Regulation of T-Type Calcium Channels in the Peripheral Pain Pathway

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ABSTRACT
Recent evidence strongly suggests that both central and peripheral T-type Ca2+ channels enhance somatic and visceral nociceptive inputs, as well as that regulation of T-type Ca2+ channel function can result in significant changes of pain threshold in a variety of animal models. Therefore, T-type Ca2+ channels in peripheral and central pain pathways, although previously unrecognized, may have great importance as targets for developing new therapies against pain. This is particularly critical in cases in which currently available treatments are limited due to serious side effects or are not consistently effective (e.g., chronic neuropathic pain). In this review, we summarize recent studies of the regulation of T-type channels in peripheral sensory neurons by means of redox agents and neuroactive steroids, as well as studies of the function of these channels in the pathophysiology of neuropathic pain.

INTRODUCTION
Acute pain is often experienced as a result of injury to peripheral tissue, where putative nerve endings of primary sensory neurons are embedded. Pain signals are transmitted from the periphery to the dorsal horn of the spinal cord, the main pain-processing region of the central nervous system (CNS). From here, pain signals travel on to higher CNS centers such as the thalamus and cortex, where final pain processing occurs and responses aimed at avoiding harmful stimuli are initiated. In contrast to the acute (noxious) pain that alerts the organism, there are also different forms of pain that do not have a primarily protective role. For example, direct injury to neural elements can result in changes in sensory pathways such as spontaneous pain and/or increased pain sensitivity. When pain-sensing nerve cells (nociceptors) are in a sensitive state, they often respond to normally nonpainful sensory stimuli in a painful fashion (allodynia) and to mildly painful stimuli in an exaggerated fashion (hyperalgesia). The electrophysiological phenomena of these altered pain responses, collectively termed sensitization, include lower thresholds for activation, increased frequency of firing in response to suprathreshold stimuli, and spontaneous firing.1,4 While sensitization of nociceptors may be present in acute pain processing, it typically contributes to a variety of disorders of sensory neurons such as those involved in chronic painful neuropathies. In spite of the great clinical importance of increased pain sensitivity, the precise cellular mechanisms underlying sensitization are unclear.

It has been well established that T-type or low-voltage-activated (LVA) calcium (Ca2+) channels can modulate neuronal activity in the peripheral and central nervous systems, causing an increase in transmission efficiency.5,6 New evidence strongly suggests that in the peripheral sensory neurons this typically results in the amplification of sensory signals, increased afferent sensory transmission and, in particular, increased pain perception (nociception).

Although, in the past, much attention was focused on the regulation of high-voltage-activated (HVA)-type Ca2+ channels, the function of T-type channels in sensory processing and, in particular, pain processing was less appreciated. This was most likely due to the fact that T-type channels in sensory neurons are resistant to modulation by traditional analgesic compounds such as opioids. However, recent in-vitro electrophysiological studies and in-vivo pharmacological, molecular, and genetic studies have firmly established that T-type calcium channels have a previously unknown supportive function in acute nociceptive processing.7,9 Despite these provocative reports, the precise contributions of T-type channels to nociception and the sensitization of pain responses...
remain poorly understood. In this review, we will describe the most recent evidence linking modulation of T-type calcium channels to peripheral pain processing. We will focus on description of the T-type channels in different subpopulations of peripheral sensory neurons and on modulation of their function by various endogenous and exogenous compounds.

**CELLULAR BASIS OF T-TYPE CHANNEL EXPRESSION IN THE PERIPHERAL PAIN PATHWAY**

Carbone and Lux\(^{10}\) provided the first detailed description of the biophysical properties of T-type Ca\(^{2+}\) channels, using patch-clamp experiments on cultured sensory dorsal root ganglion (DRG) neurons of small size. It was found that this conductance differed from previously described HVA Ca\(^{2+}\) currents in that T-type currents exhibit faster kinetics of macroscopic voltage-dependent inactivation, slower kinetics of channel closing from fully activated states (deactivation), and lower voltages at which channels become activated. Soon after these initial biophysical studies, an interesting study linking the newly discovered T-type Ca\(^{2+}\) channels with increased excitability of DRG neurons was published by White and collaborators.\(^{11}\) They demonstrated that T-type channels provide the basis for after-depolarizing potentials (ADP) in a subpopulation of medium-size DRG neurons in rats. These potentials could be crowned with repetitive burst firing of action potentials similar to phenomena previously described only in CNS neurons of the thalamus and inferior olive.\(^{3}\) The biophysical properties of T-type currents in medium-size DRG cells were very similar to those described by Carbone except that T-type current density in medium-size DRG cells was several fold higher than that in small cells. This difference raised the question of whether T-type currents in medium cells have a more prominent function in the regulation of cellular excitability.

These unique kinetic features endow neurons that have T-type channels with a lower threshold for spike firing. For example, Figure 1 shows a family of inward calcium currents in an acutely dissociated rat sensory neuron of medium size, with inactivating T-type current peaking at about -30 mV. Figure 1B presents traces from current-clamp recording in a medium-size DRG cell in which injection of a short (1 ms) depolarizing current evoked subthreshold T-type channel-dependent ADP that follows a fast Na\(^+\)-dependent action potential spike. The lower panel of this figure indicates that when same protocol was repeated at membrane potential of -85 mV, which allows more T-type channels to be available, the ADP is substantially more prominent and it is crowned with another action potential.

The pore-forming \(\alpha_1\) subunit of all known voltage-gated Ca\(^{2+}\) channels consists of four domains; each of these domains is comprised of six transmembrane sections. The second transmembrane segment forms the walls of the pore; the selectivity filter is formed by the pore loops; and the S4 segment acts as the voltage sensor. Most of the channel is thought to be intracellular, with short extracellular loops. The exception to this rule is a large segment linking IS5 to the pore loop. Early molecular studies have shown that at least three isoforms of T-type Ca\(^{2+}\) channels exist in a variety of central and peripheral nerve tissues; these are named Ca\(_{\text{v}}\)3.1 (\(\alpha_1\)G), Ca\(_{\text{v}}\)3.2 (\(\alpha_1\)H), and Ca\(_{\text{v}}\)3.3. (\(\alpha_1\)I). Although all of the isoforms of T-type channels have similar kinetic features that distinguish them from HVA channels, some unique properties in channel gating, inactivation and deactivation differentiate these related channels.\(^{6}\) Further molecular analysis has indicated that mRNA for Ca\(_{\text{v}}\)3.2 (\(\alpha_1\)H) is the most abundant isoform of T-type channels in peripheral sensory neurons of small and medium size, while Ca\(_{\text{v}}\)3.1 (\(\alpha_1\)G) and Ca\(_{\text{v}}\)3.3 (\(\alpha_1\)I) isoforms are much less frequently present in these cells.\(^{12}\) Consistent with these findings, patch-clamp recordings from acutely dissociated small sensory neurons in mice lacking the Ca\(_{\text{v}}\)3.2 gene showed a complete lack of T-type calcium currents.\(^{13}\) Thus, it appears that the Ca\(_{\text{v}}\)3.2 (\(\alpha_1\)H) T-type calcium channel underlies the major T-type current in peripheral sensory neurons.

**Small DRG cells.** Several groups of investigators have reported that small (15–30 \(\mu\)m soma diameter) DRG cells express T-type currents of moderate density.\(^{7,14-18}\) Most small DRG cells represent the somas of classically described C-type (unmyelinated, slow-conducting) peripheral nociceptors.\(^{19}\) In vitro, these cells express the functional properties of nociceptors: wide action potentials; responses to capsaicin-, heat-, proton- and ATP-gated currents; and high-threshold mecano-sensory currents.\(^{7,17}\) Thus, it is likely that these T-channel-containing small DRG cells are polymodal nociceptors capable of responding in vivo to noxious heat, chemical, and mechanical stimuli.

Recent data indicate that T-type channels have an important function in enhancing the cellular excitability of small DRG cells by reducing the threshold for action potential firing and contributing to Ca\(^{2+}\) entry during action potentials.\(^{15,20}\) Thus, in spite of their modest expression in most small DRG cells, it appears that the presence of these channels could be sufficient to increase the probability of the firing of action potentials since these cells have high input resistance. Recently, this was directly demonstrated in current-clamp recordings in rats and mice using T-type channel modulators such as redox agents.\(^{20}\)

**Medium DRG cells.** The function of medium-size (30–40 \(\mu\)m soma diameter) DRG cells having prominent T-type currents is less clear. As noted earlier, studies using acutely dissociated rat DRG cells have demonstrated that some of them express robust T-type

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**Figure 1.** T-type calcium channels in sensory neurons modulate cellular excitability. (A) Shows traces of a family of inward T-type Ca\(^{2+}\) currents evoked in representative medium-size DRG cells from by voltage steps from -90 mV (V\(_\text{m}^{-}\)) to V\(_f\), from -60 through -20 mV in 10-mV increments. Note typical criss-crossing pattern of inactivation. (B) Depicts current-clamp traces from another medium-size DRG cells. Action potentials were evoked by applying 1 ms-long 3 nA depolarizing pulse. When cell was held at membrane potential of -70 mV, it fired one fast action potential followed by a small after-depolarizing potential (ADP). When cell’s membrane was hyperpolarized to -85 mV to remove inactivation of T-type channels, the ADP was more prominent and it caused repetitive action potential firing.
The Role of T-Type Ca\(^{2+}\) Channels in Pain

**MODULATION OF T-TYPE CHANNELS IN DRG CELLS AND ANALGESIA BY NEUROACTIVE STEROID ANALOGUES**

In spite of the fact that neuronal T-type channels were first discovered in DRG cells, progress in specifically defining their function in sensory transmission and nociception has been precluded by the lack of selective and potent pharmacological blockers and modulators of these channels. It has been found that certain general anesthetics that also have significant analgesic properties (e.g., nitrous oxide, laughing gas, N\(_2\)O) and anticonvulsant agents commonly used in the treatment of chronic pain (e.g., phenytoin) induce significant blockade of the native T-type currents in small DRG neurons and of recombinant Ca\(_{\text{v}}\)3.2 currents at clinically relevant concentrations. It also has been found, in acute thermal nociceptive tests,
that anticonvulsants such as ethosuximide and phenytoin, which block T-type channels in vitro, induce analgesia in normal rats. This suggests that T-type channels in nociceptive sensory neurons may contribute, at least in part, to the analgesic effects of anesthetics and anticonvulsants. However, the maximal effect of these agents on T-type currents is achieved at generally high concentrations, raising the question of the selectivity of these compounds.

Over the past several years, promising new agents have been discovered. In 1998, a report from our laboratory demonstrated that a novel neuroactive steroid with a 5α configuration at the steroid A,B ring fusion [(+)-ECN] is a potent, voltage-dependent, selective, and partial blocker of T-type channels in small DRG neurons in adult rats (IC50 of 300 nM, maximal peak current block of about 40%). This blocking effect is strongly enantioselective, with [(+)-ECN] being about 30-fold less potent in blocking DRG T-type currents. This strongly suggests that binding of the ECN molecule to the T-type channels requires a specific structural domain rather than a nonspecific membrane interaction.

Next, with a discovery of “T-rich” cells, an excellent model was available for use in testing the effects of steroids on the cellular excitability of nociceptors. Studies showed that at concentrations that maximally blocked isolated T-type currents, [(+)-ECN] effectively inhibited ADP and T-channel-dependent burst firing in current-clamp recordings from “T-rich” cells. More recently, this work has been continued by study of the function of 5α-reduced neuroactive steroids in modulating T-type channels in nociceptors in whole animals. It has been demonstrated that other structurally related molecules, including the endogenous compound allopregnanolone (3β5αOP) and the clinically used anesthetic alphaxalone can also potently inhibit T-type currents in vitro (IC50 of about 1 μM) and induce potent peripheral analgesia to thermal radiant stimuli when intradermally injected into the hind paws of normal rats in vivo. This important study found an excellent correlation between the ability of series of 5α-reduced steroids to inhibit T-type currents in DRG cells in vitro and to induce local analgesia to noxious heat in vivo. These results strongly suggest that the potent analgesic effects of 5α-reduced steroids in vivo can, at least in part, be attributed to their effects on T-type channels in small DRG nociceptors.

In addition, it was reported that another novel steroid, a voltage-dependent blocker of T-type channels in rat sensory neurons with a 5β configuration at the steroid A,B ring fusion (3βOH), is a promising tool in functional studies of DRG T-type channels in nociception. In vitro-experiments showed that 3βOH completely blocks T-type currents (near 100% block of peak current), with a reported IC50 of about 3 μM at holding potentials of -90 mV and an IC50 of less than 1 μM at holding potentials -70 mV. Like [(+)-ECN], 3βOH in 15–20-fold higher concentrations, has little effect on voltage-gated channels that influence the excitability of nociceptors, among them HVA Ca2+ channels and voltage-gated Na+ and K+ channels. This and other 5β-reduced steroid analogues displayed excellent correlation coefficients in their action on T-type channels in vitro, as well as high correspondence in their degrees of analgesia to noxious heat as determined by thermal radiant testing in vivo (reviewed in detail). In current-clamp recordings, it has been found that 1 μM of 3βOH completely blocks ADPs and burst firing in “T-rich” nociceptors, providing a possible functional link between the inhibition of T-type channels in vitro and analgesia in vivo with 5β-reduced steroid molecules.

These in vitro and in vivo experiments have demonstrated that both 5αt and 5β-reduced neuroactive steroids are selective blockers of neuronal T-type Ca2+ channels and that, indeed, they may be much-needed tools for study of the functions of T-type channels in peripheral nociceptors. Future structure-activity studies of neuroactive steroids that are T-type channel blockers may lead to the development of novel pain therapies.

**REDOX REGULATION OF T-TYPE CHANNEL FUNCTION IN PERIPHERAL NOCICEPTORS IN VITRO AND IN VIVO**

Although the function of several ion channels, including HVA Ca2+ channels, in the pain pathway can be modulated by posttranslational modification, T-type channels were generally considered to be resistant to such modulation. However, work in our laboratory has demonstrated that these channels in small DRG neurons can be selectively and potently modulated by various reducing and oxidizing agents, including the endogenous thiol-containing amino acid, L-cysteine. Indeed, when we injected these agents into peripheral receptive fields of sensory neurons that caused, in parallel, profound effects on in-vivo thermal and mechanical nociception. This finding, for the first time suggested that DRG T-type channels have an important capacity to boost peripheral pain signals.

In vitro, native T-type currents in sensory neurons are enhanced about 2-fold when T-type channels are in a highly reduced state (e.g., after applications of L-cys or DTT). In this study, blocking T-type channels in vivo with the preferential peripheral T-type channel blocker mibebradil abolished the hyperalgesic effects of reducing agents. In contrast, T-type currents were significantly diminished (by about 50%) when T-type channels were in a highly oxidized state caused by applications of DTNB, which in vivo diminished thermal and mechanical nociception. It is noteworthy that although near-maximal potentiating effects on T-type currents in rat sensory neurons were achieved with a relatively low (100 μM) concentration of DTT and L-cys, even up to 10–20-fold higher concentrations of these reducing agents had little effect on other voltage-gated (e.g., Na+, K+, HVA Ca2+) channels or ligand-gated (e.g., ATP, heat, capsaicin, proton) channels known to shape the excitability of sensory neurons. Based on this finding, it was concluded that the in vivo effects of redox agents were mainly due to the modulation of peripheral T-type channels. Indeed, this concept recently was directly validated when L-cys was injected into the hind paws of CaV3.2 knock-out mice and wild-type littermates. Only wild-type littermates showed profound hyperalgesia to thermal radiant testing, indicating that CaV3.2 channels are required for L-cys to exert an effect on pain sensation in vivo. This strongly suggests that not only the peripheral CaV3.2 T-type channels, but also the putative redox sites on T-type channels in peripheral nociceptors, are important targets for agents that modify pain perception.

Endogenous thiol-modifying redox agents are normally present in the interstitial tissue and are present in higher concentrations in pathological conditions such as tissue injury, inflammation, and ischemia. We and our colleagues therefore recently undertook an in-vivo study of the peripheral nociceptive effects of locally injected endogenous reducing cysteine analogs (L-cysteine, D-cysteine and D, L-homocysteine) and endogenous-oxidizing cysteine analogs (L-cysteine, D-cystine and D, L-homocysteine) using an acute model of thermal peripheral nociception in intact rats. We found that the
reducing cysteine analogs induced potent dose- and time-dependent thermal hyperalgesia; conversely, the oxidizing cysteine analogs induced potent dose- and time-dependent thermal analgesia. In the presence of 3βOH, a neuroactive steroid, the hyperalgesic effects of the reducing agents were diminished, whereas the analgesic effects of the oxidizing agents were strongly enhanced, suggesting that the observed nociceptive effects were, at least in part, mediated via peripheral T-type channels. Together, these findings strongly suggest that changes in the redox state of the peripheral nociceptors, favoring either reduced or oxidized forms of cysteine molecules, function as an intrinsic local mechanism in controlling peripheral pain transmission.

In further studies of neuronal excitability, it was demonstrated that L-cys is capable of inducing burst firing in DRG cells such as T-rich and medium-size nociceptors that exhibit T-channel-dependent ADPs. Figure 2 illustrates the profound excitatory effect of 100 μM L-cys, which increased the size of the ADP and induced repetitive spike firing in a medium-size DRG cell from normal rat.

Molecular studies of redox modulation of neuronal T-type channels demonstrated that the effects of reducing agents, but not oxidizing agents, are specific for the CaV3.2 isoform of T-type channels. Thus, Nelson and collaborators have taken advantage of this isoform specificity to determine molecular mechanisms underlying the effects of reducing agents on neuronal T-type channels. They used a combination of voltage- and current-clamp recordings from native and recombinant DRG CaV3.2 channels, chimeras between redox-sensitive CaV3.2 and redox-insensitive CaV3.1 channels, and site-directed mutagenesis. First they showed in rats that reducing agents selectively decreased the excitability threshold only in small C-type nociceptors that express T-type currents. Then they demonstrated that the sensitization of these cells is achieved through a mechanism whereby reducing agents chelate metal ions (e.g., zinc) that are constitutively bound to a critical histidine residue on the external surface in domain I of CaV3.2 T-type channels. This relieves tonic channel inhibition, thus increasing the amplitude of T-type currents. Last, they showed that reducing agents sensitize CaV3.2 T-type current-containing nociceptors isolated from wild-type mice, but not nociceptors from CaV3.2 knockout mice.

The function of T-type channels in neuropathic pain resulting from partial mechanical injury to the sciatic nerve. Several in-vivo studies have strongly suggested that T-type channels may be important in the development of neuropathic pain resulting from mechanical injury to peripheral axons of sensory neurons. The occurrence of abnormalities in pain perception very similar to those in humans, such as hyperalgesia and allodynia, have been well documented in rat models of partial mechanical injury of peripheral nerves as a consequence of loose ligation of the sciatic nerve, a widely used chronic constriction injury (CCI) model.

Of particular relevance here are reports that several pharmacological blockers and modulators of T-type channels in vivo alleviate neuropathic pain in CCI. For example, it has been reported that T-type blocker (+)-ECN injected in peripheral receptive fields of sensory neurons was more potent in reversing thermal hyperalgesia in rats with CCI than in healthy rats. In support of this finding, Dogru and colleagues, in another in-vivo study, found that two other preferential T-type channel blockers, mibebradil and ethosuximide, effectively reversed hyperalgesia and allodynia in rats with CCI. Mibebradil, when locally injected into the hind paws of rats, reversed such symptoms of CCI as tactile and thermal hypersensitivity. It is noteworthy that in this study mibebradil exerted similar effects when given systemically, but not when given intrathecally. This strongly suggests that in this animal model of neuropathic pain the major site of action of mibebradil is peripheral rather than central.

It has also been reported that potent oxidizing agent DTNB, which blocks DRG T-type channels in vitro, is effective, when locally applied, in reversing CCI-induced thermal hyperalgesia in vivo. Moreover, application of the traditional T-type channel blocker Ni2+ blocks ectopic discharges from peripheral nerves in a model of segmental spinal mechanical injury. Moreover, Bourinet and colleagues reported that specific molecular knock-down of CaV3.2 T-type channels in DRG cells by means of intrathecally delivered CaV3.2 antisense reversed both thermal and mechanical hyperalgesia and mechanical allodynia in rats with CCI. This effect lasted more than 8 days, suggesting that T-type channels significantly contribute to mechanical injury-induced sensitization of pain responses. It is interesting that even though CaV3.2 antisense was intrathecally...
delivered, its major sites of action seemed to be in the somas of DRG sensory neurons. This was verified by the electrophysiological finding of diminished T-type currents in small- and medium-size DRG cells, as well as the demonstration of decreased CaV3.2 protein expression using CaV3.2 antibody and decreased Cav3.2 transcript by RT-PCR in lumbar DRG homogenates. In contrast, CaV3.2 protein expression and Cav3.2 transcripts were not affected in the spinal cord. This study provides the most direct and compelling evidence published thus far of the involvement of T-type channels in the pathophysiology of neuropathic pain resulting from mechanical injury to the sciatic nerve.

Based on these pharmacological and molecular in-vivo studies with rats, there seems to be little doubt that T-type channel modulation could be beneficial for CCI-induced pain treatment. However, in contrast to these reports, an in-vivo study using CaV3.2 knock-out mice did not find a difference in thermal and mechanical hyperalgesia in CCI-subjected and control mice.9 The exact reason for this contradiction is not known, but it is possible that developmental loss of CaV3.2 channels allows compensatory changes in some other ion channels that are not possible during acute down-regulation of channel function by pharmacological agents or antisense applications. This discrepancy could also be a consequence of the different species (rats versus mice) used in these studies. Interestingly, previous patch-clamp recordings of DRG CaV3.2 channels in CCI also gave contrasting results. Hogan and colleagues45,46 reported no change in total inward (mostly HVA) CaV2 currents in small DRG cells in rats with CCI, but found an apparent loss of T-type current in medium-size DRG cells. No explanation for these seemingly contradictory results is obvious, but it is possible that ion channels expressed on nerve endings of nociceptive DRG neurons are modified differently than are their counterparts on cell bodies in conditions associated with peripheral nerve injury.

Since the cellular and molecular basis for the putative role of T-type channels in the etiology and pathophysiology of neuropathic pain resulting from CCI remains poorly understood, future studies should focus on the possible mechanisms of T-type channel alterations in various subtypes of DRG cells expressing T-type currents by CCI and on other animal models of mechanistically induced painful neuropathy.

Cell-specific alterations of T-type CaV3 channels in sensory neurons in an animal model of streptozotocin-induced diabetic neuropathy. Currently, little is known about the function of T-type channels in the development of neuropathic pain in animal models of diabetes. Studies have demonstrated alterations in voltage-gated CaV3 channels in subpopulations of sensory neurons in various animal models of diabetes. For example, in genetically induced diabetic Bio Bred/Worchester (BB/W) rats, up-regulation of HVA CaV3 currents in small DRG cells as a result of decreased G-protein function may consecutively contribute to diminished efficacy of opioid analgesics.47 These rats, after 8 months of diabetes, also up-regulate T-type channels in small DRG cells.48 It is interesting that serum from BB/W rats increases both HVA and T-type currents in DRG cells grown in culture without alterations in T-type channel activation and inactivation kinetics.49 This suggests that unidentified circulating factors present in diabetic animals may influence the regulation of voltage-gated CaV3 channels in sensory neurons. This is important since both T-type and HVA currents are implicated in the regulation of cellular excitability of DRG cells and transmitter release in central synapses of sensory DRG and dorsal horn neurons.50 In contrast to other voltage-gated ion channels, there has been no decisive verification of the existence of accessory subunits that are T-type channel-specific. However, recent data suggest that both β and α2δ subunits of HVA CaV3 channels may increase the surface expression and current density of recombinant T-type channels.51 This is particularly important because a large increase in mRNA for the α2δ subunit has been reported in DRGs from rats with mechanically and streptozotocin-induced peripheral neuropathy.52,53 It is of note that the α2δ subunit is considered a major cellular target for the anticonvulsant medication gabapentin, which is widely used in pain clinics to relieve diabetes-induced neuropathic pain in humans.54

In spite of these provocative indications that diabetes induces abnormalities of T-type channels, no functional link between T-type channels in sensory neurons and abnormal electrogensis of DRG cells has been conclusively documented. Nevertheless, Jagodic et al.24 recently addressed this issue in a series of experiments using a behavioral model of streptozotocin-induced painful diabetic neuropathy in rats. Using voltage-clamp recordings in an early phase of disease in parallel with the development of diabetes-induced pain, they found that T-type current density increased 2-fold in medium-sized DRG cells from the lumbar ganglia of adult rats. This was accompanied by prominent changes in the biophysical properties of the channels that allowed a greater fraction of channels to be available for activation at depolarized membrane potentials. Current-clamp recordings showed that this not only correlated strongly with more frequent ADPs, but also increased cellular excitability, indicated by a lower threshold of burst-firing in DRG cells from diabetic rats than occurred in control rats. In spite of the changes in channel kinetics, a pharmacological and molecular characterization of T-type channels in DRGs from diabetic rats in this study is consistent with up-regulation of the CaV3.2 isoform. These investigators used immunohistological and pharmacological approaches to elucidate the functional consequence of these changes in T-type channels, finding that both control and diabetic medium DRG cells with ADPs stained positively for IB4, but that only diabetic cells responded robustly to capsaicin. Since increased excitability of sensory neurons may result in pathological perceptions of pain such as hyperalgesia and allodynia, Jagodic et al. hypothesized that up-regulation of T-type currents and enhanced CaV3 entry into these presumably nociceptive DRG cells could contribute to the development of symptoms in early diabetic neuropathy. Conclusive verification of the function of CaV3.2 channels in diabetic neuropathy might be done using either CaV3.2 knock-out animals or specific CaV3.2 antisense oligonucleotides as was done by Bourinet et al.8 in the model of mechanically induced peripheral neuropathy.

The function of T-type channel blockers on chemotherapy-induced painful peripheral neuropathy. Many drugs that inhibit the growth of cancer cells can frequently produce damage to peripheral nerves, thereby inducing painful neuropathies in humans. This serious side effect may limit the effectiveness of treatment in many protocols used for modern chemotherapy. Flatters and Bennett56 found that systemic intraperitoneal injections of ethosuximide, a T-type channel blocker, alleviated mechanical and cold allodynia and hyperalgesia in rats with paclitaxel- and vincristine-induced painful peripheral neuropathy. Repetitive administration of ethosuximide did not cause tolerance to its analgesic effects. In the same animal model of neuropathy, Flatters and Bennett also found that the NMDA channel blocker MK-801 was ineffective and that morphine was
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Although there are clear electrophysiological and molecular indications that T-type channels are present in sensory neurons, there is, thus far, no evidence that these channels are indeed located on the peripheral nerve endings or that the molecular entities of these channels are similar to those on the soma of the corresponding sensory neurons in DRG. Studies that might answer these questions are precluded by the small size of nerve endings (less than 1 μm). Thus, the only presently available way to prove the presence of T-type channels in peripheral nerve endings is to test the expression of these channels on peripheral nerve terminals using validated specific antibodies against CaV₃.₂ channels. Recent studies suggest the presence, predominantly of the CaV₃.₂ isoform of T-type channels, in the somas of sensory neurons in vitro. This, together with the notion that the diminished response of knock-down of CaV₃.₂ gene expression results in pronounced anti-nociception in both acute and chronic pain models, suggests that studies examining effects in the soma of sensory neurons in DRG are likely to be useful for determining the mechanisms that contribute to peripheral nociception.

It is also important to emphasize the fact that T-type channels are preferentially located on small sensory neurons likely belonging to nociceptive fibers; they are scarce on large sensory neurons, which presumably are non-nociceptive sensory fibers. This indicates that selective blockade of T-type channels may result in desirable suppression of pain sensation, but not in the alteration of other modalities of sensory transmission such as touch or vibration. By blocking pain sensation preferentially, without causing unwanted motor weakness or numbness, selective T-type channel blockers may offer an important advance in safe and efficient pain therapy.

SUMMARY

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SUMMARY

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Although there are clear electrophysiological and molecular indications that T-type channels are present in sensory neurons, there is, thus far, no evidence that these channels are indeed located on the peripheral nerve endings or that the molecular entities of these channels are similar to those on the soma of the corresponding sensory neurons in DRG. Studies that might answer these questions are precluded by the small size of nerve endings (less than 1 μm). Thus, the only presently available way to prove the presence of T-type channels in peripheral nerve endings is to test the expression of these channels on peripheral nerve terminals using validated specific antibodies against CaV₃.₂ channels. Recent studies suggest the presence, predominantly of the CaV₃.₂ isoform of T-type channels, in the somas of sensory neurons in vitro. This, together with the notion that the diminished response of knock-down of CaV₃.₂ gene expression results in pronounced anti-nociception in both acute and chronic pain models, suggests that studies examining effects in the soma of sensory neurons in DRG are likely to be useful for determining the mechanisms that contribute to peripheral nociception.

It is also important to emphasize the fact that T-type channels are preferentially located on small sensory neurons likely belonging to nociceptive fibers; they are scarce on large sensory neurons, which presumably are non-nociceptive sensory fibers. This indicates that selective blockade of T-type channels may result in desirable suppression of pain sensation, but not in the alteration of other modalities of sensory transmission such as touch or vibration. By blocking pain sensation preferentially, without causing unwanted motor weakness or numbness, selective T-type channel blockers may offer an important advance in safe and efficient pain therapy.

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T-type channels have a crucial function in the excitability of sensory neurons. The evidence reviewed here supports the concept that these channels are amplifiers of peripheral nociceptive signals such as noxious heat, as well as mechanical and chemical stimuli. In-vitro and in-vivo studies have provided a plethora of pharmacological evidence that T-type calcium channels are important in augmenting acute peripheral nociception. Figure 3 summarizes our current knowledge of the effects of different agents on the regulation of T-type channel function in peripheral sensory neurons and, consequently, on pain sensation.

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