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Is There a Role for T-Type Calcium Channels in Peripheral and Central Pain Sensitization?

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Abstract

Following tissue injury, both peripheral and central sensory neurons can become hyperexcitable, or "sensitized." Sensitization can lead to long-term pathological changes in pain sensation. Because many chronic pain conditions are refractory to most currently available treatments, there is great interest in identifying molecular targets that contribute to the sensitization of sensory neurons. Among these, several classes of ion channels have emerged as potential targets. Recent in vitro and in vivo studies have demonstrated a role for T-type Ca^{2+} channels in sensory pathways and have suggested that these channels may contribute to pain processing and sensitization. Therefore, T-type channels may represent an opportunity for the development of novel pain therapeutics and may help to address an unmet medical need.

Index Entries: Dorsal root ganglion; dorsal horn; hyperalgesia; neuropathic; nociceptors; sensitization.

Introduction

Pain experienced as a result of acute tissue injury is often termed nociciceptive pain. This type of pain serves an important, protective role by alerting organisms to the presence of potentially harmful stimuli. However, damage to afferent neuronal pathways can also result in long-term changes in sensation, such as a loss of the ability to perceive pain, spontaneous pain, or heightened pain sensitivity. Conversely to nociceptive pain, these chronic pain conditions often persist past the time of their physiological usefulness. When individuals are in a heightened state of pain sensitivity, they often respond to normally innocuous tactile or thermal stimuli in a painful fashion (allodynia) and to normally painful stimuli in an exaggerated fashion (hyperalgesia). The physiological correlates of these heightened pain responses, collectively termed peripheral

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sensitization, include spontaneous neuronal activity, a lowered threshold for neuronal activation, and an increased frequency of neuronal firing in response to suprathreshold stimuli. Additionally, peripheral sensitization can, in some instances, lead to central sensitization, which refers to an increased synaptic efficacy of dorsal horn neurons in the spinal cord as a result of, and outlasting, a barrage of nociceptor input, tissue damage, or injury to nociceptive or non-nociceptive afferent fibers *(1)*. Both peripheral and central sensitization can lead to pain hypersensitivity and are characteristic of many pain pathologies—especially neuropathic pain (pain caused by a primary lesion or dysfunction in the nervous system). However, the molecular and neurobiological mechanisms underlying the initiation and maintenance of sensitization are less clear. Recent electrophysiological, genetic, and behavioral studies have suggested a previously unrecognized contribution of low-voltage-activated (T-type) Ca^{2+} channels to both peripheral and central sensitization and have suggested that these channels may influence pain processing under various physiological and pathophysiological conditions.

Peripheral Sensitization

A large portion of the evidence for T-type channel involvement in peripheral sensitization comes from electrophysiological and behavioral studies on redox modulation of these channels in primary nociceptors. Both the synthetic reducing agent dithiothreitol (DTT) and the endogenous reducing agent L-cysteine (L-cys) significantly increase T-type currents in acutely dissociated nociceptors of the dorsal root ganglion (DRG) *(2,3).* Furthermore, reducing agents have been shown to sensitize a unique subpopulation of capsaicin-positive DRG neurons that also express a high density of T-type currents (termed T-rich cells) *(3).* T-rich cells also stain positively for the B_4-isolection (IB_4) from *Griffonia simplicifoilia*, a well-described histological marker of nociception *(3*). Although their capsaicin and IB_4 responsiveness suggests that T-rich cells contribute to nociception, it is also interesting to note that these cells display a relatively short-duration action potential, which has traditionally been associated with non-nocieptive DRG fibers in sharp-electrode studies *(4).* However, other groups *(5,6)* have shown that many subpopulations of DRG cells with short-duration action potentials also express immunohistological markers (e.g., substance P, calcitonin gene-related peptide, IB_4) and electrophysiological responses to algesic substances such as adenosine triphosphate (ATP), acid, capsaicin, and noxious heat that are relatively specific to nociceptors. These diverse observations underscore the complexity of the peripheral sensory system and the subspecialized nature of distinct nociceptor populations.

Reducing agents also induce mechanical and thermal hyperalgesia when injected into the peripheral receptive fields of both healthy and neuropathic rats in vivo *(2,7).* Conversely, oxidizing agents such as DTNB (5,5´-dithio-*bis*-[2-nitrobenzoic acid]) block T-type currents and decrease nociceptor excitability in vitro and induce analgesia to acute and neuropathic pain in vivo (Fig. 1; refs. *2,3,* and *7*). Redox modulation is a relatively common posttranslational modification and has been described for many ion channels, including high-voltage-activated (HVA) Ca^{2+} channels *(8),* Na^+ channels *(9),* K^+ channels *(10), N*-methyl-D-aspartate (NMDA) receptors *(11),* γ-aminobutyric acid (GABA) receptors *(12),* and the capsaicin receptor *(13).* Notably, however, the concentrations of redox agents that modulate T-type currents in nociceptors are an average of 1,000 to 5,000 (and in some cases 10,000–30,000) times lower than the concentrations reported to produce effects on other redox-sensitive ion channels. It has also been specifically demonstrated that the concentrations of DTT and L-cys effective in augmenting T-type currents in DRG nociceptors have no effect on other voltage- (HVA Ca^{2+}, Na^+, and K^+) and ligand-gated (capsaicin, heat, pH, and ATP) ion channels known to contribute to pain processing in the same cells.

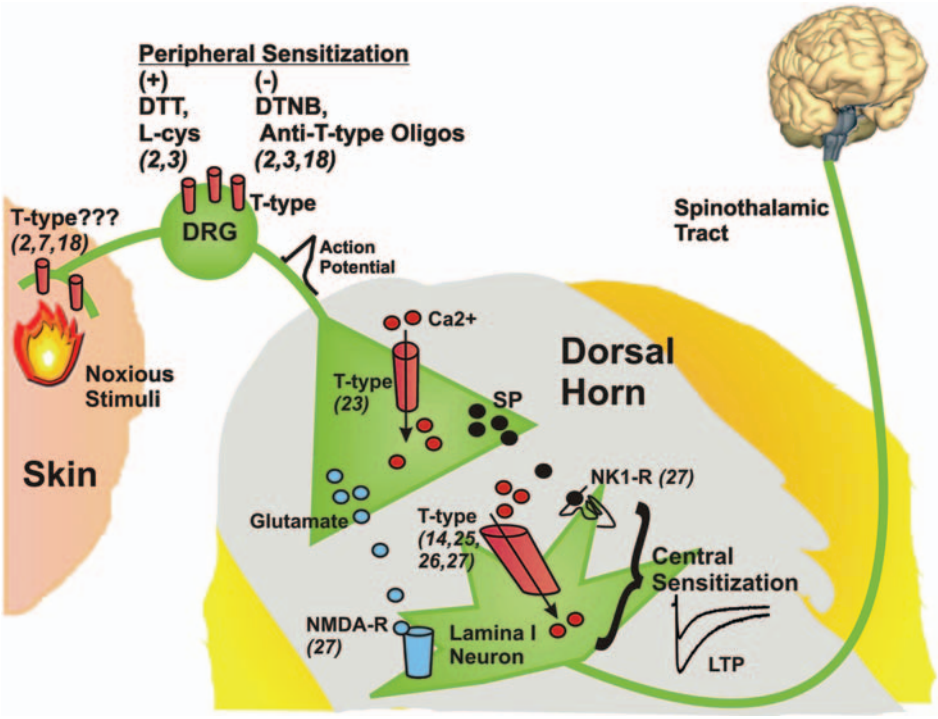

Fig. 1. Location of T-type Ca^{2+} channels in the pain pathway and their contribution to peripheral and central sensitization. Behavioral pharmacology evidence suggests putative T-type channels may contribute to the detection of noxious stimuli in the periphery, DRG T-type channels have been shown to participate in sensitization of nociceptors, limited evidence suggests presynaptic T-type channels may contribute to neurotransmitter release onto lamina I and II dorsal horn neurons, and postsynaptic T-type channels work in concert with NMDA and NK1 receptors to facilitate LTP of nociceptive synapses in the superficial dorsal horn. Reference numbers indicate supporting studies for each part of the model.

Additionally, the effects of redox agents on HEK cells expressing $Ca_V3.2$, the predominant T-type channel isoform expressed in DRG cells *(14)*, are identical to their effects on native T-type currents *(2)*.

Redox agents such as L-cys exist in blood and tissue at concentrations in a similar range to those that modulate T-type channels *(15,16)*. Therefore, it is possible that these agents are locally present and/or released at injury sites resulting in larger T-type currents and nociceptor sensitization via changes in channel gating parameters and/or gene and protein expression levels. However, this hypothesis has not been conclusively demonstrated. The observation that many redox agents are weakly or slowly membrane-permeable but their effects on T-type channels are very rapid *(2)* suggests the existence of putative extracellular redox sites located directly on T-type channels. However, additional studies are needed to establish this and to elucidate the exact molecular composition of the T-type channel redox site/s.

Similarly to reducing agents, serum from diabetic rats has been shown to increase T-type currents in DRG neurons. Ristic et al. *(17)* found that DRG cells isolated from healthy rats had T-type currents that were up to threefold larger when they were cultured for 18 to 24 h in media containing 10% serum from rats with diabetic neuropathy compared to serum from nondiabetic, age-matched controls. Although

the specific mechanisms responsible for the upregulation are not entirely clear, it is intriguing to speculate that conditions associated with chronic pain such as diabetic and chemotherapeutic neuropathy may result in the upregulation of T-type channels or the accumulation of endogenous modulators that enhance T-type currents and contribute to the severe sensitization that often accompanies these pain pathologies.

In addition to pharmacological studies, Bourinet et al. *(18)* found that genetic knockdown of T-type channels in small- and medium-size (presumably nociceptive) DRG cells using antisense oligonucleotides reduced peripheral sensitization to mechanical and thermal stimuli induced by chronic constriction of the sciatic nerve, a well-established neuropathic pain model. The authors also showed that intrathecal injections of anti-$Ca_v3.2$ antisense oligonucleotides, but not anti-$Ca_v3.1$ or -$Ca_v3.3$, resulted in an approx 80% decrease in T-type currents in DRG cells, with a concomitant decrease in acute pain thresholds as well as the aforementioned effects on neuropathic pain. These data further support a role for $Ca_v3.2$ T-type channels as boosting nociceptive signals in the periphery.

Conversely to these findings, other studies have documented no change *(19,20)* or a decrease *(21,22)* in the density of somatic T-type currents in medium-size DRG neurons following sciatic nerve injury. Although a clear reason for this discrepancy is not readily apparent, it is possible that putative T-type channels on sensory nerve endings may be differently remodeled compared to their counterparts in the neuronal soma following peripheral nerve injury. It is also possible that different cells expressing T-type currents have different remodeling responses to neuronal injury.

Finally, T-type channels have been implicated in mediating synaptic transmitter release from a subpopulation of DRG nerve terminals onto dorsal horn neurons in the superficial laminae (I and II) of the spinal cord *(23).* This is interesting because laminae I and II are the central termination sites for the majority of DRG nociceptors. These data indicate that conditions resulting in upregulated T-type currents and peripheral sensitization in nociceptors may also result in increased neurotransmitter release at the spinal synapse, a phenomenon that could potentially contribute to the induction of central sensitization.

Central Sensitization

T-type channels are present in a myriad of central nervous system neurons, where they are involved in the regulation of cell excitability *(24).* Numerous studies have revealed the presence of T-type channels in dorsal horn neurons of the spinal cord *(14,25,26,27),* but their role in spinal processing remains uncertain. Interestingly, several studies have described the interaction of dorsal horn T-type channels and the pro-inflammatory peptide substance P. Following the detection of noxious stimuli, some nociceptors release substance P from their central terminals in the spinal cord, where it can bind to NK_1 receptors on dorsal horn neurons and activate central pain pathways. Ryu and Randic *(26)* showed a significant increase in dorsal horn T-type currents following application of substance P. Additionally, more recently, Ikeda et al. *(27)* reported that NK_1-mediated signaling pathways, NMDA receptors, and T-type channels synergistically facilitated activity- and calcium-dependent long-term potentiation of nociceptive synapses in the superficial dorsal horn. This suggests that a novel form of T-type channel-mediated synaptic plasticity may contribute to the development of hyperalgesia and central sensitization in the dorsal horn. Additionally, the T-type currents in this study were sensitive to low concentrations of Ni^{2+}, indicating that similarly to DRG nociceptors, they most likely arise largely from $Ca_v3.2$ (Fig. 1; ref *24*).

There is also evidence implicating T-type channels in visceral sensitization. Kim et al. *(28)* showed that mice homozygous for a null mutation of the $Ca_v3.1$ T-type channel gene lacked burst-firing in thalamocortical relay neurons and displayed hyperalgesia to acute

visceral pain but showed no defects in peripheral nociception. The mutant mice showed enhanced pain behavior upon intraperitoneal injections of acetic acid and $MgSO_4$. Additionally, wild-type mice administered intrathalamic injections of mibefradil, a nonselective T-type channel blocker, mimicked the mutant phenotype. These data suggest that thalamic $Ca_v3.1$ T-type currents function to inhibit visceral nociception. The authors suggest that $Ca_v3.1$ channels in thalamic relay neurons are activated following repeated nociceptive influx from the viscera and subsequently function as sensory filters to inhibit the central processing of pain signals. These data present a role for thalamic $Ca_v3.1$ channels that is functionally distinct from the proposed role of peripherally located $Ca_v3.2$ channels and suggests that nociceptive processing may involve multiple T-type channel isoforms in various neurons, depending on the nature and location of the painful stimulus.

Conclusions

Sensitization is a common feature of many debilitating pain pathologies. It is a complex process that involves a diverse array of cells and subcellular components, including various voltage- and ligand-gated ion channels. Recent studies have indicated that T-type Ca^{2+} channels are present throughout the pain pathway and may contribute to both peripheral and central sensitization. Additionally, T-type channels are largely absent in non-nociceptive primary afferent fibers as well as motor neurons of the brain stem. Together, these observations suggest that pharmacological blockade of T-type channels may attenuate pain without significant effects on other sensory or motor modalities, which would be a distinct therapeutic advantage.

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