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Role of Calcium Permeable Channels in Pain Processing

Célio Castro-Junior, Luana Ferreira, Marina Delgado,
Juliana Silva and Duana Santos

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Abstract

Calcium-permeable channels control intracellular calcium dynamics in both neuronal and nonneuronal cells to orchestrate sensory functions including pain. Calcium entering the cell throughout these channels is associated with transduction, transmission, processing, and modulation of pain signals. Clinic, genetic, biochemical, biophysical and pharmacological evidence points toward calcium-permeable channels as the key players in acute and persistent pain conditions. Ligand-gated calcium channels such as TRP channels or some subtypes of voltage-gated calcium channels shows abnormal functioning in persistent pain states. Also, NMDA receptors can be unlocked from their physiological Mg^{2+} blockade under persisten pain states to culminate with central sensitization. The primary goal of this chapter is to present an overview of the functioning of different classes of calcium-permeable channels and how they become altered to modulate the sensation of pain in acute and chronic states. The most important evidence from classical and recent studies will be discussed trying to depict ways of modulating those channels as a strategy for better pain control.

Keywords: pain, sensitization, calcium channels, NMDA receptors, VGCC's, TRP channels

1. Introduction

1.1. Overall pain neurobiology

For a better understanding of how calcium channels regulation is involved in pain states, it is relevant define pain and discusses overall aspects of the transmission and modulation of pain signals throughout the nervous system. Pain is defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of

such damage". This definition was proposed by Harold Merskey in 1964 and was adopted by the International Association of the Study of Pain (IASP) and since 1979 is the most accepted definition for pain worldwide. That definition clearly mention the sensory aspect of pain. As an organic sensorial modality, pain processing relies on electrical signal transmission throughout the nervous system. Despite the fact that emotional features of an individual directly interferes with final pain interpretation, this chapter is focused on the sensory aspects (also called nociception) of pain and how calcium-permeable channels are involved in pain processing.

The macroscopic pathway of pain signals comprises a well-accepted route throughout the peripheral and central nervous system (**Figure 1A**). Detection of noxious stimuli may occur in skin, muscle, joints, and internal organs. Generated signals travel from periphery to the spinal cord through axons of sensory afferent fibers. Once those fibers enter the spinal cord through the dorsal horn, they made synapses with second order neurons that convey synaptic release of neurotransmitter into new action potentials. That action potential travels up along the spinal cord mainly through spinothalamic trait until the thalamus where a new synapse occurs and pain can be initially perceived by its intensity. From the thalamus, signals diverge to different brain areas mainly the somatosensory cortex and limbic systems allowing a more broad interpretation and association of pain with emotional experiences (**Figure 1**). This ascending path is counterbalanced by a descending circuitry that connects with the ascending fibers to facilitate or reduce the traffic of electrical pain signals to the brain.

1.2. Calcium channels in the pain pathway

Importantly, the ion channels present at the membrane of neurons of the above pathway functionally orchestrate the generation and processing of pain signals (**Figure 1B**). Although calcium channels are the focus of this chapter, sodium and potassium channels also holds prominent contribution of signal transmission mainly on the conduction of the bioelectrical pain signals. Noxious stimuli are initially transduced into electrical signals by the peripheral end of sensory neurons. Those terminals convey diverse sensory modalities such as pain, itch, discriminative touch into action potentials. Some terminals belong to a subset of fibers that are specialized on the detection of noxious stimuli (delta and C fibers). Some of the markers associated with specific nociceptive fibers include calcium-permeable channels such as the cold/menthol receptor TRPM8, the heat vaniloid receptor TRPV1, the mustard oil receptor TRPA1 and the purinergic receptor P2X3.

Those markers consist of cation-permeable channels that activate in response to noxious stimuli (e.g. TRPA1 respond to noxious cold and TRPV1 to heat) to allow mainly sodium and calcium influx into the nerve terminal contributing for action potential generation. At the central terminal of sensorial afferent neurons, the action potential triggers the opening of voltage-gated calcium channels and the consecutive influx of calcium ions cause exocytosis of excitatory neurotransmitter (glutamate) which further depolarize a second order neuron in the spinal cord. The expression pattern of N-type voltage gated calcium channels at the dorsal horn of spinal cord is consistent with the role of VGCC's in afferent/spinal synapses. Furthermore, null mice for the N-type channels show higher threshold for thermal and mechanical
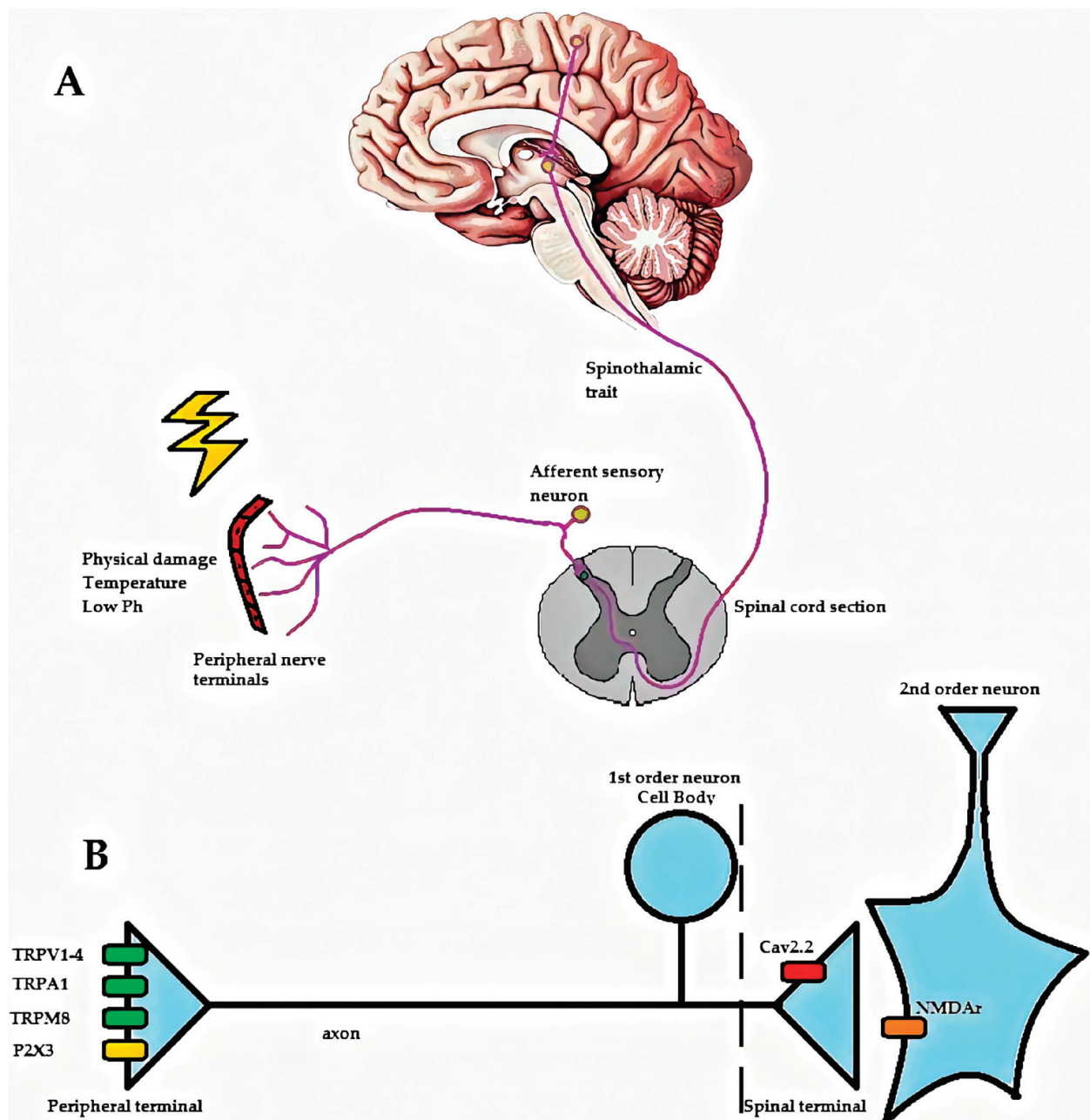
Figure 1. Afferent processing of nociceptive signals: (A) peripheral nerve terminals trigger action potential in response to harmful stimuli. Electrical potentials reach the spinal cord throughout axons from sensory afferent fibers. At the dorsal horn of spinal cord those afferent fibers make synapse with 2nd order neurons that project to thalamus and then to other brain areas to allow central processing of pain; (B) differential cellular distribution of distinct calcium permeable channels in the ascending pain pathway.

sensitivity. Nevertheless, other subtypes of VGCC's also contribute to this process but N-type channels are the most relevant and studied in this process. The propagation of action potential through thalamus depends on the summation of synaptic potentials received by the 2nd order neuron. Glutamate released on those synapses activate NMDA and AMPA receptors to allow calcium and sodium entry providing a rapid onset depolarization process, thus 3rd and consecutive neurons can be activated (**Figure 2**).
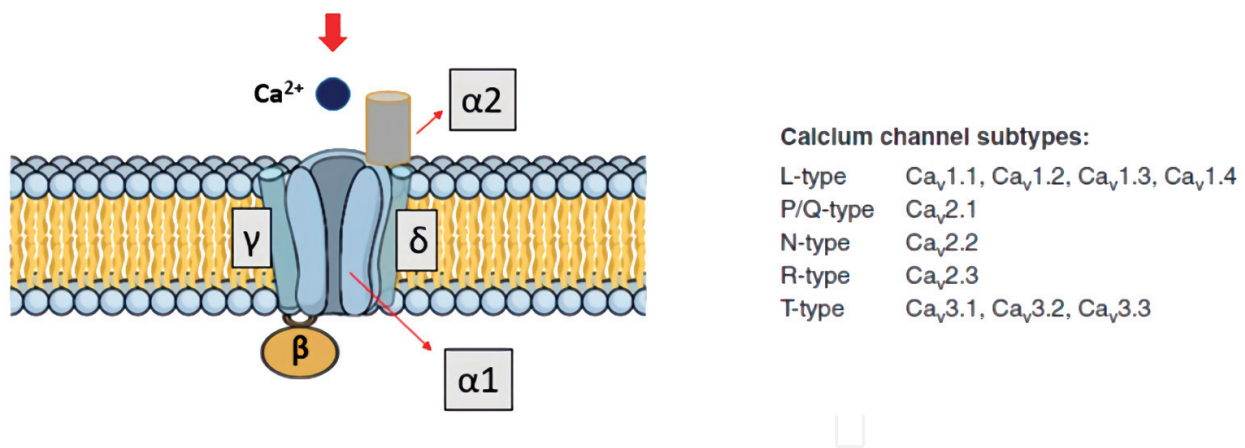
Figure 2. Overall molecular assembly and classification of a voltage gated calcium channels.

Normal pain has an evolutionary purpose of protecting us from harmful environments. Thus, the neurochemical cascade of normal pain relies on the proper function of calcium and other ion channels to allow a physiologic functioning of our sensorial system. However, when pain goes untreated, or when some pathological process goes embedded, maladaptive changes can occur. Ion channels may become differentially expressed, badly recycled, differentially phosphorylated or even unblocked by endogenous ions, therefore, activation thresholds are altered, or ion conductance is increased for a given input, so bioelectrical facilitation occurs with pain signals culminating in a state known as sensitization. The sensitization is present in several chronic pain conditions transforming pain in a worldwide public health problem whose treatment is largely inefficient and challenging. The precise role of calcium channels in the pain process is a very pursued scientific theme. The currently available tools for genetic and molecular studies are unraveling new putative targets that could be controlled in order to promote new options for better pain management.

This chapter presents an overview of current state of art of the knowledge on calcium-permeable channels associated to pain processing. Their divisions, classifications, and way of control are discussed in the view of signaling transmission in pain. Finally, we present how the current understanding of this theme may turn into therapeutic opportunities to treat pain.

2. The role of voltage-gated calcium channels (VGCC) in pain

As the name says, these channels are able to respond to variations in the electric field that trigger changes in its conformation, which allows these channels to transit between open or closed states [1]. Therefore, calcium can flow into the intracellular space at depolarized voltages within the peak of action potentials. In this topic, we will give an overview of the updated data involving VGCC's and how they contribute to the pain pathway. Specific details of molecular composition and classification are given in previews chapters of this book.

In resume, VGCC can be classified as L, N, P/Q, R and T and are distinguished by their different sensitivity for some pharmacological agents and their channel conductance kinetics based on their voltage activation properties. The VGCC classes can be further divided into two groups by

their voltage activation properties: the high-voltage activated type (L, N, P/Q and R-type) and the low voltage activated the T-type channel [2, 3]. Besides their biophysical and pharmacological features, different calcium channel isoforms show distinct cellular and subcellular distributions to fulfill specific functional roles. These diverse functional roles ultimately pose a challenge when drawing new calcium channel modulators with low risk of adverse effects.

Several evidences suggest that some subtypes of VGCC have greater involvement in pain pathways than others. The action potentials of neurons are able to reach the central terminals of a sensory neuron, activating voltage-gated calcium channels and allowing calcium influx in the cell, which triggers synaptic vesicle exocytosis containing the neurotransmitters. In sensory neurons of the pain pathway, neurotransmitters such as glutamate, substance P, and CGRP are released after activation of Ca^{2+} channels, mainly the L, N and P/Q type [4]. Due to their prominent role in pain processing signals, Calcium channels are considered important targets for the treatment the treatment of pain [5].

2.1. N-type ($Ca_{V2.2}$)

This channel is a hetero-oligomeric complex consisting of α_{1B} β, and $\alpha_2\delta$ subunits (**Figure 2**). N-type channels are present in synaptic terminals of the dorsal horn of spinal cord and in DRG neurons [5]. These channels are not restricted to pain pathways; they are in fact widely distributed in the central nervous system. Some studies show that these channels are proportionally more expressed in small than in large sensory neurons. One of the important functions of N-type is the control of neurotransmitter release. A region in the linker between domains II and III of N-type channel forms a binding site for the proteins coupled to the membrane, allowing the release of neurotransmitters [6, 7].

In animal models of neuropathic pain, N-type channel has been shown to underlie significant changes in their levels and composition. N-type channels are considered the main targets for the development of new analgesics. Studies have shown that knockout mice for N-type channels display higher thresholds for pain perception when compared to their wild-type [8]. The calcium channel blocker known as ziconotide shows proven clinical efficacy against pain when administered intrathecally [9].

2.2. L-type ($Ca_{V1.1}$ $Ca_{V1.2}$ $Ca_{V1.3}$ $Ca_{V1.4}$)

L-type $Ca_{v1.2}$ and $Cav_{1.3}$ have been reported to be up or down regulated in DRG of neuropathic pain [10]. Studies on the antinociceptive effect of L-type calcium channel blockers combined to opioids have reported significantly higher antinociceptive effect [11]. In sensory neurons, L-type Ca^{2+} channels appear to be involved in nociception since nifedipine, a L-type blocker, inhibits the release of substance P induced by inflammation [12].

2.3. P/Q-type ($Ca_{V2.1}$)

The $Ca_V2.1$ subunit drives both P-type and Q-type currents. This channel is expressed in Purkinje and Granular cells but is not restricted to neurons only. In glutamatergic and GABAergic synapses the P/Q currents are essential for the release of neurotransmitters [13].

P/Q-type have an important role in the regulation of neurotransmitter release at central neurons. Although the role of P/Q-type calcium channels in migraine is well established, the participation of these calcium currents in pain signaling is much less understood. The Nagoya mutant mouse carries a loss of function mutation in P/Q channels and shows reduced inflammatory pain phenotype. Although complete deletion of P/Q channels leads to hyposensitivity to neuropathic pain, they paradoxically show increased thermal acute nociception. Indeed, mice that completely lack P/Q presents motor deficit that compromises normal life spam. Therefore, although P/Q may contribute to pain signaling, they have a much more limited role than N-type and T-type channels.

2.4. T-type ($Ca_{V3.1}$, $Ca_{V3.2}$, $Ca_{V3.3}$) low voltage

T-type calcium channels evoke secretion from the neuroendocrine cells and are capable of associating with the synaptic vesicle release machinery. These channels are activated by membrane potentials close to the resting potential, with low threshold; its inactivation is rapid and reactivation requires a strong hyperpolarization. Due to their hyperpolarized activation range T-type is notoriously associated with the regulation of neuronal excitability. Their main role is probably in the rhythmic action potentials of muscle cells and neurons [14]. T-type channel can be found at dorsal horn of spinal cord, in various subpopulations of primary afferent neurons suggesting, thus, a role of these channels in pain signaling. The activity of T-type is increased in the afferent fibers in chronic pain conditions, such as traumatic nerve injury, metabolic nerve diabetic neuropathy or toxic neuropathies induced by chemotherapy [15]. Conversely, Ethosuximide, a T-type channel blocker, produce analgesia in pain models in rodents.

2.5. R-type ($Ca_{V2.3}$)

R-type channels contribute to neurotransmitter release at certain synapses and are strongly involved in memory and neuronal learning [16] but are also linked to the regulation of neuronal excitability in a number of neuronal subtypes including DRG neurons. Cav2.3 channels contribute to pain signaling mechanisms; however, the exact roles of these channels remain to be clarified. These channels are present in the somatosensory neurons of the peripheral ganglia, implying them as components of the pain pathways. Genetic research approaches confirmed this, with R-type knockout mice exhibiting reduced pain perception [8]. Like N-type channels, R-type are upregulated in neuropathic pain associated with nerve damage. SNX-482 is a synthetic peptide derived from the venom of the tarantula *Hysterocrates gigas* that specifically blocks R-type channels. Intrathecal administration of SNX-482 causes analgesia in models of neuropathic pain [17].

3. TRP family and pain

TRP channels represent an extended protein family consisting of more than 30 distinct subtypes of channels. TRP channels were firstly characterized on the *Drosophila melanogaster* eye, in which they act depolarizing the photoreceptor cells in response to light. These channels have characteristics of

polymodal activation since temperature changes, pH alterations and chemicals (ex. Capsaicin) can activate those channels. Once activated, calcium and sodium flow from the extracellular space through these channels to convert the stimuli into locally spreading membrane depolarizations, propagating action potentials to the spinal cord and higher brain centers.

Since the cloning of the first vanilloid receptor (TRPV1), six subfamilies have been described: vanilloid (TRPV), canonical (TRPC), melastatin (TRPM), ankyrin (TRPA), polycystin (TRPP), and mucolipin (TRPML) [18]. The members of the above superfamily participate in the molecular mechanisms of pain signaling by acting as transducers of harmful thermal, mechanical and chemical stimuli, for review see [19]. TRP channels are found not only in neurons but also in a wide variety of cell types, including smooth muscle, epithelial, and immune cells. Since this book chapter is focused on calcium channels and pain, special attention will be given for the role of TRP channels mainly those expressed in sensorial neurons. Therefore, we will discuss the role of TRPV1, TRPA1, TRPV4, and TRPM8 in more details since they are the most well characterized so far regarding their role in the pain pathway. For a more broad view of all TRP members and their respective functions see [20].

Although TRP channels share little similarity between subfamilies, they exhibit a similar membrane topology. Four subunits are required to form a TRP functional channel. Each subunit contains six membrane-spanning helices (termed S1–S6) as well as a pore-forming loop between S5 and S6 that enables the distinct cation selectivity and permeability among TRP channels. Details of the structural and biochemical characterization of TRP channels are presented in previews chapters from this book.

In addition to their pivotal role in the transduction of harmful stimuli into membrane depolarization, a growing number of evidence have been shown that these channels are regulated by pro-inflammatory mediators, such as, serotonin, bradykinin, prostaglandins, proteases, chemokines, and growth factors, confirming how essential these channels are for the sensitization of the afferent pain pathway [21]. Given that these channels are of tremendous importance in somatosensory perception, their dysregulation, as well as the increased expression and sensitivity, is often associated with inflammatory and neuropathic pain [22].

3.1. TRPV1 channel

The first characterized nociceptive TRP channel was the transient receptor potential vanilloid type 1 (TRPV1) that was cloned in 1997 using an expression-cloning screening strategy (138). TRPV1 is a cation permeable channel that is expressed in nociceptive fibers and is responsible for the detection of noxious stimuli from the periphery such as low pH, temperature rises (>42°C), osmolality changes, arachidonic acid metabolites, second inflammatory messenger and capsaicin (irritant compound of the chili). TRPV1 channel expression has been reported in small to medium neurons, widely expressed in the central and peripheral nervous system, gastrointestinal tract, bladder epithelium and skin [23]. Because of locating pattern as well as activating properties, the TRPV1 receptors have a key role in the pain transmission.

Topically applied capsaicin and related vanilloid compounds produce burning pain by depolarizing specific subsets of C and Aδ nociceptors due to TRPV1 expression on those fibers.

Type II Aδ nociceptors have a much lower heat threshold, but a very high mechanical threshold. The activity of this afferent almost certainly mediates the "first" acute pain response to noxious heat. The unmyelinated C fibers are also heterogeneous. Like the myelinated afferents, most C fibers are polymodal, that is, they include a population which is sensitive to both heat and mechanical stimuli [24]. Those of greater interest are the heat-responsive, but mechanically insensitive, unmyelinated afferents (so-called silent nociceptors) that develop mechanical sensitivity only in the setting of injury [25].

Studies have shown that other inflammatory mediators such as prostaglandin E2 (PGE2) trigger sensitization of TRPV1 channels via phosphorylation, leading to development of thermal hyperalgesia [26]. Conversely, the lack of such sensitization in TRPV1-knockout mice provides genetic evidence for the idea that TRPV1 is a key component of the mechanism through which inflammation produces thermal hyperalgesia [27]. The interaction result in a deep decrease in the channel's thermal activation threshold, as well as an increase in the magnitude of responses at suprathreshold temperatures—the biophysical equivalents of allodynia and hyperalgesia, respectively.

TRP channels are activated or positively modulated by phospholipase C-mediated cleavage of plasma membrane phosphatidylinositol 4,5 bisphosphate (PIP2). Of course, those are many downstream consequences of this action, including a decrease in membrane PIP2, increased levels of diacylglycerol and its metabolites, and increased cytoplasmic calcium, as well as consequent activation of protein kinases. In the case of TRPV1, the most, if not all, of these pathways have been implicated in the sensitization process.

Nevertheless, TRPV1 modulation is one of most relevant to tissue injury-evoked pain hypersensitivity, particularly in the development of inflammation. This would include conditions such as sunburn, infections, rheumatoid or osteoarthritis, and inflammatory bowel disease. Another interesting example includes pain from bone cancer, where tumor growth and bone destruction are accompanied by tissue acidosis, as well as the production of cytokines, neurotrophins, and prostaglandins that can, altogether modulate TRPV1 to cause sensitization.

Several studies have proposed a fundamental role of TRPV1 in hypersensitivity states that result from tissue inflammation, including thermal and mechanical hyperalgesia [28]. Due to its high expression in nociceptors, TRPV1, therefore, blockers of TRPV1 have been shown to have analgesic properties. However, while capsaicin is able to produce central and peripheral sensitization associated with secondary hyperalgesia, prolonged or repetitive administration of capsaicin locally on the epidermis results in channel desensitization, a condition that can result in analgesia [29]. Surprisingly, attempts to develop TRPV1 antagonists have been less successful than the use of TRPV1 agonist such as capsaicin. Given the role of this channel in the regulation of body temperature [30], most of the antagonists tested in preclinical and human studies presented hyperthermic side effects [31].

3.2. TRPA1 channel

TRPA1 receptor was originally identified and cloned by Jaquemar et al. in 1999 [32]. In both humans and rodents, TRPA1 is expressed in a subpopulation of small-diameter peptidergic

nociceptors of the dorsal root, nodose, and trigeminal ganglia, along with TRPV1. TRPA1 channel is expressed in vagal and primary afferent fibers innervating the bladder, the pancreas, the heart, the respiratory tract, and the gastrointestinal tract [33].

This channel is activated by a variety of noxious stimuli, including cold temperatures, pungent natural compounds, and environmental irritants as menthol, mustard oil, wasabi and horseradish [34]. TRPA1 is a major effector of the known proinflammatory mediator bradykinin, which elicits sensory neuron excitation *ex vivo* and hyperalgesia *in vivo* [35]. TRPA1 is also modulated indirectly by proalgesic agents, such as bradykinin, which act by PLC-coupled receptors. Studies have evidenced that TRPA1-deficient mice show dramatically reduced cellular and behavioral responses to all of these agents, as well as a reduction in tissue injury-evoked thermal and mechanical hypersensitivity [36].

Genetic evidence in humans also point toward a role of TRPA1 in pain signaling. A recent study described a gain-of-function mutation in humans suffering from episodic pain syndromes. This autosomal dominant mutation occurs in the fourth transmembrane domain of TRPA1 and generates normal pharmacological profile of this receptor but increases inward current at resting potentials. Since cold temperature is a trigger of enhanced pain perception in that human cohort study, the authors confirm the role of the TRPA1 channel as a noxious cold sensor as well as an irritant sensor [37].

Phα1β, the peptide purified from the venom of the armed spider *Phoneutria nigriventer*, which previously has been shown to exhibit antinociceptive effects [38, 39], and its recombinant form (CTK 01512–2) have now been identified as selective and potent TRPA1 channel antagonist with antihyperalgesic effects in a relevant model of neuropathic pain [40]. These findings, in addition to the reinforcing the role of TRPA1 channels in pain transmission, suggest Phα1β and CTK 01512–2 as novel strategies for the treatment of painful conditions where TRPA1 channels might be involved.

3.3. TRPV4 channel

TRPV4 has been considered the main molecular candidate for sensing osmotic changes, pressure, and shear stress in neurons and muscle tissue thus contributing to pain transduction associated to these stimuli [41, 42]. TRPV4 regulates intracellular calcium signaling, temperature sensing, osmotic and mechanic transduction, as well as maintenance of cell volume and energy homeostasis [43]. It is present in various cell types, including endothelial and epithelial cells, chondrocytes, and adipocytes. For being expressed in DRG and trigeminal ganglia neurons, has suggested a role in pain responses to mechanical stimuli in somatic tissue and visceral organs [33]. Like TRPV1 and TRPA1, TRPV4 is also activated by polyunsaturated fatty acids. Metabolites of arachidonic acid activate TRPV4 by an indirect mechanism involving the cytochrome P-450, therefore, suggesting a role of TRPV4 in inflammatory associated sensitization process.

3.4. TRPM8 channel

TRPM8 channel is considered as the primary sensor of cold in mammalian. TRPM8 channels are present in 10% of small DRG and trigeminal ganglia neurons, is activated by temperature

of below 25°C and by agents as menthol and eucalyptol and that do not express the classical markers of nociceptors such as TRPV1 and CGRP, suggesting that TRPM8 is a cold thermosensor for non-noxious temperatures [44]. However, studies have reported that acute activation or inhibition of TRPM8 can have analgesic effects either on visceral or neuropathic pain [45, 46].

4. Ionotropic glutamate receptors

Numerous ions channels of many types of receptors, including the ionotropic glutamate receptors, contribute to the detection and processing the pain signals. The function of these channels is detection the information from primary afferent neurons of mechanical and chemical insults, generation of action potentials, regulation of neuronal firing patterns, provide the initiation of neurotransmitter release at the dorsal horn synapses and the ensuring activation of spinal cord neurons that project to pain centers in the brain. The ionotropic glutamate receptors are involved in the mechanisms underlying peripheral and central sensitization and are important for pain sensation and pain perception. Changes in channel expression and function of NMDA receptors are thought to contribute to chronic pain states.

NMDA and AMPA receptors are expressed in secondary sensory neurons of the spinal cord, some interneurons and in neurons of supraspinal central nervous system. The ionotropic glutamate receptors include three pharmacologically and genetically distinct receptor types, named N-methyl-D-aspartate receptor (NMDA), α-amino-3-hydroxy-5-methilixazole-4-propionic acid receptor (AMPA) and kainate receptors.

At the molecular level, seven homologous genes code for NMDA subunits and are categorized into three major classes: GluN1/NR1, (Grin 1), GluN2/NR2 (Grin 2A, Grin2B, Grin2C, Grin2D) and GluN3/NR3 (Grin 3A, Grin 3B). The GluN1 subtype is essential for NMDA function and is expressed in the majority of the central nervous system, while differential expression of Glu2 subtype variants accounts for differences in the functional properties of NMDA receptors. A typically functional NMDA receptor contains two GluN1 and two GluN2 genetically encoded subunits (for review see [28]).

Glutamate and aspartate are the principal excitatory neurotransmitters that act on postsynaptic ionotropic glutamate receptors in response to noxious stimulation [47]. As the most studied inotropic glutamate receptor in pain is the NMDA receptor, it will be described in more details. NMDA receptors represent the most recognized postsynaptic source of calcium rise in the neurons, regulated by many kinases, phosphatases, and other enzymes. Moreover, NMDA activation is the major component of inflammatory and neuropathic pain. These receptors have independent mechanisms for facilitating excitatory and boosting synaptic transmission and, in this way, are important in the pain system [48].

The pore of NMDA receptor is permeable to monovalent cations such as sodium and potassium and divalent cations including calcium. Activation of NMDA receptors requires the binding of L-glutamate and glycine as an obligatory co-agonist. However, at physiological resting membrane potential, the pore is largely blocked by extracellular magnesium ions
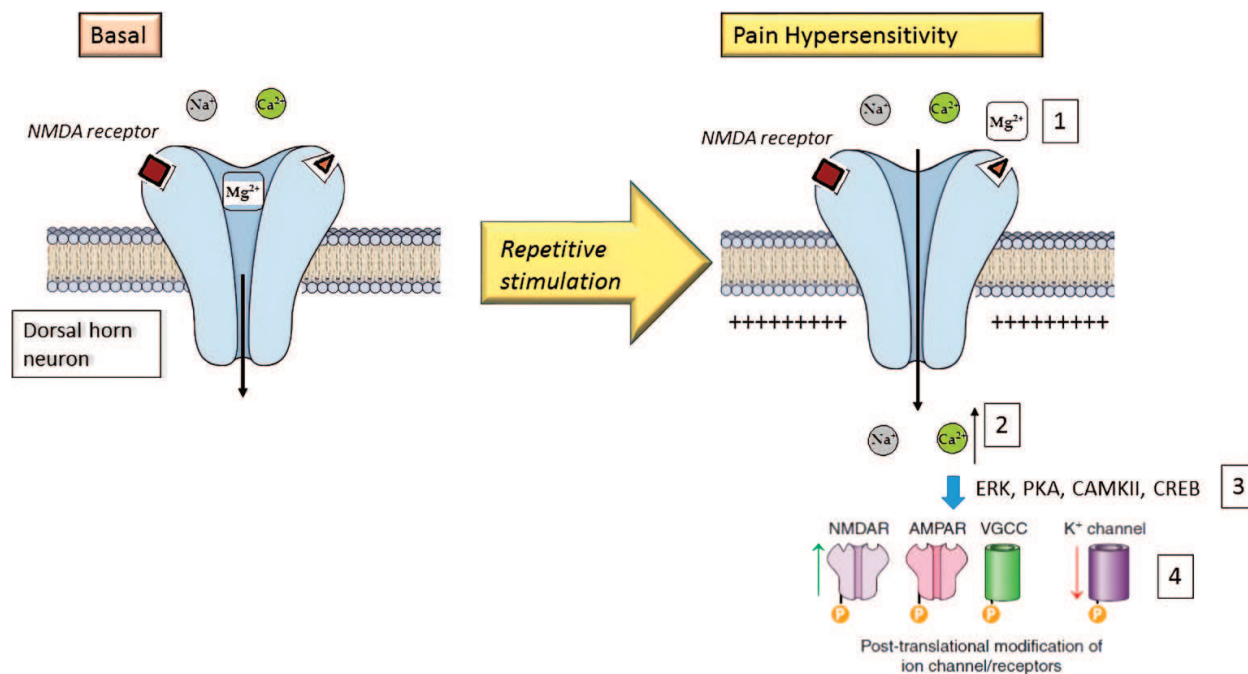
Figure 3. NMDA receptor activation and dorsal horn neuronal windup. Left: under basal physiological conditions, NMDA receptor is in a non-potentiated, with reduced sodium and calcium inward currents due to the blockage of magnesium ions from the extracellular space. Right: repetitive stimulation from pre synaptic afferent inputs causes relief of Mg^{2+} blockage (1). Calcium elevation in cytosol (2) activates kinase cascades including ERK, PKA, CAMKII (3) that leads to an upregulation in the activity of excitatory ion channels through posttranslational modifications (4) culminating in persistent changes in dorsal horn excitability.

(**Figure 3**). The cumulative depolarization produced during central sensitization leads to a relief of NMDA receptors blockade by magnesium causing an increase in intracellular calcium, synaptic depolarization and transmission and neuronal excitability. The process known as windup is the initial activity-dependent event that increases the synaptic responses and triggers central sensitization. Wind up is a form of physiological pain characterized by a successive increase in the output of a dorsal horn neuron produced by repetitive noxious stimuli [28].

Windup and Cumulative depolarization activates convergent signaling cascades from NK1, G protein-coupled metabotropic receptors (mGluR) and tyrosine kinase receptors, all present in the superficial dorsal horn leading to suppression of magnesium blockade of NMDA channels and enhance NMDA channels gating and function [48]. In dorsal horn neurons, NMDA receptors are known to be regulated by AMPA channel and unregulated by tyrosine kinase family (Src). During central sensitization, the Src enhance the NMDA receptor function raising intracellular calcium and activating de calcium/calmodulin-dependent kinase II (CaMKII) and protein kinase C (PKC) [48].

Downstream to the Mg^{2+} unblock of NMDA receptors, the flow of sodium and calcium ions through NMDA receptors leads to depolarization of the synaptic membrane and facilitate the excitatory postsynaptic potentials (EPSPs) and cause an increase of intracellular calcium concentrations. The intracellular calcium causes activation of kinases, including protein kinase A (PKA), protein kinase C (PKC) and extracellular signal-regulated kinase (ERK). The

NMDA-dependent activation of these kinases can, in turn, increase the excitability of voltage-gated-calcium channels as well as inhibit the voltage-gated-potassium channels (for review, see [28]). Furthermore, calcium entry through AMPA receptors may also contribute to the downregulation of inhibitory glycinergic synaptic function in spinal cord neurons culminating with even more facilitation of the ascending of pain signals. **Figure 3** shows the activation of NMDA receptors.

Central sensitization is a result from activity-dependent changes in spinal neuronal function and involves both long-term potentiation of individual synapses as well as the increased excitability of neurons within the spine cord dorsal horn [48]. Most of the excitatory input to pain pathway neurons is subthreshold, and increased gain results in the recruitment of these inputs to the output of the neurons, causing them to fire to normally ineffective inputs. These changes constitute central sensitization and are responsible for pain produced by low threshold afferent inputs and the spread of hypersensitivity to regions beyond injured tissue [48]. As it has been stated, NMDA receptors are considered the most validated molecular player responsible for sensitization process at the spinal cord.

5. Therapeutical opportunities

The prominent role of calcium permeable channels in the pain pathway represents the opportunity of controlling those channels for improving pharmacotherapeutic pain management. To date, several analgesic agents exert their effects by functionally interacting with calcium channels. The most well-recognized strategies that are already in use comprehends pore inhibition of voltage-gated calcium channels—with focus on N-type channels; inhibition of VGCC's by binding on $\alpha_2\delta$ auxiliary subunit; inhibition or desensitization of vanilloid receptors mainly TRPV1 and inhibition of NMDA receptors. Although there is an ever-growing number of substances discovered to act on the above targets, the number of those with use approved for humans is still small.

Ziconotide (Prialt™) was developed and approved as a first-in-class synthetic version of ω-conotoxin MVIIA, a peptide blocker of $Ca_{V2.2}$ channels. It was approved by US Food and Drug Administration and European Medicines Agency for the management of severe chronic pain associated with cancer, acquired immune deficiency syndrome (AIDS) and neuropathies—refractory to other current pain medications. Despite the clinical efficacy of ziconotide to treat severe pain, its impracticalities of intrathecal administration, low therapeutical index, and severe neurotoxic effects have restricted its use to rare circumstances. Ziconotide is the first and unique drug, so far, derived from an animal toxin to be approved for use in pain management, in humans [49].

The consecutive need for a more favorable ratio of anti-nociception to side effect has led to the discovery of new conotoxins that already translated from basic bench to bed side research (for review, see [50]). CVID is another ω-conotoxin with remarkable analgesic actions. AM-336 (leconotide) is the synthetic version of CVID that has been tested as a therapeutic agent and has completed phase II clinical trial. Compared with ziconotide, CVID has improved specificity for

N-type VGCCs showing, thus, improved efficacy and fewer cardiovascular side effects. Similar to CVID, CVIE and CVIF are ω-conotoxins capable of blocking N-type channels and completely and reversibly relieve mechanical allodynia in rodent models of neuropathic pain. Due to their inherently large size and hydrophilic nature, peptides, in general, are unable to cross the blood-brain barrier. Therefore, the methods for administering ω-conopeptides are limited to intrathecal delivery. Therefore, alternative strategies are needed, for example, the use of nonpeptidic small molecules that has limited systemic degradation. Another strategy is the development of state-dependent channel blockers who preferentially inhibits the calcium channel when they are in activated state which appears in highly active pain fibers. A few numbers of state-dependent blockers is currently under development, for review see [51], but all of them are still in a pre-clinical phase of testing.

Several pharmaceutical companies have T-type voltage gated calcium channels on their list of targets to manage pain. It has been shown that T-type channels are expressed in a subset of primary afferent neurons and have been implicated in synaptic release in the spinal cord suggesting a role of these channels in pain processing. Consistent with this idea, systemic or intrathecal administration of ethosuximide of mibefradil (T-type calcium channel blockers) mediates analgesia in rodents [52]. Indeed, in the recent years, a new generation of both state-dependent and state-independent T-type blockers appeared (ex. TTA-P2, TTA-A2, and Z123212) and both mediate analgesia in rodent models of pain [14]. Near future will show if clinical drugs emerge.

The most frequently prescribed calcium channels modulation for neuropathic pain are the gabapentinoids. These drugs were initially designed to perform as analogues of GABA and originally indicated for the treatment of epilepsy. However, it becomes empirically evident that this class of drugs is very efficient in attenuating pain in postherpetic neuralgia, diabetic neuropathy, and fibromyalgia. Thus, initial off-label use of gabapentinoids turns into approved used for palliative care neuropathic pain states. The main clinically used gabapentinoids include gabapentin and the newer derivative pregabalin. Gabapentinoids were identified as ligands for the auxiliary voltage-gated calcium channel subunit $\alpha_2\delta$ although they also bind to GABA receptors but with lower affinity. The interaction of gabapentinoids with the $\alpha_2\delta$ subunit is required for the antinociceptive activity of gabapentinoids given the lack of correspondent efficacy in null mice [53]. By binding to $\alpha_2\delta$ subunit, gabapentinoids acts as inhibitors of $\alpha_2\delta$ subunit-containing VDCC's therefore inhibiting neurotransmitter release. At the cellular level, it is unclear how gabapentinoids inhibit neurotransmitter release. It is suggested they inhibit axonal trafficking of $\alpha_2\delta$ subunit and thus the recycling of calcium channel complexes, which is elevated in injured primary afferents.

Blocking N-methyl D-aspartate (NMDA) receptors inhibits the wind-up phenomenon of spinal dorsal horn neurons. Given this phenomenon is a key event in the pain sensitization process, NMDA ionophore antagonists have been shown to have potent attenuating effects in pain states, though not approved, in humans. Numerous NMDA antagonists have been employed for preclinical work. These include channel blockers such as ketamine, and memantine [54]. NMDAR antagonists e.g. ketamine and dextromethorphan, are generally effective in patients with neuropathic pain such as complex regional pain syndrome and painful diabetic neuropathy [55]. Main observed side effects of these antagonists include neuronal toxicity and

profound psychotomimetic effects. Then, current usage of these class of drugs to treat pain is off-label usage.

TRP channels are also promising targets for drug discovery. The initial focus of research was on TRP channels that are expressed on nociceptive neurons. Indeed, a number of potent, small-molecule TRPV1, TRPV3, and TRPA1 antagonists have already entered clinical trials as novel analgesic agents but this is a rapidly expanding and changing field. Although there has been considerable excitement around the therapeutic potential of this channel family since the cloning and identification of TRPV1 channels as the capsaicin receptor more than 20 years ago, only modulators of a few channels have been tested clinically. TRPV1 channel antagonists have suffered from side effects related to the channel's role in temperature sensation. Paradoxically, high dose formulations of capsaicin have reached the market and shown therapeutic utility. A number of potent, small molecule antagonists of TRPA1 channels have recently advanced into clinical trials for the treatment of inflammatory and neuropathic pain, and TRPM8 antagonists are following closely behind for cold allodynia. Other TRP channels such as TRPV3, V4, and TRPM2 have also attracted significant attention [56].

6. Conclusions

The calcium flow throughout ion channels in the membrane of sensory neurons convey information about how pain signals are transduced, transported and interpreted by the nervous system. Regarding the gating control of calcium channels, the voltage-gated (mainly N-type) and the ligand-gated (mainly vanilloid receptors and NMDAR) are the most well characterized to show a close association with pain processing. The normal function of these channels helps to control pain in their primitive purpose that is the protection from harmful environment. However, maladaptive changes of these channels (eg. altered expression levels or modulation by intracellular phosphorylative cascades) may end up with chronification or even exacerbation of pain signals transforming it into a public health problem. Although the majority of clinical trials are disappointing so far, a progressive approach to clinical trials designs with calcium channels modulators will be key to the success of future therapeutic approaches. Alternative approaches include the rational search for drug combination regimens applying calcium channels modulators associated with other drugs. Concurrently, basic research may also help to identify novel targets, for example, splice variants of calcium channels that have a more specific role in pain processing. Therefore, new target-specific drugs could also improve the efficacy and toxicity profiles for pain management.

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Conflict of interest

All the authors declare they have no conflicts of interest.

Appendices and nomenclature

TRP   Transient receptor potential

VGCC   Voltage gated calcium channels

NMDAR   N-methyl D-aspartate receptor

GABA   Gamma aminobutyric acid

DRG   Dorsal root ganglion

ERK   Extracellular signal regulated kinase

PKA   Protein kinase A

CAMK   Calcium calmodulin kinase

EPSP   Excitatory postsynaptic potentials

PLC   Phospho lipase C

CAPES   Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

FAPEMIG   Fundação de Amparo a Pesquisa de Minas Gerais

Author details

Célio Castro-Junior*, Luana Ferreira, Marina Delgado, Juliana Silva and Duana Santos

*Address all correspondence to: celiojunior@santacasabh.org.br

Neuropharmacology Laboratory, Institute for Education and Research, Hospital Santa Casa, Belo Horizonte, MG, Brazil

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