excitability of the neuron. Dephosphorylation of glycine receptor by PG E2 produced during inflammation diminishes glycine receptor-mediated inhibition at the spinal cord (32,33). Excessive stimulation of sensory afferents modulates the inhibitory interneurons by releasing anandamide. Anandamide activates the cannabinoid 1 (CB1) receptors, which are selectively expressed in the GABAergic interneurons (30).

Microglia and astrocytes are involved in chronic pain conditions. Substances released by these non-neuronal cells can modulate neuronal excitability and synaptic transmission by various mechanisms. Recent studies implicate microglia in this response by altering the chloride gradient by diminished expression of chloride co-transporter KCC-2. The chloride concentration builds up inside the cell resulting in the alteration of chloride gradient. In this condition, activation of chloride channels results in loss inhibition on the projection neuron carrying nociceptive information (34). Activated glia produces various proinflammatory cytokines such as tumor necrosis factor-α, interleukin-1β (IL-1β), and IL-6 (35). These factors participate in central sensitization by enhancing the function of NMDA receptors thereby increasing Ca^{2+} influx and augmenting the production of reactive oxygen and nitrogen species (RO/NS), nitric oxide, PGs, and BDNF (31). Cytokines also activate p38 mitogen-activated protein kinase and extracellular signal-regulated kinase and transcription factors such as nuclear factor-κB. Astrocytes and microglia also express several pattern-recognition receptors including toll-like receptors (TLRs). TLR2 and TLR4 are predominantly expressed in microglia and are able to release cytokines (6,36). Purinergic receptor type 4 (P2X4) is selectively expressed in microglia and is upregulated by the activation of TLR4 by fibronectin (36). Opioids have been shown to bind to TLR4 and cause activation of microglia that may be involved in opioid-induced hyperalgesia, tolerance, and withdrawal. Furthermore, activation of glia by heat shock proteins is via TLRs (36).

DIFFERENT PAIN STATES OR MODALITIES OF PAIN

Pain can be classified as acute nociceptive pain (pain that occurs immediately after an insult without tissue damage), allodynia (pain associated with innocuous stimuli), and hyperalgesia (pain following tissue damage and inflammation). Chronic pain or neuropathic pain manifests as painful sensation with or without specified peripheral inputs (pain including spontaneous pain following nerve damage or nerve degeneration or amputation).

Acute Pain or Nociceptive Pain

Pain experienced at the moment of an insult is acute pain. It could be thermal, mechanical, or chemical. Pain experienced by moderate heat insult (50–70°C) or cold (0–10°C), bumping against an object, or exposure to dilute pungent chemicals are some forms of acute pain and can be resolved within a matter of seconds or minutes.

Allodynia

Allodynia is a pain state associated with innocuous stimuli in a manner nociceptors are activated at normal body temperatures, by the blowing wind, by the contact of one's own clothing, or enhanced sensation to nonpungent chemicals (4). Therefore, allodynia can occur from either increased responsiveness of spinal cord relay neurons (central sensitization) or lowering of nociceptor activation threshold (peripheral sensitization) by the release of proinflammatory agents. A simple experiment in humans demonstrates this effect. Subcutaneous injection of capsaicin induces nociceptive pain; however, over time, surrounding areas become sensitive to light touch.

It has been suggested that this phenomenon is due to sensitization and recruitment of Aβ fibers by inflammatory substances released from peripheral and central sensory nerve terminals (16).

Inflammatory Pain

If the intensity of the stimuli is greater and results in tissue damage, a cascade of inflammatory events is initiated. For example, following tissue damage caused by extreme heat/cold, bumping against an object, or exposure to concentrated pungent chemicals, inflammatory mediators are released, and nociceptive ion channels are sensitized. Vascular permeability is increased causing extravasation and swelling, thereby activating sensitized mechanosensitive channels. Since it is an injury, the natural immune response is triggered by recruiting leukocytes, macrophages, fibroblasts, mast cells, neutrophils, and platelets, which results in a full-fledged inflammatory response. The components of inflammation, termed the "inflammatory soup," can alter sensory neuronal excitability either by directly activating ion channels (protons, ATP, serotonin, and glutamate) or through second messengers produced by activation of metabotropic receptors (glutamate, serotonin, ATP, SP, CGRP, NPY, BK, NGF, BDNF, and PGs) (20). During inflammation, RO/NS are produced and activate transcription factors and lead to upregulation of the nociceptive ion channels (37,38). Furthermore, nociceptive TRP channel, TRP ankyrin 1 (TRPA1), is directly activated by reactive molecules produced during oxidative stress. The enhanced neuronal activity from periphery can cause changes in the spinal cord level by releasing proinflammatory substances and neuropeptides. Enhanced neuronal activity can induce LTP by enhancing Ca^{2+} influx into postsynaptic neurons (16) and decrease inhibitory transmission by activating Gi-coupled receptors such as CB1 receptors, selectively expressed in inhibitory neurons (30). Activation of CB2 receptors present in immune cells can diminish inflammation in the periphery. Nonsteroidal anti-inflammatory agents have been proven to be very effective in producing relief from inflammatory pain (39).

Chronic or Neuropathic Pain

Neuropathic pain occurs as a result of neuronal plasticity and neuronal rewiring following traumatic, viral, surgical, metabolic, or drug-induced damage to the neurons. The pain associated with phantom limb, postherpetic neuralgia, diabetic peripheral neuropathy, HIV-associated neuropathy, and chemotherapy-induced neuropathy can be included under neuropathic pain conditions (19,40). The components involved in development and maintenance of neuropathic pain are far from clear. The question is whether enhanced peripheral inputs from the primary afferent neurons are required or normal inputs from the periphery are amplified at the second and third order neurons. The changes that occur causing enhanced neuronal activity resulting in spontaneous pain are still not fully understood. Phantom pain after an amputation is commonly referred to as neuropathic pain because there are no peripheral inputs. Pain associated with diabetic peripheral neuropathy is due to nerve terminal degeneration. However, the severed neurons develop neuromas that can function as trigger points to generate spontaneous action potentials (16,41). Changes that occur in the second and third order neurons to become hyperactive include modulation of synaptic transmission and induction of LTP. Neuropathic pain is relieved by morphine acting at peripheral, spinal, and supraspinal structures, whereas fluoxetine and duloxetine (serotonin NE transport inhibitor) act by facilitating descending pathway and gabapentin by modulating Ca^{2+} channels at peripheral, spinal, and supraspinal sites, suggesting the involvement of different levels of pain neuroaxis. In neuropathic pain, the challenge is to identify the locus or loci of the defect. Most of the drugs that are effective in neuropathic pain have a global action, in that the whole neuroaxis is affected including the areas that control affective and cognitive states.

International association for the study of pain has classified complex regional pain syndrome (CRPS) as CRPS I and CRPS II. CRPS I, previously known as reflex sympathetic dystrophy, is considered as a neuropathic pain syndrome characterized by continuing pain, allodynia, hyperalgesia, vasomotor (edema), and sudomotor abnormalities. It involves both peripheral and central sensitization along with regional inflammation. CRPS II, formerly known as causalgia, is a pain syndrome that starts after a nerve injury and can distribute over several other areas with time (42).

MORPHINE AND ITS ANALOGS AS THERAPIES FOR CHRONIC PAIN CONDITIONS

The mainstay of treatment for chronic and neuropathic pain conditions is morphine and its analogs. Morphine activates three types of receptors, namely, MOR, DOR, and kappa (κ) opioid receptor (KOR). It is important to know the distribution of these receptors to understand morphine actions and the associated adverse effects. MOR is expressed in the heat-sensitive TRPV1 expressing peptidergic (CGRP and SP containing) small diameter unmyelinated nociceptors that form synapses at lamina I and inner lamina II of the spinal cord. DORs are expressed in mechanosensitive myelinated and nonpeptidergic primary afferents terminals that form synapses at the lamina I and outer lamina II (11,43,44). DORs are also diffusely distributed in the spinal dorsal horn neurons, and KORs are distributed in outer lamina of the spinal cord segments, which receives inputs from the visceral structures (43,44). The RVM and the PAG areas of the brain stem express high levels of MOR and reasonable levels of DOR and KOR (43,45). These two brain stem nuclei neurons are also involved in the descending pathway, activation of which decreases pain transmission and induces analgesia (7,17,18). Notably, fluoxetine and duloxetine that inhibit 5HT and NE transporters facilitate this pathway by increasing the levels of 5HT and NE.

The analgesic actions of morphine and its analogs are mainly mediated by their activation of MORs; however, some analogs may also act on DOR and KOR. Seven different types of MOR have been identified and characterized (46). Opioid drugs may have different affinities on these specific subtypes of MOR. Since tolerance is a major problem with opioid treatment, it can be overcome by using agonists that have selectivity on different type of MOR. For example, it has been shown that methadone action on DOR may underlie its efficacy in patients who have developed tolerance to morphine (46).

Chronic use of morphine is associated with (1) tolerance (requiring higher concentrations to induce the same response), (2) hyperalgesia (patients develop increased sensitivity to pain), (3) dependence, (4) respiratory depression,

and (5) constipation. The variability in the efficacy of morphine, development of psychological dependence, and tolerance to a certain degree can be attributed to genetic polymorphisms in opioid receptors and other proteins that may be associated with morphine action (43–47). There are two genetic variants that affect MOR transcription, and internalization has been identified. Tolerance can occur as a result of enzyme induction by morphine and by other drugs that enhances the metabolism of morphine. Hence, higher doses are required to cause the same degree of analgesia. Since opioid receptors are inhibitory G-protein-coupled receptors, the receptor binding and the associated second messenger production can undergo changes and manifest as tolerance. The inhibitory action on the neurons is mediated by decreased cAMP levels that inhibit calcium currents and enhance potassium currents, resulting in decreased transmitter release (48). The high affinity agonists such as fentanyl occupy fewer receptors thereby reducing internalization of the receptors and delayed development of tolerance.

Paradoxically, chronic use of morphine is capable of inducing hyperalgesia and allodynia. It has been shown that morphine-induced hyperalgesia has different characteristics than the original pain for which the patient is being treated for (43). Morphine-induced hyperalgesia is unrelated to withdrawal effects of morphine (49). The proposed mechanisms underlying morphine-induced hyperalgesia include the activation of NMDA type glutamate receptor and the cholecystokinin (CCK)-induced enhancement of the descending pathway from the brain stem. NMDA receptors are involved in the sensitization of the spinal cord neurons as evidenced by their antagonists to reverse morphine-induced hyperalgesia (43,49). CCK released from brainstem RVM neurons causes enhanced release of dynorphin. Although dynorphin is a KOR-specific agonist, the effect of dynorphin in hyperalgesia is unrelated to KOR activation (49). Both CCK and dynorphin increase the excitability of spinothalamic track neurons and augment nociceptive transmission (43,49,50).

Opioid dependence and some of the most distressing opioid withdrawal symptoms are related to changes that involve the locus ceruleus (LC). Neurons in this region release NE, which governs wakefulness, breathing, blood pressure control, and general alertness. Activation of MOR present in LC suppresses the release of NE, resulting in drowsiness, slowed respiration, and low blood pressure. However, with repeated exposure to opioids, the LC neurons adjust to compensate for lower NE levels. On cessation of therapy, excessive release of NE triggers jitters, anxiety, muscle cramps, and diarrhea. Other brain areas such as limbic areas, in addition to the LC, also contribute to the production of withdrawal symptoms (51).

Central nervous system depression, with associated respiratory depression, can severely limit the physician's ability to manage acute and chronic pain states. Independent of the route of administration, opioid-induced respiratory depression leads to hypoventilation and hypoxemia and has produced irreversible neurologic injury and death (43). Evidence from experiments in animals has suggested that this is a consequence of MOR-mediated blockade of specialized respiratory neurons in the brainstem (43,52).

Constipation is a serious side effect of narcotic analgesics. It can be either acute (typical of short-term narcotic use) or chronic (common with long-term use). The pathophysiology of constipation due to opioids is well described. Possible effects include increased anal sphincter tone, reduced peristalsis in the small intestine and colon, increased electrolyte and water absorption, and impaired defecation response. Two opioid receptors, MOR and DOR, are located on gut smooth muscle and play a significant role in gastrointestinal motility (53).

NOCICEPTIVE TRP CHANNELS AS TARGETS FOR NEXT GENERATION ANALGESICS

Nociceptive ion channels are targets for developing antagonists for pain relief. Sensory nerve endings (both peripheral and central) express ion channels that respond to chemical, thermal, and mechanical stimuli, which include acid-sensitive ion channels, degenerin/epithelial sodium channels, purinergic ATP-gated ion channels (P2X), and TRP channels (54–57). Over 30 members of the TRP family of ion channels have been cloned, and their functional properties have been studied (18,57,58). Nociceptive TRP channels that respond to chemical, thermal, and mechanical stimuli include TRPV1, TRPV4, and TRPA1 (58–62). The ion channels that transduce direct mechanosensitivity have not been identified (60,62–64). Sensory TRP channels are sensitized by proinflammatory agents that are coupled to intracellular signaling pathways and mediate heightened pain sensitivity (20,65). In order to obtain effective pain relief, it is first necessary to understand the molecular structures that respond to physical (temperature and mechanical force) and chemical stimuli that are responsible for initiating a receptor potential and generating an action potential.

It is becoming evident that TRP channels can be considered as molecular thermometers. In sensory neurons, a variety of TRP channels are expressed and are activated by specific temperature range or, in some cases, at a particular temperature precisely. TRPA1, TRPV4, and TRPV1 are activated by temperatures <15°C, >27°C, >42°C, respectively. Although it is not clearly documented that TRPA1 is activated by cold, TRP melastatin 8 is activated by temperatures <23°C (62). It is also interesting that some of these channels are activated by gustatory agents. For example, TRPA1 is activated by mustard oil, ingredients from garlic, wasabi, and horseradish; and TRPV1 is activated by capsaicin, an ingredient in hot chili peppers and by acidic pH of vinegar. It is an extremely painful sensation when inadvertently a mouthful of piping hot spicy chili dish garnished with vinegar is taken. With repeated exposure of agonists, the receptors are desensitized with time, a mechanism that may underlie the ability to tolerate and enjoy spicy food and the effectiveness of topical application of capsaicin.

Expression and Functions of TRPV1

A subset of neurons in DRG, TG, and nodose ganglia expresses TRPV1, a nonselective cation channel with high calcium permeability (66). These are peptidergic, small to medium diameter neurons that give rise to unmyelinated C-fibers and thinly myelinated Aδ-fibers. TRPV1 is also expressed in neurons that are labeled for D-galactosyl-binding lectin IB4 and expresses the ionotropic ATP receptor, P_2X_3

(54,62,65). TRPV1 is expressed on the peripheral and central terminals of small diameter sensory neurons (62,65,66). It functions as a polymodal receptor at the peripheral nerve terminals and modulates synaptic transmission at the first sensory synapse between DRG/TG and dorsal horn/CSTN neurons (10,65). TRPV1 has also been shown to modulate synaptic transmission in certain regions of the brain (65). TRPV1 is activated by heat (>42°C), protons, anandamide, AA metabolites, *N*-arachidonyl dopamine (NADA), capsaicin (pungent ingredient of hot chili peppers), and resiniferatoxin (RTX; obtained from *Euphorbia resinifera*) (58,65,66).

TRPV1 knockout mice are able to sense normal temperature with some deficiency but lack thermal hypersensitivity following inflammation (67,68). Results from knockout mice studies reveal several nonsensory functions of TRPV1. TRPV1 knockout mice show reduced anxiety-related behavior and exhibit a form of synaptic depression in the hippocampus. In addition, the knockout mice demonstrated deficits in developing LTP (69). Recently, it has been reported that TRPV1-expressing pancreatic sensory neurons control islet inflammation and insulin resistance in diabetes (70,71).

Expression and Functions of TRPV4

TRPV4 is a putative mechano/osmosensitive channel expressed in many cell types including sensory neurons. It is a homologue of the *Caenorhabditis elegans* osmosensory channel, OSM-9, that is expressed in sensory neurons, hypothalamus, vascular smooth muscle cells, kidney, trachea, cochlear hair cells, endothelial cells, and keratinocytes in vertebrates (60–62,72). TRPV4 is sensitive to cell swelling and shear stress, thus functioning as a putative mechanosensor (72–74). TRPV4 is activated by hypotonicity, heat (>27°C), DAG, PKC-activating (phorbol 12-myristate 13-acetate) and non-activating phorbol esters (4α-phorbol 12,13-didecanoate), and 5′,6′-epoxyeicosatrienoic acid derived from anandamide and AA (60,72), and is involved in nociception (60–62). Recently, it has been shown that TRPV4 can be activated by hypotonic solution in a system, independent of polyunsaturated fatty acids (162), and by mechanical force in excised membrane patches (75,76).

In TRPV4 knockout mice, the sensitivity of the tail to pressure and acidic nociception are reduced as compared to wild-type mice. It was surprising that there was no change in von Frey hair test between TRPV4 knockout and wild-type mice. However, TRPV4 is necessary for the normal response to changes in osmotic pressure and functions as an osmotic sensor (61,72). TRPV4 knockout results in the loss of shear stress-induced vasodilation, a response pattern critically dependent on endothelial TRPV4 expression (77). Thus, Ca^{2+}-influx through endothelial TRPV4 channels contributes significantly to endothelial mechanotransduction. Recent studies show that TRPV4 is also involved in hearing (78).

Expression and Functions of TRPA1

TRPA1 is a nonselective, Ca^{2+}-permeable cation channel, first isolated from human fibroblasts (79). It is unique in its structure among TRP channel for having a large number (17) of ankyrin repeat domains, which may impart a spring-like action to proteins. It is activated by allyl isothiocyanate (mustard oil, horseradish, and wasabi), allicin, diallyldisulfide (in garlic extract), cinnamaldehyde (in cinnamon oil), acrolein (in tear gas and car exhaust), and *N*-methyl maleimide through modification of cysteine residues. It is also activated by tetrahydrocannabinoid, WIN 55,212-2, and BK (62,80–83). Finally, physical stimuli like noxious cold (<18°C) temperatures and mechanical force have been proposed to activate the channel (1,62,80–85). TRPA1 has been suggested to be a sensor for mechanical stimuli because its *Drosophila* homologue, *painless*, participates in mechanical nociception and belongs to the TRP family (86). TRPA1 is also selectively activated by endogenous chemicals produced during oxidative stress including H_2O_2/hydroxyl radicals, aldehydes, such as 4-hydroxynonenal, cyclopentenone PGs such as 15d-PGJ2, and hypochlorite (87).

Behavioral studies in mice lacking TRPA1 ($TRPA1^{-/-}$) confirmed its role in nociception to pungent substances (88,89). Treatment with TRPA1 antisense oligodeoxynucleotides reduced behavioral hypersensitivity to cold after CFA-induced inflammation or sciatic nerve injury and decreased cold hyperalgesia following L5 spinal nerve ligation (90,91). Kwan *et al.* (88) showed that TRPA1 knockout mice exhibited impaired behavioral responses to a cold plate maintained at 0° C. However, Bautista *et al.* (89) failed to demonstrate this effect. Kwan *et al.* (88) reported that $TRPA1^{-/-}$ mice showed a deficiency in sensing noxious punctate cutaneous mechanical stimuli; these mice had higher mechanical thresholds and reduced response to a series of suprathreshold stimuli when compared to wild-type mice, suggesting a potential role in the transduction of high-threshold mechanical stimuli. On the other hand, Bautista *et al.* (89) reported no difference in mechanical thresholds between TRPA1 knockout and wild-type mice. Recent studies using TRPA1 knockout mice have confirmed that there is no difference in acute cold sensation, but mechanical sensitivity is significantly altered. It has been shown that low threshold Aβ and D-hair mechanoreceptive fiber characteristics have also changed (92,93).

DEVELOPMENT OF TRPV1 CHANNEL AGONISTS AND ANTAGONISTS FOR TREATMENT OF PAIN

Since TRPV1 is the earliest member of the TRP channel family that was cloned in 1997, TRPV1 has been extensively studied. It is becoming clear that both TRPV1 agonists and antagonists are capable of inducing pain relief. The use of antagonists is a logical approach because they inhibit the receptor and prevent the generation of a receptor potential at the peripheral terminals. However, to justify the use of agonists, the mechanism of action must be delineated. Since TRPV1 is a highly Ca^{2+}-permeable channel and Ca^{2+} is known to cause desensitization and is expected to inhibit the generation of receptor potential and subsequently the action potential. Since desensitization is a function of concentration, the concentration of the agonist is critical to induce desensitization. The other possible mechanism is related to depolarization block induced by TRPV1 agonists at the nerve terminals and thereby inhibiting nociceptive transmission. Nondesensitizing and nonpungent concentrations of potent agonists can depolarize the nerve terminals in a ramp-like manner leading to depolarization block by maintaining the voltage-gated Na^+ channels in an inactivated state. This will

inhibit nociceptive transmission in the short-term; however, since TRP channels are highly Ca^{2+} permeable, sustained influx of Ca^{2+} can cause nerve terminal degeneration in the long-term leading to long-lasting pain relief. This is an elegant approach to relieve pain arising from inaccessible areas such as interior of the bone in bone cancer and large visceral mass in abdominal cancers, where the TRP channels expressed in the spinal cord can be targeted. The role of TRP channels expressed in the spinal cord is not fully understood, but may become relevant in chronic pain conditions (10,19,20,38,65).

Usefulness of the TRPV1 blockade has been demonstrated to be beneficial in pain induced by Herpes zoster, diabetic peripheral neuropathy, bone cancer, arthritis, inflammatory bowel disease, and migraine (60,62,65). Intrathecal administration of RTX has been used to ameliorate painful conditions (10,94,95). Intriguingly, bone cancer induced by inoculation of carcinoma cells mainly results in altered mechanosensitivity, yet TRPV1 antagonists have been found to be useful (65).

TRPV1 Agonists

In earlier studies, a single intrathecal injection of capsaicin depleted SP from primary sensory neurons and caused a reduction in thermal and chemical sensitivities in rats, but there was no apparent change in responses to mechanical sensitivity (10,65). Recent experiments have demonstrated that TRPV1 is selectively expressed in the sensory nerve terminals at the DH of the spinal cord (laminae I and II) and CSTN. Administration of RTX causes a sustained increase in sEPSCs as compared to capsaicin which induced a desensitizing response (10,65). Recording of evoked synaptic currents reveals synaptic failures that are likely to be due to depolarization block of presynaptic terminals from sustained TRPV1 activation. This effect is expected to cause reduced nociceptive transmission and short-term analgesia. In the long-term, RTX leads to ablation of TRPV1 expressing nerve terminals as a result of sustained Ca^{2+} influx, which is indicated by complete loss of TRPV1 staining. Furthermore, intrathecal administration of RTX reduced selectively inflammatory thermal hypersensitivity without altering acute thermal sensitivity. This further confirms that TRPV1 expressing central nerve terminals in the dorsal horn are involved in inflammatory thermal hypersensitivity (10). This is in consensus with recent studies that demonstrates that MORs are expressed in heat-sensitive neurons, and DOR are expressed in mechanosensitive neurons (11). Immunostaining studies have shown that TRPV1 in DRG neuronal cell bodies and peripheral terminals are preserved, suggesting that sensory efferent functions such as TRPV1-mediated CGRP and SP release at the peripheral nerve terminals are expected to be intact (10). Vasoactive peptides, such as CGRP and SP have been shown to be essential for controlling microvascular circulation in perineurial capillaries and coronary vessel (65). The specificity of targeting TRPV1 by intrathecal administration of RTX has been shown by the observation that RTX did not affect mechanosensitivity tested using von Frey filament and the staining of TRPV4; a putative mechanosensor was intact in the dorsal horn (10).

Targeting TRPV1 expressing peripheral nerve terminals in bladder has proven to be useful in treating urinary bladder hyperreflexia. Intravesical administration of RTX results in the reduction in TRPV1 immunoreactivity and leads to long-lasting reduction in bladder pain and incontinence (65,96). Interestingly, intravesical administration of RTX does not induce suprapubic pain as compared to capsaicin, possibly suggesting that slow and sustained activation of TRPV1 by RTX causes depolarization block (10,65).

TRPV1 is also involved in regulation of body temperature. Subcutaneous injection of capsaicin decreases body temperature by 2–3°C and permanently reduced the capacity of rats to withstand a hot environment (65,97). TRPV1 antagonists increase the body temperature to the same extent. Several TRPV1 antagonists are in clinical trials, and hyperthermia poses a serious limitation to their usefulness (97). The promise of TRPV1 antagonists to treat painful conditions may not become a reality. Selective targeting of spinal segments may be achieved by slow infusion of RTX using osmotic mini pumps that may spare thermoregulatory centers in the hypothalamus and avoid hyperthermia.

Another advantage of RTX is that it appears to be selective. Even when administered intraperitoneally, it specifically ablates TRPV1-expressing nociceptors. TRPV1 has been implicated in diverse function such as release of the potent vasodilator CGRP and maintaining microvascular circulation, including the coronaries and regulation of insulin secretion (65,70,71). Therefore, intrathecal administration of TRPV1 agonists may be superior than selectively targeting TRPV1 expressed in peripheral nerve terminals.

TRPV1 Antagonists

The well-studied TRPV1 antagonist is capsazepine, which is a competitive antagonist. Capsazepine is a weak antagonist with IC_{50} values of 0.2–4 μM and exhibits species-dependent difference in inhibiting TRPV1. It has been demonstrated to block other receptors and channels, which include voltage-gated calcium channels and acetylcholine receptors (1,3,58,65,85,98).

Iodo-resiniferatoxin (iodo-RTX), one of the most potent antagonist of TRPV1, has been reported to inhibit capsaicin-induced currents in *Xenopus laevis* oocytes expressing TRPV1 in nanomolar range and inhibited capsaicin-elicited pain responses when administered intrathecally. Furthermore, iodo-RTX has been demonstrated to inhibit both heat- and proton-evoked responses in sensory neurons (1,3,58,65,85,98).

There is a great interest by pharmaceutical companies to develop potent TRPV1 antagonists, because it has become an important target for a variety of painful conditions. A non-vanilloid TRPV1-antagonist, *N*-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carbox-amide (BCTC), has been synthesized. BCTC can block acid-mediated activation of rat TRPV1. Oral administered BCTC has been demonstrated to inhibit both mechanical and thermal hyperalgesia without affecting normal/acute nociception (65,85,98).

SB-366791 is a competitive inhibitor of TRPV1 that inhibits capsaicin, acid, and noxious heat-mediated activation of the receptor. It is superior to capsazepine as it blocks acid-mediated activation of rTRPV1. Spontaneous and miniature excitatory synaptic currents (sEPSC and mEPSC), recorded

from the neurons of the spinal cords obtained from CFA-injected animals, were decreased in the presence of SB-366791 (1,3,65,85,98).

A-425619 is a competitive, potent, and highly selective antagonist of TRPV1 (99). It inhibits increases in intracellular calcium influx evoked by capsaicin, NADA, anandamide, or pH in HEK cells heterologously expressing hTRPV1 as well as capsaicin-induced inward currents in cultured rat DRG neurons. Furthermore, capsaicin-mediated intracellular influx was inhibited by A-425619 at much lower concentrations, when the hTRPV1 heterologously expressed in HEK cells is sensitized by a PKC activator, PDBu, or low pH (6.0). A-784168 and A-795614 have comparable efficacy in *in vitro* assays but have significant differences in their ability to permeate the central nervous system. Interestingly, both of these agents demonstrated equal efficacy when administered intrathecally; however, when administered orally, A-784168, which crosses the blood–brain barrier, was found to be more effective than A-795614 (99). This study demonstrates that spinal and supraspinal structures are involved in TRPV1 antagonists-induced pain relief.

A piperazinylpyrimidine analog (AMG517) is in phase 1 clinical trials. Amgen has also developed other potent compounds (AMG0347, AMG8163, and AMG9810) (1,3). Neurogen/Merck has launched NDG-8243/MK-2295 in phase II clinical trials. The latest compound to enter clinical trials is GRC-6211 from Glenmark/Eli Lilly pharmaceuticals. Other TRPV1 antagonists including JNJ-17203212 (Johnson and Johnson) and JYL1421 (Pacific Corp.) have been shown to be effective in bone cancer pain (1,3,65).

CONCLUDING REMARKS

Pain is perceived when action potentials generated in the nociceptors are transmitted to the appropriate regions in the somatosensory cortex. However, the involvement of regions of the brain that control cognitive and affective states have to be considered because of their role in chronic neuropathic pain conditions. The stimuli that generate a receptor potential to trigger an action potential are thermal, mechanical, and chemical in nature. The threshold for the activation of nociceptive ion channels can be drastically reduced, when they are sensitized by proinflammatory agents or overexpression of receptor proteins by transcriptional upregulation in such a manner that innocuous thermal, mechanical, and chemical stimuli are able to generate a receptor potential. It must also be considered that the resting membrane potential can be in a depolarized state that can initiate spontaneous action potentials. The strategies to prevent generation of action potentials at the periphery are to reduce the intensity of thermal, mechanical, and chemical stimuli to prevent the sensitization of the nociceptors and to maintain the resting membrane potential in a hyperpolarized state. The effectiveness of nonsteroidal anti-inflammatory drugs (NSAIDs) in inducing pain relief can be explained by their ability to prevent sensitization.

When NSAIDs become ineffective, the strategy is to prevent nociceptive transmission at the level of the spinal and supraspinal areas by inhibiting neurotransmission. Action potentials generated at the peripheral terminals of the primary afferent neuron invade the primary afferent terminal in the spinal dorsal horn laminae I and II and release glutamate that binds to the postsynaptic receptors and induces an excitatory postsynaptic potential. As a result of temporal and spatial summation, when the membrane potential reaches the threshold in the second order neuron, an action potential is generated, and the nociceptive information is transmitted to higher pain centers. Primary afferent central terminal is an excellent target to prevent nociceptive transmission. An important aspect is being clarified with respect to the selectivity of neurons that carry specific sensory modalities. For example, nonmyelinated, peptidergic fibers carry inflammatory thermal hypersensitivity, whereas myelinated nonpeptidergic fibers carry mechanical hypersensitivity. MORs are expressed in nonmyelinated C and Aδ fiber terminals, and DORs are expressed in myelinated Aβ fibers. A mechanism by which nociceptive transmission can be reduced is to prevent long-term changes in synaptic strength as seen with LTP. The potency of morphine and its analogs can be explained by their ability to inhibit synaptic transmission and to prevent the development of LTP associated with nociceptor activity. One of the mechanisms of morphine tolerance is due to decreased expression of MOR and DOR at the first sensory synapse. Furthermore, loss of inhibition at the spinal cord is an important mechanism, and strategies can be adopted to prevent this. The hyperalgesia also can be explained by the interference with the descending pathway and respiratory depression by its action on the respiratory centers.

Recently, several nociceptive ion channels have been cloned, and their distribution in both peripheral and central terminals of the nociceptors makes them ideal targets to develop analgesics. Since their distribution is predominantly in the primary afferent nociceptors, they may not induce serious side effects such as sedation, addiction, and respiratory depression. It is also intriguing that the distribution the TRP channels mirrors is the distribution of MOR and DOR in the sensory neurons. Targeting both TRPV1 and TRPA1 can be a strategy to reduce both thermal and mechanical pain sensitivity. Unfortunately, drugs that have been developed to block the nociceptive ion channel TRPV1 have encountered a serious side effect of hyperthermia. The challenge is to develop compounds without this side effect. TRP channels are involved in functions other than sensory in nature, and some of the TRP channels are distributed throughout neuroaxis. Antagonizing TRP channels can lead to decreased release of vasoactive peptides and affect cardiovascular functions. Since TRP channels are highly Ca^{2+} permeable, sustained activation by agonists can also lead to Ca^{2+}-dependent nerve terminal degeneration. TRP channels are involved in bladder function; therefore, possible adverse effects associated with bladder function must be taken into consideration. Furthermore, use of TRP channel agonists is a useful strategy, because they are able to desensitize or internalize the receptor and also cause depolarization block and prevent nociceptive transmission.

Voltage-gated Na^{+} and Ca^{2+} channels and several ionotropic and metabotropic receptors are being considered as potential targets for treatment of chronic pain conditions. The selectivity of action may be a concern because these receptors are expressed globally. Recently, activated

microglia and the associated substances (neurotrophins and neuropeptides) released have been implicated in chronic pain conditions. Neurotrophins and neuropeptides can be targeted by developing antibodies, and their respective receptors can be targeted by small molecule agonist/antagonists.

The pressing questions are as follows: "Are the effects of morphine on the peripheral and spinal cord level sufficient to cause pain relief without the supraspinal action?," "Is the activation of reward pathway necessary for the pain relief?," "How do anticonvulsants and antidepressants induce pain relief?," and "Can TRP channel agonists/antagonists be considered as alternatives to morphine and its analogs?"

Finally, the severity of adverse effects must be evaluated, and the balance between the beneficial and the adverse effects should be cautiously and pragmatically considered. Using agents that selectively target nerve terminals at the periphery or at the level of the spinal cord may avoid some of the systemic adverse effects. In any case, TRP channels are potential novel targets for next generation analgesics, and if proven to be successful in clinical trials, their agonists and antagonists may become as alternative to morphine to treat certain modalities pain.

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