Calcium channel functions in pain processing

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Voltage-gated calcium channels (VGCC) play obligatory physiological roles, including modulation of neuronal functions, synaptic plasticity, neurotransmitter release and gene transcription. Dysregulation and maladaptive changes in VGCC expression and activities may occur in the sensory pathway under various pathological conditions that could contribute to the development of pain. In this review, we summarized the most recent findings on the regulation of VGCC expression and physiological functions in the sensory pathway, and in dysregulation and maladaptive changes of VGCC under pain-inducing conditions. The implications of these changes in understanding the mechanisms of pain transduction and in new drug design are also discussed.

Introduction

Pain information processing starts from activation of peripheral nociceptors, causing action potentials to propagate along the primary afferent nerve fibers into sensory neurons in dorsal root ganglia (DRG). They are further relayed to the spinal dorsal horn primary afferent nerve fibers into sensory neurons in dorsal root ganglia (DRG). They are further relayed to the spinal dorsal horn through the central axons of sensory neurons. The action potentials reaching the central terminals of sensory afferents can cause membrane depolarization, activation of voltage-gated calcium channels (VGCCs), and calcium influx, which triggers synaptic vesicle exocytosis. This then leads to the release of excitatory neurotransmitters including glutamate, pain-inducing peptides such as substance P and calcitonin gene-related peptide (CGRP) into the synaptic cleft. These neurotransmitters can then cause activation of post-synaptic dorsal horn projection neurons and interneurons, leading to spinal modulation of sensory signals. Certain types of VGCC can also regulate the excitability of DRG primary sensory neurons and dorsal horn neurons. In addition, VGCCs may contribute to ascending and descending modulation of sensory signals. Thus, changes in expression and functions of VGCCs in pain-inducing conditions can be potential targets for chronic pain management.

VGCCs can be classified based on their voltage activation characteristics as high- or low-voltage activated channels. The VGCCs can be further subdivided based on their structural similarities of the channel-forming α₁-subunit (Ca₁.1, Ca₁.2, Ca₁.3) or their sensitivity to blockade by pharmacological agents (L, N, P/Q, R and T-type). Collectively, the high-VGCCs include L-(Ca₁.1, Ca₁.2, Ca₁.3, Ca₁.4), P/Q-(Ca₂.1), N-(Ca₂.2) and R-(Ca₂.3) type channels, while the low-VGCCs include T-type (Ca₃.1, Ca₃.2, Ca₃.3) channels. The high-VGCCs typically form heteromultimers that consist of the channel-forming α₁-subunit along with auxiliary β₁-, α₂δ and γ-subunits.¹ Even through conclusive findings are not yet available, data from co-expression and electrophysiological recording experiments support that the low-VGCCs seem to be α₁-subunit monomers.²

So far, ten α₁-subunits have been identified in mammals and are encoded by distinct genes.¹ This subunit is also subjected to alternative splicing.³,¹¹ The α₁-subunit consists of four homologous domains (I–IV), each having six transmembrane helices (S1 through S6), which together form the calcium conduction pore, voltage sensors and gating apparatus.¹² The S4 transmembrane domain contains positive charged amino acids for voltage sensing. There are four known β-subunits (β₁ through β₄), which are intracellular proteins that enhance cell surface expression of the α₁-subunits and modulate the gating properties through their interactions with the channel-forming α₁-subunit and intracellular signaling molecules.¹³-¹⁵ Four α₂δ-subunits have been identified (α₂δ-1 through α₂δ-4), each consisting of two disulfide-linked peptides (α₂ and δ) that are encoded by the same gene.¹⁶,¹⁷ Similar to the β-subunit, α₂δ subunits promote and stabilize cell surface expression of VGCCs.¹⁸,¹⁹ Eight γ-subunits have been identified and appear to act as glycoproteins with four transmembrane segments, but the exact function of the γ-subunit is not well defined.²⁰,²¹ Together, the auxiliary subunits modulate the functional properties of the α₁-subunit.

The biophysical properties of the channel-forming α₁-subunit and tissue-specific distribution of VGCCs play a critical role in governing different pathophysiological functions of VGCCs.¹ Regulation of VGCC function by various signaling molecules and pathways adds another level of control. In addition, alternative splicing could control the coupling of VGCCs to signaling pathways and ultimately their functions.³,⁶,⁸,¹¹,¹²,²⁷ For example, Ca₁.1 VGCCs and Ca₂.2 VGCCs are regulated by protein phosphorylation and G-proteins, respectively.⁸ Ca₃.3 VGCCs are regulated by various kinases and G-protein pathways as summarized by recent reviews in references 21 and 29. Based on the functional diversity, tissue specific distribution and coupling to different signaling pathways, these VGCCs play distinct roles in physiological processing of sensory information. In addition, they could become the target of maladaptive neuroplasticity under pain-inducing conditions, leading to the development of...
sensory hyperexcitability and behavioral hypersensitivity (pain). Therefore, correcting VGCC dysregulation and maladaptive changes in the sensory pathway represents an attractive approach for the development of therapeutic agents for tailored pain management under different pain-inducing etiologies. Accordingly, the focus of this review is on our recent understanding of VGCC regulation and function in the sensory pathway under normal and pain-inducing conditions and the potential contributions of changes in VGCC expression/function to pain processing.

L-type Voltage-gated Calcium Channels

L-type channels are widely distributed in the central nervous system, cardiac muscles, smooth and skeletal muscles, retina, sinoatrial node and cochlear hair cells. In the superficial dorsal horn, L-type channels are expressed mainly on neuronal cell body and dendrites, mediating the activation of calcium-dependent enzyme activities, gene transcription, synaptic signaling and plasticity, as well as the activation of other ion channels such as calcium-activated potassium channels. Membrane depolarization with high voltages results in prolonged activation of L-type channels due to slow inactivation kinetics, which lead to extended calcium influx over a long period of time. Increased intracellular calcium in neuronal cell body and dendrites can lead to subsequent alterations in dorsal horn neuron excitability due to calcium-dependent activation of signaling pathways, receptors/ion channels and altered gene transcription. As a consequence, these changes can lead to enhanced excitability of dorsal horn projection neurons, excitatory interneurons, and/or reduced excitability of inhibitory interneurons, which can cause behavioral hypersensitivity, leading to increased pain perception.

Four different isoforms exist for L-type channels (Ca_{1.1–1.4}), with Ca_{1.2} and Ca_{1.3} being strongly expressed in neurons. The biophysical properties of Ca_{1.3} differ from other isoforms, in that they are activated faster and at more negative membrane potentials. Thus, Ca_{1.3} can contribute to spontaneous neuronal firing. Dysregulation of Ca_{1.3} under pain-inducing pathological conditions could thus potentially contribute to enhanced dorsal horn neuron hyperexcitability. The regulation of L-type channel functions in neurons by intracellular signaling molecules has been reviewed recently. It has been shown that regulation of L-type channel α_{1B}-subunits by cAMP-dependent protein kinase or protein kinase A can enhance current activity. In contrast, both calmodulin and calcium binding proteins can regulate calcium-dependent inactivation and calcium-dependent facilitation of Ca_{1.3} channels.

In neuropathic pain models, L-type channels are shown to be dysregulated in DRG and the spinal cord. For example, Ca_{1.2} and Ca_{1.3} are downregulated in rat DRG neurons following chronic constriction injury of the sciatic nerve, and Ca_{1.2} is upregulated in the spinal cord post spinal nerve ligation in a manner that is correlated with behavioral hypersensitivity. Ca_{1.2} channels are also expressed within the anterior cingulated cortex, and may be involved in fear learning and behavioral pain responses. Knockdown of the Ca_{1.2} L-type channel via intrathecal peptide nucleic acid based anti-sense strategies and small interfering RNA (siRNA) reverses dorsal horn neuron hyperexcitability and behavioral hypersensitivity in the spinal nerve ligated neuropathic pain model, supporting that Ca_{1.2} dysregulation may contribute to chronic pain maintenance. These findings contradict previous findings showing that intrathecal administration of L-type channel blockers (verapamil, diltiazem and nimodipine) had no effect on pain behaviors in neuropathic pain models derived from spinal nerve ligation, diabetic and vincristine-induced neuropathies. These discrepancies may be due to Ca_{1.1} subtype selectivity of the agents.

L-type channels are also implicated pharmacologically in morphine-induced analgesia and chronic tolerance. Administration of L-type channel antagonists nifedipine and verapamil enhances morphine analgesia and attenuates the development of morphine tolerance, which is in agreement with the notion that increased calcium entry is associated with morphine tolerance. Chronic nimodipine and morphine co-administration has synergistic effects in reducing morphine antinociception effects. However, there are some discrepancies regarding the contribution of VGCC subtypes to morphine analgesia and tolerance in biochemical studies. Co-administration of nimodipine and morphine leads to a decrease in Ca_{1.2} and an increase in Ca_{2.2} channel expression while chronic morphine administration causes an increased expression of both channels in the superficial dorsal horn. Western blot data from another study demonstrate a decreased level of Ca_{1.3}, but not Ca_{1.2} and Ca_{2.2} channels in brain stem after chronic morphine treatment. It is likely that morphine analgesia and tolerance are mediated by distinct VGCC subtypes at different locations, which might be distinguished by local treatment with subtype-selective pharmacological agents. Identifying VGCC subtypes underlying morphine analgesia and tolerance with other means would be critical for further advancement in the field.

P/Q-type Voltage-gated Calcium Channels

Ca_{2.1} P/Q-type channels are expressed at the pre-synaptic terminals in the spinal dorsal horn and may play a role in neurotransmitter release (reviewed in ref. 49). Ca_{2.1} is encoded by a single gene, and P- and Q-type channels differ in their ω-agatoxin IVA sensitivity and inactivation kinetics. It is hypothesized that alternative splicing of the Ca_{2.1} gene results in the phenotypic variants of P- and Q-type channels, and P-type channels derive from post-translational modifications or modulation of putative proteins.

The involvement of P/Q-type channels in pain processing is not well understood. P/Q-type channels show little colocalization with substance P and treatment with ω-agatoxin IVA, a specific inhibitor of P/Q-type channels, has no effect on the release of either substance P or CGRP from peptidergic sensory neurons. This suggests that P/Q-type channels are not involved in the release of these pain-inducing neurotransmitters from primary afferents. Instead, P/Q-type channels are highly expressed in dorsal horn laminae II-VI pre-synaptic terminals, where polysynaptic inputs exist. It has been suggested that P/Q-type channels may be involved in the release of excitatory
and inhibitory transmitters in spinal dorsal horn. Data from electrophysiology studies have revealed that ω-agatoxin IVA has a minimal effect on monosynaptic C- and Aδ-fiber inputs, but a strong effect on polysynaptic nociceptive transmission. Together, findings from these studies suggest that P/Q-type channels are likely localized on interneurons and play a role in modulating synaptic transmission in the spinal dorsal horn.

In animal models, data regarding the role of Ca$_{2.1}$P/Q-type channels in pain processing are not consistent. In neuropathic pain models, intrathecal administration of ω-agatoxin IVA to block P/Q-type channels has no effect on mechanical allodynia and thermal hyperalgesia. Deleting Ca$_{2.1}$ in mice results in no change in nociceptive responses to non-injurious noxious thermal stimuli. These data suggest an anti-nociceptive role of P/Q-type channels. However, mice with spontaneously occurring P/Q-type channel mutations show decreased sensitivity to nociceptive stimuli, and deletion of Ca$_{2.1}$ in mice results in reduced nociceptive responses in inflammatory and neuropathic pain models. For visceral pain states, Q-type channels are found to be important for acute bladder nociception at the spinal level.

Together, it is likely that the role of P/Q-type channels on pain processing depends on the etiology of nociception.

**N-type Voltage-gated Calcium Channels**

Ca$_{2.2}$ N-type channels are highly concentrated in neuronal cells including those involved in sensory signal processing such as spinal dorsal horn neurons, dorsal root ganglion cell bodies and their central terminals that form synaptic connections with spinal dorsal horn neurons. The colocalization of these channels with pain-inducing neurotransmitters and functional blockage of substance P, CGRP and glutamate release by N-type channel antagonists suggest a major role of the N-type channel in controlling synaptic transmission in pain processing, especially in C- and Aδ-nociceptors. It has been reported that Ca$_{2.2}$ N-type channels are upregulated in spinal dorsal horn during the initiation and maintenance stages of pain states after peripheral nerve injury. Thus, blocking synaptic transmission via N-type channels could serve as a prime target for reducing pain signal transmission to the central nervous system.

Small peptide inhibitors of N-type channels from cone snail toxins have been used to study the functional roles of these channels in pain processing. ω-conotoxins MVIIA (SNX-111, ziconotide or Prialt) and GVIA were used to examine their interaction with specific gating states of N-type channels. Originally, ω-conotoxins are thought to block the N-type channel pores completely by binding to amino acid residues just outside the pore to diminish calcium influx, which may lead to adverse side effects. Recent studies, however, show that ω-conotoxin GVIA is able to modulate the gating properties of N-type channels, thereby leading to a reduction in action potential-induced calcium influx by ~50% without blocking the pore. In addition, ω-conotoxin GVIA binding can destabilize the open state and alter gating transitions between closed states of N-type channels, which can reduce pain-inducing neurotransmitter release in the dorsal horn. This is in contrast with findings that ω-conotoxin MVIIA blocks all gating states. This discrepancy may derive from the differences between the secondary binding sites for these toxins. Thus, modulation of N-type channels by ω-conotoxin GVIA and its derivatives may provide a proof of concept for the development of new state-dependent N-type VGCC blockers for pain management.

Omega-conotoxin MVIIA (Ziconotide or Prialt, Elan Pharmaceuticals Inc., San Diego, CA) is the first N-type channel antagonist approved by the US Food and Drug Administration and European Medicines Agency for management of chronic severe pain refractory to other current pain medications. Data from pre-clinical studies have shown that intrathecal administration of ω-conotoxin MVIIA inhibits hyperalgesia and allodynia in neuropathic and inflammatory pain models. Due to the wide-distribution of N-type channels and the peptidergic nature of the drug, its application is limited to intrathecal delivery (reviewed in ref. 71). A recent study introduced a new small molecule, N-triazole oxindole TROX-1, an inhibitor of Ca$_{2.2}$ N-type channels that can be administered orally. TROX-1 is able to reverse inflammatory-induced hyperalgesia and nerve-injury induced allodynia to the same extent as current anti-inflammatory and neuropathic pain drugs. A substantial effort has been made towards developing small molecule N-type channel blockers that may be efficacious in pain management post systemic administration (reviewed in ref. 73).

Modulation of N-type channels for sensory information processing can occur through voltage-dependent inhibition by the Gβγ subunit of G-proteins, and voltage-independent inhibition by protein tyrosine kinase in DRG neurons. Inhibition of N-type channel currents upon Gβγ binding can be reversed by protein kinase C-dependent phosphorylation of the Gβγ binding site of the N-type VGCC. Interestingly, activation of μ-opioid receptors results in inhibition of N-type channels through Gβγ subunits. Similar activation of ORL1 (opioid receptor-like 1) receptors (also known as nociceptin receptors) has also been shown to inhibit N-type channels through a G-protein mediated mechanism in the absence of the ligand nociceptin. In addition, ORL1 receptors have been shown to heterodimerize with μ-opioid receptors and associate with N-type channels, resulting in internalization of N-type channels. By doing so, the ORL1 receptor appears to act as a physical link between μ-opioid receptors and N-type channels to modulate opioid receptor mediated regulation of channel activity and trafficking. Prolonged exposure to the ORL1 receptor agonist nociceptin leads to protein kinase C-dependent internalization of N-type channel complexes and consequently downregulated calcium entry, a regulatory means that could have significant implication in controlling N-type channel functions in sensory pathway.

Identification of alternative splicing of the N-type VGCC α$_{3}$-subunit, such as exon37a and exon37b splice variants, may lead to improvement of drug specificity for modulating N-type channel activity in pain processing. Both exon37a and exon37b are mutually exclusive and encode 32 amino acids of the proximal c-terminal region of the N-type channel that differ by 14 amino acids. Exon37a has been shown to be almost exclusively expressed in capsaicin-sensitive nociceptive DRG neurons.
and support increased N-type current densities. Specifically, exon37a containing channels remain open longer upon activation compared to those containing exon37b. Silencing exon37a via siRNA in vivo reduces basal thermal nociception and development of thermal and mechanical hyperalgesia during inflammatory and neuropathic pain states. Voltage-dependent G protein inhibition of N-type channels is indistinguishable between exon37a and exon37b isoforms. However, exon37a appears to confer a greater susceptibility to voltage-independent inhibition of N-type channel currents through a mechanism involving Gi/o subunits and kinase-dependent phosphorylation. A tyrosine encoded within exon37a, but not exon37b, acts as a molecular switch in controlling N-type channel current density and voltage-independent inhibition that ultimately leads to modulation of nociception. Furthermore, exon37a regulates the extent of μ-opioid receptor-mediated inhibition of N-type channels, and the absence of exon37a results in reduced morphine-induced analgesia without affecting basal response to noxious thermal stimuli.

R-type Voltage-gated Calcium Channels

Cα,2.3 R-type channels are classified as being “resistant” to inhibitors of other high-voltage-activated L-, N-, P- and Q-type channels. The R-type channels are found in neuronal cells and may play a role in regulating neurotransmitter release and neuronal excitability. CaV2.3 channels have been suggested to contribute to pain transmission by regulating both nociceptive and anti-nociceptive behaviors through spinal and supraspinal mechanisms as shown in mutant mice lacking the Cα,2.3 R-type channels. Data from a recent study have shown that SNX-482, a selective R-type channel antagonist from tarantula venom, inhibits Cα and Aδ-fiber-mediated dorsal horn neuronal responses and neuropathic pain states in nerve-injured rats, suggesting that R-type channels may contribute to central sensitization in the spinal cord during neuropathic pain processing. This is supported by data from a tissue injury model in which SNX-482 treatment increased behavioral sensitivity during the second phase of formalin-induced pain response but produced analgesic effects in the second phase of the formalin-test, which is considered a centrally mediated nociceptive response.

T-type Voltage-gated Calcium Channels

Unlike Cα,1 and Cα,2 channels, Cα,3 T-type channels activate at hyperpolarized levels, close to resting potentials (low voltage thresholds). The T-type channels are expressed in tissues throughout the body, including the heart, smooth muscles, pancreas, kidney and neuronal tissues. It appears that T-type channels consist of α1-subunits that do not associate with auxiliary subunits. Sensory pathways, T-type channels are located on primary afferent terminals and dorsal root ganglia, with Cα,3.2 being the most abundant isoform in the DRG, and thus the T-type channel subtype capable of the most prominent role in nociception. Mechanisms underlying T-type channel regulation and neuronal functions have been reviewed recently. Data from electrophysiology studies suggest that T-type channels are involved in shaping action potentials, regulating neuronal firing patterns, lowering action potential thresholds, promoting burst firing, oscillatory behavior and enhancing synaptic excitation. T-type channel activation close to the resting potential allows calcium influx in response to sub-threshold synaptic input when the cells are at rest, and enhances the neuronal excitability by boosting synaptic inputs and lowering the threshold for high-threshold spike generation. In addition, CaV3 T-type channels form complexes with low-voltage-activated A-type potassium channels, allowing the potassium channels to regulate neuronal firing at a subthreshold membrane potential range. Thus, blocking the T-type channel can lead to overall reduction of neuronal excitability.

Data from animal studies suggest that functional contribution of T-type channels to pain processing varies based on their modalities, subtypes and anatomical locations. Increased T-type channel currents are found in small DRG neurons following chronic constriction injury of the sciatic nerve, but in medium-size DRG neurons following chemical-induced diabetic neuropathy. These changes may lead to increased excitability (lowered activation threshold) of sensory neurons that can contribute to the pathological pain responses such as mechanical allodynia and thermal hyperalgesia observed in both models. T-type channels are implicated in the development of chronic musculoskeletal pain syndromes, as mice deficient in Cα,3.2 fail to develop acid-induced chronic mechanical hyperalgesia. Moreover, mice lacking Cα,3.2 show a hypoalgesic response to acute, somatic, visceral and tonic inflammatory insults, altogether suggesting that Cα,3.2 T-type channels play a pro-nociceptive role in processing of noxious stimulation. This is further supported by data indicating that Cα,3.2 expression is increased in DRG in diabetic neuropathy and neuronal nerve injury models, and knock down of Cα,3.2 by siRNA or anti-sense oligonucleotides results in anti-nociceptive effects in these pain models. In addition, Cα,3.1 deficient mice show a reduction in nerve injury-induced behavioral hypersensitivity, suggesting that Cα,3.1 may also be a contributor to neuropathic pain processing. However, Cα,3.1 deletion in mice leads to an increase in visceral pain, similar to what is observed following thalamic infusion of T-type channel blockers. This suggests that Cα,3.1 T-type channels in the thalamus are anti-nociceptive.

Since T-type channels are subjected to dysregulation under some pain-inducing conditions, normalizing dysregulated T-type channels thus represents an attractive alternative strategy in developing novel pain medications. It has been reported that inhibiting T-type channels using non-selective T-type channel antagonists such as ethosuximide and mibefradil, effectively blocks and reverses both tactile hypersensitivity and thermal hyperalgesia in pain models. These non-selective antagonists inhibit input spikes, indicative of diminished synaptic activity, probably through a reduction in exocytosis of neurotransmitter from primary afferent neurons. Furthermore, Cα,3.2 T-type channel-dependent activation of extracellular signal-regulated kinase (ERK) in the anterior nucleus of paraventricular thalamus correlates with acid-induced chronic hyperalgesia, and inhibiting ERK activation in...
wild-type mice prevents chronic mechanical hyperalgesia.\textsuperscript{106} The endogenous reducing agent, L-cysteine, selectively and potently enhances T-type channel currents and promotes cutaneous thermal and mechanical hyperalgesia. In contrast, the oxidizing agent, 5,5’-dithio-bis-(2-nitrobenzoic acid), inhibits T-type channel currents in small dorsal root ganglia and alleviates hyperalgesia in pain models.\textsuperscript{115,116} Hydrogen sulfide, a gasotransmitter, has also been implicated as a neuromodulator in sensory transmission by activating Ca\textsubscript{\textalpha}\textsubscript{2},3,2 channels in primary afferents and in spinal nociceptive neurons, thus leading to sensitization of nociceptive processing and hyperalgesia.\textsuperscript{117} Treatment with inhibitors of T-type channels or of cystathionine-\gamma-lyase (CSE), an enzyme involved in hydrogen sulfide formation, causes a reversal of hyperalgesia and allodynia in spinal nerve injured rats that show an upregulation of Ca\textsubscript{\textalpha}\textsubscript{2},3,2, but not CSE, in injured DRG. This suggests that endogenous hydrogen sulfide may activate or sensitize elevated T-type channels that contribute to neuropathic pain maintenance.\textsuperscript{108} The Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} subunit of high VGCC is the binding site for gabapentin and pregabalin, another class of drugs with pain relief properties in preclinical and clinical studies. These gabapentinoid drugs are not calcium channel blockers, but may play an important role in normalizing VGCC malfunctions in disease states such as pain and epilepsy. The direct actions of gabapentinoids on ion channels are reviewed by Dr. Osvaldo Uchitel in this issue.\textsuperscript{108} Recently, a panel of experts recommended these gabapentinoid drugs as the first line treatment for neuropathic pain conditions.\textsuperscript{109} Even though these drugs were designed to mimic the structure of the inhibitory neurotransmitter \gamma-aminobutyric acid (GABA), later studies have found that their actions are not mainly mediated through the GABAergic system (reviewed in ref. 120). Since expression of Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} proteins is dysregulated in DRG and spinal dorsal horn in neuropathic pain models,\textsuperscript{121,125} uncovering their contributions to pain processing and the actions of these drugs in pain relief is critically important. Data from biochemical studies have supported that increased expression of this subunit leads to increased calcium channel currents in DRG neurons, dorsal horn neuron hyperexcitability and behavioral hypersensitivity. Gabapentin can normalize these abnormal behaviors without affecting normal activities in the control groups. This supports that dysregulation of Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} proteins in the sensory pathway contributes to dorsal horn neuron hyperexcitability and pain processing, and that the actions of gabapentin in pain relief are mediated by normalizing these maladaptive changes.\textsuperscript{126} In contrast, upregulated Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} proteins may interfere with the antinociceptive effects of \alpha-conotoxins.\textsuperscript{128} At the peripheral level, it is noteworthy that calcium currents are actually reduced in DRG neurons from nerve-injured rats that show DRG Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} upregulation and behavioral hypersensitivity.\textsuperscript{123,129-131} This contradicts data from Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} overexpressing transgenic mice in which increased calcium currents in isolated DRG neurons correlate with behavioral hypersensitivity.\textsuperscript{126} This discrepancy may be due to additional modulatory effects from other injury factor(s) that are missing in the Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} overexpressing mice (without injury). At the spinal level, it appears that elevated Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} mediates behavioral hypersensitivity through enhanced excitatory pre-synaptic input that activates glutamate receptors at post-synaptic dorsal horn neurons.\textsuperscript{131} The mechanism of this neuroplasticity in pain processing is not yet clear, but data from recent studies have suggested that injury-induced increase of Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} in DRG leads to increased trafficking of Ca\textsubscript{\textalpha}\textsubscript{2}\textdelta\textsubscript{1} proteins to the pre-synaptic terminals that may cause increased VGCC expression and VGCC-mediated neurotransmission.\textsuperscript{121,122,139,134} This process may ultimately lead to abnormal synaptogenesis.\textsuperscript{135,136} Since both processes are sensitive to blockade by gabapentinoids,\textsuperscript{121,133,136} normalizing these cellular maladaptive changes in pain-inducing conditions may underlie a chronic mechanism of gabapentinoid drugs in pain relief.

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### Summary

VGCCs are widely expressed and distributed throughout the body and play an obligatory role in important physiological functions. The specificity of VGCC functions derives from tissue-specific distribution of VGCC subtypes, their coupling to unique intracellular signaling pathways and interactions with other proteins, that are critical in mediating cellular functions. Preclinical data have implicated the involvement of dysregulation and/or malfunctions of VGCC subtypes in pain processing. This includes, but is not limited to, changes in expression and regulation of VGCC subtypes/subunits, and alterations in functional interactions of VGCC subtypes with other proteins or cellular molecules/co-factors that directly or indirectly modulate abnormal VGCC functions in pain pathways. Thus, normalizing these pathological maladaptive changes in different pain-inducing etiologies represents an attractive approach for designing the next generation of pain medications that should be more target-specific and have fewer side effects. This could also be achieved by modifying existing VGCC drugs to improve their efficacy and toxicity profiles. As discussed in a recent review in reference 137, some of these drugs are in the pipeline and our better understanding of VGCC functions in pain processing will eventually lead to more promising pain medications.

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