REVIEW

T-type voltage-gated calcium channels as targets for the development of novel pain therapies

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It is well recognized that voltage-gated calcium (Ca^{2+}) channels modulate the function of peripheral and central pain pathways by influencing fast synaptic transmission and neuronal excitability. In the past, attention focused on the modulation of different subtypes of high-voltage-activated-type Ca^{2+} channels; more recently, the function of low-voltage-activated or transient (T)-type Ca^{2+} channels (T-channels) in nociception has been well documented. Currently, available pain therapies remain insufficient for certain forms of pain associated with chronic disorders (e.g. neuropathic pain) and often have serious side effects. Hence, the identification of selective and potent inhibitors and modulators of neuronal T-channels may help greatly in the development of safer, more effective pain therapies. Here, we summarize the available information implicating peripheral and central T-channels in nociception. We also discuss possible future developments aimed at selective modulation of function of these channels, which are highly expressed in nociceptors.

Abbreviations

3βOH, [(3β, 5β, 17β)-3-hydroxyandrostane-17-carbonitrile]; ADP, after-depolarizing potential; CCI, chronic constrictive injury; CNS, central nervous system; DH, dorsal horn; DRG, dorsal root ganglia; DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid); ECN, [(3β, 5α, 17β)-17-hydroxyestrane-3-carbonitrile]; GABA, γ-amino-butyric acid; HVA, high voltage-activated; KO, knock out; LTP, long-term potentiation; LTS, low threshold calcium spike; LVA, low voltage activated; mEPSCs, miniature excitatory postsynaptic currents; MK-801, 5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate; NMDA, N-methyl-D-aspartate; NPP, neuropathic pain; nRT, nucleus reticularis thalami; PKC, protein kinase C; PNS, peripheral nervous system; ROCK, Rho-associated kinase; STZ, streptozotocin; TC, thalamo-cortical; T-channels (T)-type Ca^{2+} channels; TTA-A2, [2-(4-Cyclopropylphenyl)-N-((1R)-1-{5-[(2,2,2-trifluoroethyl)oxo]-pyridin-2-yl}ethyl)acetamide]; TTA-P2, 3,5-dichloro-N-[1-(2,2-dimethyl-tetrahydro-pyran-4-ylmethyl)-4-fluoro-piperidin-4-ylmethyl]-benzamide; TTX, tetrodotoxin; VGCCs, voltage-gated calcium channels; WT, wild type

The function of T-channels in the pain pathway

Pain is often a result of the activation of neurons (nociceptors) that can sense damaging stimuli by acute events subsequent to the injury of peripheral tissue such as the skin and internal organs. This form of pain, called nociceptive pain, is self-protective and typically evokes actions aimed at limiting or avoiding further tissue damage. In contrast, neuropathic pain, which results from primary dysfunction of peripheral nociceptive, as well as non-nociceptive nerves or central nervous system ('central pain'), typically outlasts the initial stage of injury and frequently leads to debilitating disorders that are not amenable to conventionally available drug therapies. Many *in vitro* and *in vivo* studies have identified the

functions of diverse subtypes of voltage-gated Ca^{2+} channels (VGCCs) in both peripheral and central sensory pathways to nociception. These channels can contribute to the development of both nociceptive and neuropathic pain. In this review, we summarize the most recent evidence linking T-type Ca^{2+} channels (T-channels) to central and peripheral pain processing. We also discuss the potential development of pain therapies aimed at these channels, which are abundantly expressed in nociceptors.

Molecular pharmacology of T-channels

Based on the membrane potential at which they gate ion currents, VGCCs are classified as high-voltage activated (HVA) or sustained currents, and low-voltage activated (LVA) or transient currents (T-type) (Catterall, 2000). Based on their sensitivity to pharmacological agents, HVA channels are further classified into at least five subtypes: L-, N-, P-, Q- and R-. While HVA calcium channels function in fast synaptic transmission in the central nervous system (CNS) (Miller, 1998; Catterall, 2000), it is thought that T-currents have a unique function in neuronal excitability (Llinas, 1988; Huguenard, 1996; Perez-Reyes, 2003). This notion is based on the unique ability of T-currents to become activated after a small depolarization of the cell membrane, which allows them to function at near-resting membrane potentials. Neuronal T-channels have been shown to promote Ca^{2+}-dependent burst firing and low-amplitude intrinsic neuronal oscillations, as well as Ca^{2+} entry and amplification of weak dendritic synaptic signals in a myriad of CNS neurons. Moreover, the function of T-channels can be altered by pathological conditions such as absence epilepsy, which, in turn, can decrease the threshold for the initiation of seizure activity (Kim *et al.*, 2001; Song *et al.*, 2004; Zhang *et al.*, 2004). Cloning of pore-forming α1 subunits of T-channels has demonstrated the existence of at least three subtypes, α1G (Ca$_v$3.1) (Perez-Reyes *et al.*, 1998), α1H (Ca$_v$3.2) (Cribbs *et al.*, 1998) and α1I (Ca$_v$3.3) (Lee *et al.*, 1999a). Post-translational modifications such as alternative splicing of all three genes encoding the α1 subunit of Ca$_v$3 can occur in tissues (Monteil *et al.*, 2000; Chemin *et al.*, 2001b; Murbartián *et al.*, 2002; 2004; Latour *et al.*, 2004; Emerick *et al.*, 2006; Zhong *et al.*, 2006), further contributing to the diversity of native T-currents. Indeed, such modifications may underlie the biophysical and pharmacological heterogeneity of T-currents previously described in native neuronal, cardiac and endocrine cells (Herrington and Lingle, 1992; Todorovic and Lingle, 1998).

In electrophysiological experiments, T-currents, because of their unique biophysical properties, can be relatively easy identified; that is, these currents have characteristically slow voltage-dependent activation and almost complete inactivation, which typically generate a criss-crossing pattern when the family of currents is generated at progressively more positive voltages (Figure 1A). In addition, T-currents have several-fold slower deactivation (transition from open to closed states) than any HVA subtype does (Figure 1B). However, in spite of the progress made in recent years, pharmacological tools for the study of T-currents are still very limited (see also other reviews by McGivern, 2006 and Lory
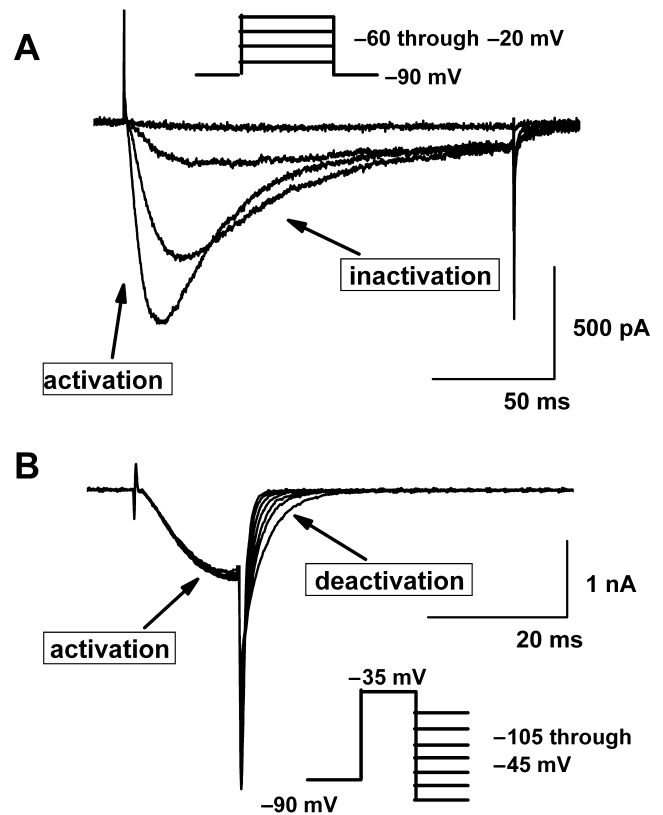

Figure 1

(A) A family of inward T-currents evoked in small, acutely dissociated dorsal root ganglion (DRG) cells from holding potential of −90 mV with 200 ms-long depolarizing steps of −60 mV through −40 mV. Note that current waveforms at more positive potentials exhibit faster activation and inactivation kinetics as evidence by 'criss-crossing' pattern. (B) Typical slow deactivation of tail currents in another small DRG cell generated with a short 15 ms-long depolarizing steps from −90 mV to −35 mV, followed by incremental hyperpolarizing steps of 10 mV starting from −105 mV through −45 mV. Insets on both panels show voltage stimulation protocol used to generate these currents.

and Chemin, 2007). While there are many natural toxins or venom components that could be used to study the multiple HVA currents, only recently have substances been identified that can selectively and potently block T-currents. For example, a scorpion toxin, kurtoxin, which is a potent submicromolar blocker and gating modifier of cloned T-currents, also, with similar potency, affects voltage-gated sodium currents (Chuang *et al.*, 1998) and native HVA Ca^{2+} currents (Sidach and Mintz, 2002). Traditional compounds previously used to investigate T-channels, such as ethosuximide (Coulter *et al.*, 1989) and amiloride (Tang *et al.*, 1988), can block T-currents, but only at fairly high concentrations [inhibitory concentrations that block 50% of the maximal current (IC$_{50}$s) in excess of 100 μM]. At such concentrations, these compounds are also likely to block other ion channels, thus limiting their value for determining the function of T-channels both *in vitro* and *in vivo*.

Mibefradil, marketed by Hofmann-La Roche (Basel, Switzerland), is a peripherally acting antihypertensive drug. Even

at low micro- and nanomolar concentrations, mibrefradil has been shown preferentially to block T-channels over HVA channels in vascular smooth muscle (Ertel *et al.*, 1997) and cerebellar Purkinje cells (McDonough and Bean, 1998) and thus, for a while, was considered to be a promising selective T-channel blocker. Further studies have shown that mibefradil exhibits voltage-dependent inhibition of T-currents in acutely dissociated small dorsal root ganglia (DRG) cells *in vitro* by stabilizing the channel into inactivated states (Todorovic and Lingle, 1998). This is important since binding to inactivated states is an important property of drugs that modulate ion channels as it can provide selectivity to their action, and it implies that drugs preferentially affects actively-firing neurons. Consistent with this, mibefradil has moderate analgesic properties in healthy rats (Todorovic *et al.*, 2001a) but completely reverses symptoms of neuropathic pain from chronic constrictive injury (CCI) of sciatic nerve in rats *in vivo* (Dogrul *et al.*, 2003). Particularly exciting was the fact that the drug was marketed in Europe as an antihypertensive agent and was well tolerated, presumably due to poor penetration into the CNS. Unfortunately, it was soon withdrawn from the market due to drug–drug interactions. Moreover, studies have since indicated that this molecule blocks not only L-type HVA-type Ca^{2+} currents, but also other voltage-gated currents (e.g. I_{Na+} and I_{K+}) at low µM concentrations, thus casting doubt on its selectivity and usefulness in selectively studying T-channels (Jimenez *et al.*, 2000; McNulty and Hanck, 2004; Coste *et al.*, 2007). However, an interesting compound (NNC 55-0396) was recently generated by modifying the molecule of mibefradil that preserves its potency in blocking T-channels while greatly diminishing its potency on HVA currents (Huang *et al.*, 2004). Towards this end, R(-) efonidipine has been shown to exhibit greater selectivity for T-type versus HVA currents in hippocampal neurons, although with more than 10-fold lower potency when compared with mibefradil (Shin *et al.*, 2008). Thus, it is still possible that more potent and selective peripherally acting analogues of mibefradil will be developed and will prove useful not only in studies of the function of T-channels, but in the development of clinical analgesics.

Divalent cations such as nickel (Ni^{2+}), at concentrations up to 30 µM (Lee *et al.*, 1999b), as well as low micromolar concentrations of zinc (Zn^{2+}) and copper (Cu^{2+}), preferentially inhibit the $Ca_V3.2$ isoform of T-channels in preference to other T-channel isoforms and HVA channels (Todorovic and Lingle, 1998; Nelson *et al.*, 2007a,b; Traboulsie *et al.*, 2007). This is particularly important, given that metal ions such as Zn^{2+} and Cu^{2+} may be endogenously present in neural tissue and may act as natural modulators of T-channel function. Some agents used in human medicine are particularly interesting in that, at clinically relevant concentrations, they may alter the function of T-channels. Nitrous oxide (N_2O; laughing gas) is a potent analgesic but a weak anaesthetic; at clinically relevant concentrations up to 80%, it selectively inhibits T-type over HVA-type Ca^{2+} currents in acutely dissociated DRG neurons (Todorovic *et al.*, 2001b). Like Ni^{2+}, N_2O selectively blocks the α1H ($Ca_V3.2$), but not the α1G ($Ca_V3.1$) or α1I ($Ca_V3.3$) isoforms of T-channels (Todorovic *et al.*, 2001b; Joksovic *et al.*, 2005). In contrast, recent study showed that N_2O may also inhibit in smaller extent recombinant $Ca_V3.1$ currents (Bartels *et al.*, 2009). The apparent reason for

inconsistency with the above mentioned study is not obvious. However, it is possible that different methods of application of N_2O in these studies contributed to this discrepancy. However, it is well established at these concentrations N_2O also blocks the neuronal N-methyl-D-aspartate (NMDA) subtype of glutamate receptors (Jevtović-Todorović *et al.*, 1998) and activates two-pore domain background potassium conductance (Gruss *et al.*, 2004).

Neuroleptics are another major group of clinically used drugs that potently block T-channels at the same concentrations as those used for other cellular targets such as D_2 dopamine receptors. Santi *et al.* (2002) showed that two neuroleptics, diphenylbutylpiperidine pimozide and penfluoridol, are particularly potent blockers of recombinant T-channels, with estimated IC_{50} values of about 30–50 nM and 70–100 nM, respectively.

Some neuroactive steroids with a 5α configuration at the steroid A, B ring fusion, could be promising pharmacological agents for the study of T-channels. For example, (+)-ECN [(3β, 5α, 17β)-17-hydroxyestrane-3-carbonitrile] is a potent, albeit partial voltage-dependent blocker of T-channels in acutely dissociated rat sensory neurons (IC_{50} of 300 nM). It has no significant effect on other voltage-gated channels (e.g. Na^+, K^+ or HVA-type Ca^{2+} channels) or ligand-gated ion channels [e.g. glutamate and gamma amino-butyric acid (GABA)-gated channels; Todorovic *et al.*, 1998]. The selective pharmacological actions of ECN and related structural steroid analogues provide a unique opportunity to test their role in peripheral somatic pain transmission *in vivo*. When peripheral thermal nociception was studied by locally injecting a steroid into the peripheral receptive fields of a rat hind paw, it was found that the peripheral analgesic effects of 5α-reduced neuroactive steroids depended on their T-channel-blocking potential (Pathirathna *et al.*, 2005a). For example, structural modifications, which eliminated T-channel blocking capacity, resulted in almost the complete loss of steroid-induced analgesic activity *in vivo*. On the other hand, ECN was the most potent and enantioselective peripheral analgesic in the thermal pain paradigm.

Another addition to the potentially useful T-channel-selective blockers is a novel neuroactive steroid with a 5β configuration at the steroid A, B ring fusion, [(3β, 5β, 17β)-3-hydroxyandrostane-17-carbonitrile] (3βOH), which is an effective voltage-dependent blocker of T-currents in acutely isolated DRG cells (with an IC_{50} of 2.8 µM at a holding potential of –90 mV and IC_{50} 0.8 µM at holding potentials of –70 mV). However, this steroid has little effect on HVA-type Ca^{2+} voltage-gated Na^+ and K^+ channels or $GABA_A$-gated channels at concentrations from 1 to 10 µM (Todorovic *et al.*, 2004a). To evaluate the ability of 5β-reduced neuroactive steroids to induce analgesia *in vivo*, we injected various doses of each of these steroids into the peripheral receptive fields of sensory neurons. We found that 5β-reduced neuroactive steroids induce potent dose- and time-dependent antinociceptive responses in rats. These responses correlated closely with the ability of the steroids to block T-currents in acutely dissociated rat sensory neurons.

In further search for T-channel selective compounds, Merck and Co (Whitehouse Station, NJ, USA) has synthesized and examined the *in vitro* effects of 3,5-dichloro-N-[1-(2,2-dimethyl-tetrahydro-pyran-4-ylmethyl)-4-fluoro-piperidin-4-

ylmethyl]-benzamide (TTA-P2) and related derivatives of 4-aminomethyl-4-fluoropiperdine on T-currents in recombinant cells (Shipe *et al.*, 2008). It was found that these compounds are highly selective, state-independent and potent blockers of all recombinant T-channel isoforms (IC$_{50}$s ranging from 20 to 100 nM), which makes them some of the best new tools available for the study of T-channels. However, the potency and selectivity of these drugs in native neurons, including those in pain pathways, have not been well studied. Of note, a related compound and state-dependent blocker of T-channels TTA-A2 [2-(4-Cyclopropylphenyl)-N-((1R)-1-{5-[(2,2,2-trifluoroethyl)oxo]-pyridin-2-yl}ethyl) acetamide], when given systemically, suppressed *in vivo* active waking and promoted slow-wave sleep in wild-type mice, but not mice with double knockout of Ca$_V$3.1 and Ca$_V$3.3 (Kraus *et al.*, 2010). Thus, CNS side effects of pan-T-channel blockers that promote sedation may impede the use of these drugs as clinical analgesics.

Modulation of T-type channels

Recent evidence indicates that T-channels may be potently modulated by various classes of endogenous and experimental agents.

Redox modulation
T-channels in peripheral sensory or DRG neurons can be selectively and potently modulated by various reducing and oxidizing agents, including the endogenous sulphur-containing amino acid L-cysteine. Recent studies have shown that T-currents in acutely dissociated sensory neurons are significantly enhanced when T-channels are in a reduced state. However, these currents are largely inhibited when T-channels are in an oxidized state, strongly suggesting the importance of putative redox-sensitive sites on peripheral T-type channels (Todorovic *et al.*, 2001a). This, in turn, can profoundly affect cellular excitability by enhancing Ca^{2+} influx during action potential of at least three different subtypes of nociceptors (Nelson *et al.*, 2005; 2007b; Jagodic *et al.*, 2007); it can also affect the excitability of cells of the reticular thalamic nucleus (Joksovic *et al.*, 2006). A computational modelling study has demonstrated that an increase in the cellular excitability of putative nociceptive 'T-rich' cells in DRGs can be achieved by hyperpolarizing gating shifts in T-channels by L-cysteine (Nelson *et al.*, 2005). Thus, both an increase in the amplitude of T-current and alterations in the gating parameters of T-channels in nociceptors may increase the probability of spike firing. Further studies using Markov-state models have shown that L-cysteine induces gating alterations of T-currents in nociceptors by increasing the transition rate from resting to open, and decreasing the transition rate from open to inactivated states (Nelson *et al.*, 2010).

L-cysteine induces potent hyperalgesia in rats when injected into the peripheral receptive fields of sensory neurons *in vivo* (Todorovic *et al.*, 2001a). This was recently confirmed by the demonstration that hyperlagesia induced by local peripheral injections of L-cysteine is present only in wild-type mice, not Ca$_V$3.2-knock-out mice (Nelson *et al.*, 2007b). Furthermore, molecular mutagenesis studies have

shown that histidine 191 in repeat I of Ca$_V$3.2 is critical for the action of reducing agents that chelate off endogenously bound divalent metal ions (e.g. Zn^{2+}), which appears to exert tonic inhibition of T-channels both *in vitro* and *in vivo* (Nelson *et al.*, 2007b; Kang *et al.*, 2010). A related phenomenon was recently described for gaseous transmitter hydrogen sulfide (H$_2$S), an endogenous compound formed from L-cysteine. It was found that H$_2$S facilitates Ca$_V$3.2 currents *in vitro*, probably via chelation of zinc, consequently leading to both peripheral and central sensitization of acute somatic (Kawabata *et al.*, 2007; Maeda *et al.*, 2009) and visceral (Matsunami *et al.*, 2009; Nishimura *et al.*, 2009) nociception, as well as maintenance of chronic neuropathic pain (Takahashi *et al.*, 2010). Thus, reducing agents and other endogenous metal chelators present in the body (e.g. albumin, histidine, H$_2$S) may serve as natural agonists for the Ca$_V$3.2 T-channels.

Surprisingly, further experiments with H191 demonstrated that this site is crucial for the inhibitory modulation of peripheral and central T-channels by another endogenous reducing agent, L-ascorbic acid (ascorbate, vitamin C). Ascorbate, a ubiquitous redox agent, may act as both an antioxidant and a prooxidant. At nanomolar concentrations (IC$_{50}$ 10 nM for human and 25 nM for rat channels), ascorbate selectively inhibited recombinant Ca$_V$3.2-based T-currents but, even at 1000-fold higher concentrations, had no significant effect on Ca$_V$3.1 or Ca$_V$3.3 T-current (Nelson *et al.*, 2007b). At physiologically relevant concentrations, ascorbate selectively inhibited native DRG and thalamic T-currents and diminished T-channel-dependent burst firing in reticular thalamic cells. This highly subtype-specific effect was achieved via metal-catalysed oxidation of critical metal-binding and Ca$_V$3.2-unique H191 residue. Furthermore, using recombinant Ca$_V$3.2 channels and Ca$_V$3.2 knockout-mice, it was recently demonstrated that N$_2$O-induced analgesia in mice formalin test is likely mediated by metal-catalysed oxidation of H191 of Ca$_V$3.2 (Orestes *et al.*, 2010). This strongly suggests that the development of novel inhibitors of T-channels may focus on the H191 of repeat I of T-channels.

Phosphorylation and G-protein modulation
Recent evidence from experiments involving recombinant T-channels indicates that G-protein β–γ subunits selectively inhibit the function of Ca$_V$3.2 channels by interacting with the intracellular loop connecting domain II and II of Ca$_V$3.2 (Wolfe *et al.*, 2003) and by decreasing single-channel open probability without affecting voltage-dependent kinetics of the channel (DePuy *et al.*, 2006). In contrast, corticotrophin-releasing factor interacts with G-protein β–γ subunits to selectively inhibit Ca$_V$3.2 channels by causing hyperpolarizing shift in half-inactivation potential (Tao *et al.*, 2008). Furthermore, activation of Gαq/11-coupled muscarinic acetylcholine receptors leads to their interaction with domains II and IV of human Ca$_V$3.3, selectively inhibiting Ca$_V$3.3 (Hildebrand *et al.*, 2007).

It recently has been proposed that a form of modulation involving phosphorylation reactions may modify native and recombinant T-currents, thus having an important effect on the function of T-channels. For example, recombinant Ca$_V$3.2 T-channels are stimulated by Ca^{2+}/calmodulin-dependent kinase II (Wolfe *et al.*, 2002) via phosphorylation of a critical Serine$_{1198}$ residue in the II-III intracellular loop of Ca$_V$3.2

(Welsby et al., 2003) and modulated by protein kinase A (Chemin et al., 2007b), by phosphorylation of the Serine$_{1107}$ residue on the II-III intracellular loop of Ca$_V$3.2 (Hu et al., 2009). Furthermore, all three isoforms of recombinant T-channels can be stimulated by protein kinase C (PKC) (Park et al., 2006; Chemin et al., 2007b; Joksovic et al., 2010), presumably by phosphorylation reactions, although the specific amino acid residues targeted in the channel have not yet been identified. Interestingly, stimulation of PKC with phorbol esters inhibits T-currents in small DRG cells in culture (Schroeder et al., 1990), and thalamo-cortical (TC) cells in intact brain slices (Cheong et al., 2008). Iftinca et al. (2007) have found that different recombinant and native T-currents may be modulated by phosphorylation reactions involving Rho-associated kinase (ROCK). Notably, this was associated with the up-regulation of T-currents in small DRG cells and a depolarizing shift in voltage-dependent activation and inactivation. In this study, site-directed mutagenesis demonstrated that the ROCK-mediated effects involve two distinct phosphorylation consensus sites consisting of serines and threonines in the domain II-III intracellular linker. However, the physiological relevance of G-proteins and phosphorylation reactions that may modify native T-currents and their contribution to the function of the cells expressing these channels has not been conclusively demonstrated. It is tempting, nonetheless, to speculate that posttranslational modifications of T-channels in the pain pathway by phosphorylation reactions or G-protein-mediated modulation modulates pain signalling, as this has been demonstrated for other nociceptive ion channels, including HVA Ca^{2+} channels (reviewed in Bhave and Gereau, 2004).

Endocannabinoids and arachidonic acid

Local release and accumulation of arachidonic acid metabolites in the peripheral tissues may be involved in direct sensitization of peripheral nociceptors during inflammatory reactions. Interestingly, arachidonic acid and related compounds have been shown to modulate recombinant T-channel isoforms. For example, arachidonic acid inhibits, with similar potency, both recombinant Ca$_V$3.1 (IC$_{50}$ 3 μM; Talavera et al., 2004) and Ca$_V$3.2 T-channels (10 μM blocks about 80% of current; Zhang et al., 2000). Further, an arachidonic acid-related compound and anandamide, an endogenous endocannabinoid agonist, were recently shown to cause direct inhibition of all three isoforms of recombinant T-type channels, with Ca$_V$3.2 (IC$_{50}$ 330 nM) and Ca$_V$3.3 (IC$_{50}$ 1.1 μM) more potently inhibited than Ca$_V$3.3 was (IC$_{50}$ 4.1 μM; Chemin et al., 2001a; 2007a). Importantly, Barbara et al. (2009) have provided functional evidence directly implicating endogenous lipoamino-acid inhibition of T-channels in DRG cells in nociception. They found that the inhibitory effects on T-current persisted in cell-free patches, strongly suggesting direct interactions of lipoamino acids with T-channels. Thus, lipoamino acid and related compounds may be important targets for the development of future pain therapies.

T-channels in primary sensory neurons of dorsal root ganglia (DRG)

The nociceptive system is based on highly regulated relay mechanisms that connect the periphery (e.g. skin, mucosa,

viscera, muscles and joints) to the higher pain-processing regions in the CNS. Primary sensory or DRG neurons are bipolar cells. Their long processes originate in the periphery, whereas their somas are located in trigeminal or spinal (dorsal root) ganglia; their central process terminates in the dorsal horn of the spinal cord. There, these cells synapse with second-order nociceptive neurons.

Many different types of sensory receptors originate in the periphery; they can be classified based on the anatomy of the nerve fibres and the type of activating stimuli. Low-threshold mechanoreceptors have large myelinated fibres (Aα and Aβ), conduct fairly fast (10–100 m·s^{-1}), and convey mechanosensations of light touch, proprioception and vibration. High-threshold mechanoreceptors have thinly myelinated (Aδ) fibres with conduction velocities ranging from 2 through 8 m·s^{-1}; unmyelinated (C-type) sensory fibres conduct most slowly (less than 1.4 m·s^{-1}) (Burgess and Perl, 1967; Bessou and Perl, 1969). While both Aδ and C-type sensory fibres respond to intense mechanical and noxious thermal stimuli, C fibres are also sensitive to a variety of chemical irritants, and thus are often called polymodal nociceptors. DRGs contain the somas of thin Aδ- and C-type sensory fibres, which typically are less than 31 μm in average diameter, as well as the bodies of Aα- and Aβ-type fibres, which typically are larger than 45 μm in average diameter; medium-size neurons (between 32 and 45 μm) could belong with either group (Lee et al., 1986; Scroggs and Fox, 1992).

In the last several years, pharmacological, electrophysiological and genetic studies have begun to demonstrate how ligand- and voltage-gated channels specify functions for subsets of sensory neurons in the pain pathway. These ion channels, if they serve as pain transducers, can activate nociceptors; they also can control transmitter release from either peripheral or central endings, or modulate the excitability of nociceptors (Caterina and Julius, 2001).

The possible functional relationship between T-channels and other ligand-gated channels thought to mediate the excitability of sensory neurons has not been determined. Most acutely dissociated small DRG cells (<31 μm in diameter) expressing T-currents are sensitive to capsaicin and have the electrical properties of classically described nociceptors with wide action potentials (Cardenas et al., 1995; Coste et al., 2007; Nelson et al., 2007b). T-channels also have been reported to co-exist with ATP- and heat-gated currents in small, acutely dissociated DRG cells (Todorovic et al., 2001a) and to co-express with GABA$_A$ channels in medium-size sensory neurons (White, 1990). T-currents are present in small- and medium-size cold- and menthol-sensitive sensory neurons (Viana et al., 2002), but their function in the excitability of cold-sensitive neurons has not been studied. We recently described novel sub-population of DRG cells (soma size 26–31 μm) that expresses robust T-currents and essentially no HVA currents ('T-rich cells', Nelson et al., 2005). Furthermore, most small and 'T-rich' DRG cells expressing T-currents are sensitive to capsaicin, express tetrodotoxin-resistant Na$^+$ currents, are IB$_4$ positive and generate high-threshold mechanosensory currents, all of which strongly suggest their nociceptive function (Nelson et al., 2005; 2007b; Coste et al., 2007). In addition, T-channels also are present in a subpopulation of medium-size acutely dissociated sensory neurons (soma diameter 32–45 μm) that are either putative

Table 1

Summary of ionic current signatures in different subpopulations of acutely dissociated sensory neurons

Isolated DRG soma diameter (μm)	Sensory fibre type	I_{TCa}^{2+}- current density	I_{HVACa}^{2+}- current density	$I_{Na+TTX-R}^{-}$- current density	I_{TRPV1}^{-}- current density	Threshold for mechano-sensory currents	Expression of IB$_4$ imuno	Presumed function *In vivo*
Small 15–25	C-type Classic	++	++++	++++	++++	High	++	Nociceptive
Small 26–31	C-type T-rich	++++	None	++++	++	High	+++	Nociceptive
Medium 32–45	A-δ	++	++++	++++	++++	n.a.	++++	Nociceptive
Medium 32–45	D-hair	++++	++	+	None	Low	n.a.	Mechanosensory
Large >45	A-α/β	++	++++	+	None	Low	None	Mechanosensory

Data are based on the following references: Nelson *et al.* (2005), Jagodic *et al.* (2007), Coste *et al.* (2007) and Wu *et al.* (2004). Note that T-currents are expressed in three subpopulations of putative nociceptive and two subpopulations of putative mechanosensory non-nociceptive neurons.

++++, very high; +++, high; ++, moderate; +, low; n.a., not applicable; I_{TCa}^{2+}, T-type Ca^{2+} currents; I_{HVACa}^{2+}, high-voltage-activated Ca^{2+} currents; $I_{Na}^{+}{}_{TTX-R}$, tetrodotoxin-resistant voltage-gated Na$^+$ currents; I_{TRPV1}, transient receptor potential vanilloid 1 (capsaicin-gated) currents; IB$_4$, isolectin B$_4$; DRG, dorsal root ganglion.

nociceptors as they bear IB$_4$ antigen (Jagodic *et al.*, 2007) or that subserve touch sensation mediated by D-hair mechanoreceptors (Shin *et al.*, 2003; Dubreuil *et al.*, 2004). Prominent Ca$_V$3.2 T-currents in 'T-rich' and medium-size DRG cells generate visible after-depolarizing potential and high-frequency burst firing in current-clamp recordings. Furthermore, amplification of sub-threshold membrane depolarizations via T-channels may decrease the threshold for action potential firing in DRG cells that express T-channels (Nelson *et al.*, 2005; 2007b; Jagodic *et al.*, 2007). This is important since these patterns of membrane firing may contribute to symptoms of neuropathic pain such as hyperalgesia (exaggerated pain sensation) and allodynia (non-painful stimulus is perceived as painful). Furthermore, conditions associated with axonal injury may lead to a change in peripheral fibre function and a previously mechanosensitive fibre may turn into a pain conducting fibre, which is one of the physiological bases of mechanical allodynia. Table 1 summarizes the properties of T-currents and expression of other ionic currents in acutely dissociated putative nociceptive and non-nociceptive DRG neurons.

Studies using *in situ* hybridization have demonstrated that mRNA for Ca$_V$3.2 (α1H) is the most abundant isoform of T-channels in peripheral sensory neurons of small- and medium-size neurons (Talley *et al.*, 1999). Patch-clamp recordings from small, acutely dissociated sensory neurons in mice lacking the Ca$_V$3.2 gene essentially showed a complete loss of T-currents (Chen *et al.*, 2003; Nelson *et al.*, 2007b). Consistent with this, Ca$_V$3.2 knockout mice phenotype is characterized with profound attenuation of acute pain responses (Choi *et al.*, 2007). Bourinet *et al.* (2005) recently examined pain thresholds to both thermal and mechanical hyperalgesia in adult rats with molecular knockdown of Ca$_V$3.2 T-channels in DRG neurons. Intrathecally administered anti-Ca$_V$3.2 antisense oligonucleotides led to significant depression of pain responses in both acute thermal and mechanical pain testing. Importantly, this resulted in a concomitant decrease in T-current density in corresponding lumbar DRG neurons, diminished expression T-channel immunoreactivity in DRG tissue homogenates, and decreased Ca$_V$3.2 mRNA transcript expression in DRGs. Thus, this study confirmed that the major function of T-channels in DRG sensory neurons is to support acute nociceptive signals.

T-channels in dorsal horn of the spinal cord

The spinal dorsal horn (DH) contains the central terminals of peripheral afferent fibres, projection neurons of the spinothalamic and other ascending tracts, and local inhibitory GABAergic neurons (interneurons). Little is known about the function of T-channels in the complex spinal DH circuitry. In one study, ethosuximide depressed excitability to both low- and high-intensity mechanically and thermally evoked neuronal responses recorded extracellularly from an anaesthetized rat with a spinal nerve injury (Matthews and Dickenson, 2001). However, the required concentrations were in a range up to 100 mM, at which ethosuximide was likely to affect various voltage-gated currents (e.g. Na$^+$ currents) (Leresche *et al.*, 1998). In a behavioural study, Dogrul *et al.* (2001) intrathecally injected mibefradil, which enhanced opioid-induced analgesia. Interestingly, mibefradil applied alone did not change baseline pain transmission.

T-channels are present in moderate density in DH neurons located in superficial lamina (Ryu and Randic, 1990). Ikeda *et al.* (2003), using acute spinal cord slice preparations, showed that nickel-sensitive T-currents in projection neurons of lamina I of the spinal DH work synergistically with neurokinin-1 (substance P) and NMDA receptors to enhance excitatory synaptic strength after brief (1 s) but high-frequency stimulation (100 Hz) of sensory afferents. This is important because lamina 1 of the DH receives synaptic input from small-diameter fibres that conduct pain signals from peripheral nerves. This study demonstrated that T-channels are crucial in raising intracellular calcium levels to the critical threshold necessary for activity- and calcium-dependent long-term potentiation (LTP) of synaptic activity resulting from stimulation of the peripheral nociceptors. However,

high-frequency stimulation of afferent neurons may not be a physiologically relevant stimulus. Thus, Ikeda *et al*. (2006) followed with a demonstration that low-frequency stimulation of peripheral nociceptors such as that caused by inflammation can induce lasting synaptic plasticity in the form of LTP in another group of projection neurons in the spinal DH; these neurons also critically depended on T-channels. Furthermore, these investigators identified a whole signalling cascade that, in addition to T-channels, includes NMDA receptors, substance P (neurokinin) receptors, PKC, Ca^{2+} calmodulin-dependent kinase, intracellular calcium stores and nitric oxide.

Bao *et al*. (1998) showed, using intact spinal-cord slice preparations, that T-channels could be important in the presynaptic regulation of the spontaneous release of miniature excitatory synaptic currents (mEPSCs) in superficial spinal lamina, which receive their main sensory input from peripheral nociceptors. The frequency, but not the amplitude, of mEPSCs was diminished by mibefradil and nickel, but not by classical blockers of HVA-type Ca^{2+} currents. However, the major caveat of this study is that both mibefradil and nickel may, with similar potency, block R-type calcium channels (reviewed in Perez-Reyes, 2003). Thus, direct evidence of the function of T-channels in DH neurons is lacking. Attempts to obtain such evidence should be undertaken using newer, more selective drugs and/or knockout animals.

T-channels located both presynaptically and postsynaptically in DH neurons may have important functions in neuronal plasticity. This possibility is supported by the increase in synaptic strength, cellular excitability, and consequent susceptibility to sensitization of DH neurons, particularly in superficial laminas. These neurons could be especially important in mediation of the abnormal sensitivity to pain that occurs during inflammatory processes or as a result of peripheral nerve injury. This possibility is supported by the expression of transcripts for $Ca_V3.3$, $Ca_V3.2$ and $Ca_V3.1$ subtypes of T-channels in DH neurons (Talley *et al*., 1999), even though the function of the transcripts in these channels has not been clearly demonstrated. Isoform-specific antisense down-regulation of $Ca_V3.2$ and $Ca_V3.3$, but not $Ca_V3.1$ in spinal cord relieved mechanical allodynia and thermal hyperalgesia in rats with chronic compression of DRG (Wen *et al*., 2006). In contrast, mice lacking the $Ca_V3.1$ gene have attenuated pain responses in a neuropathic pain model resulting from L5 spinal nerve ligation (Shin *et al*., 2008). Additional studies will be needed to address the molecular basis of T-channel-mediated nociceptive processing in the DH of the spinal cord.

T-channels in the thalamus

Pain signals from the DH of the spinal cord traverse through the thalamus, the main sensory relay station in the CNS, before reaching higher CNS structures. T-currents in TC cells and the nucleus reticularis thalami have a critical function in the generation of low-amplitude oscillations involving reciprocally interconnected cortical and thalamic neurons. T-currents also are strongly implicated in the states of arousal and sleep, as well as absence seizures (Huguenard, 1996; Perez-Reyes, 2003). T-channel-dependent bursting activity and underlying low-threshold calcium spikes (LTS), which normally are associated with sleep, are accompanied

by slow-frequency and high-amplitude electroencephalographic patterns that rarely occur during awake states.

Given the major function of the thalamus in sensory processing and the abundance of T-channel variants in different thalamic nuclei, is it possible that the alteration of their function leads to changes in nociception? Extracellular recordings from the medial thalamus of patients with neurogenic pain have shown abnormalities of LTS-mediated bursts that could at least contribute to persistent pain (Jeanmonod *et al*., 1996). However, slow-frequency oscillations in the lateral thalamus of patients with various chronic pain syndromes were found not to differ from those in the lateral thalamus of healthy patients (Radhakrishnan *et al*., 1999). These results suggest that the bursting activity of thalamic neurons, possibly mediated by distinct isoforms of T-channels, exhibits different patterns in a variety of chronic pain syndromes.

Through a combination of pharmacological, genetic and electrophysiological studies, it has been shown (Kim *et al*., 2003) that T-channels in ventroposterolateral thalamic relay neurons have an important function in visceral pain. Moreover, mice lacking the $Ca_V3.1$ gene did not exhibit low-threshold bursting in the thalamus. These mice also exhibited hyperalgesia to visceral pain induced by intraperitoneal injections of acidic solutions, a finding that was reproduced by the intra-thalamic infusion of mibefradil. Further studies have shown that both $Ca_V3.1$ T-type and L-type calcium channels in thalamic neurons, respectively, regulate burst and tonic firing mode of these cells, in PKC-dependent pathways (Cheong *et al*., 2008). Thus, in contrast to the pronociceptive function of the peripheral $Ca_V3.2$ T-channels in DRG and the spinal DH, activation of thalamic $Ca_V3.1$ T-channels has an anti-nociceptive effect.

Recent evidence also supports pro-nociceptive roles of $Ca_V3.2$ isoforms of T-channels in the thalamus during the development of acid-induced chronic muscle pain. Chen *et al*. (2010) found that that intramuscular injection of acid induces chronic mechanical hyperalgesia in wild-type and $Ca_V3.1$-deficient mice, but not in $Ca_V3.2$-deficient mice. Furthermore, administration of the T-channel blocker ethosuximide either intraperitoneally or intracerebroventricularly, but not intramuscularly or intrathecally, abolished mechanical hyperalgesia in wild-type mice. The development of mechanical hyperalgesia in wild-type mice requires a signalling cascade involving $Ca_V3.2$-dependent activation of extracellular signal-regulated kinase in the anterior nucleus of the paraventricular thalamus.

The function of T-type channels in animal models of chronic neuropathic pain

When nociceptors are in an increased state of responsiveness, they often respond to normal sensory stimuli as though they were painful (allodynia) and to mildly painful stimuli as though they were acutely painful (hyperalgesia). The electrophysiological correlates of these altered pain responses, collectively termed 'peripheral sensitization', include lower thresholds of activation, increased frequency of firing in

response to suprathreshold stimuli and spontaneous firing (Coderre *et al.*, 1993; Bhave and Gereau, 2004). Although sensitization of peripheral nociceptors contributes to various pain pathologies, the precise cellular mechanisms underlying sensitization are unclear. However, recent studies of different animal models of neuropathic pain support the importance of T-channels in initiating and maintaining chronic neuropathic pain. Thus, blockers of T-channels may provide much-needed new treatments for intractable neuropathic pain symptoms such as hyperalgesia and allodynia.

Chronic constrictive injury to the sciatic nerve

Jagodic *et al.* (2008) has shown that T-current density is significantly increased in small DRG cells in rats with CCI. Redox agents, locally injected, modulate thermal hyperalgesia by similar means, though to different degrees, in healthy rats and rats with CCI (Todorovic *et al.*, 2004b). For example, L-cysteine given to rats with CCI induced thermal hyperalgesia of significantly smaller magnitude and shorter duration than the same doses in intact or sham-operated rats did. However, an oxidizing agent, 5,5′-dithio-bis-(2-nitrobenzoic acid), transiently abolished thermal hyperalgesia in neuropathic pain (NPP) rats and caused a higher degree of analgesia than it did in intact or sham-operated animals. These results suggest that the putative redox-sensitive modulatory site on T-channels in peripheral nociceptors may be in a reduced state after peripheral nerve injury. Similarly, Dogrul *et al.* (2003) found that local intraplantar injections of mibefradil abolished the progression and reversed CCI-induced mechanical and thermal hyperalgesia. In this study, mibefradil exerted similar effects when given systemically, but not intrathecally. Since mibefradil does not cross the blood-brain barrier, this argues that the major site of its antihyperalgesic effect in the CCI model is peripheral. Dogrul and colleagues found, in the same study, that systemic injections of ethosuximide reversed NPP in the rat CCI model.

Pathirathna *et al.* (2005b) reported that ECN, a neuroactive steroid and T-channel blocker, was about fivefold more potent in alleviating thermal hyperalgesia in neuropathic pain in rats with CCI than it was in sham-operated rats. Specific molecular knockdown of $Ca_V3.2$ T-currents in rat nociceptors in the CCI model provided complete relief from thermal and mechanical hyperalgesia and allodynia for up to 7–8 days (Bourinet *et al.*, 2005). Conversely, up-regulation of $Ca_V3.2$ T-channels by endogenous H_2S contributes to the maintenance of neuropathic pain in a spinal nerve injury model (Takahashi *et al.*, 2010).

Chemotherapy-induced peripheral neuropathy

Many drugs that are effective in suppressing the growth of tumour cells also cause painful peripheral neuropathies that limit their use in cancer therapy. Flatters and Bennett (2004) found that systemic administration of ethosuximide alleviated mechanical and cold allodynia and hyperalgesia in rats with paclitaxel- and vincristine-induced painful peripheral neuropathy. Furthermore, chronic use of ethosuximide did not cause tolerance to analgesic effects. These authors reported that the administration of

morphine and/or 5S,10R)-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate, an NMDA blocker, had little effect on paclitaxel-induced neuropathic pain.

Diabetic neuropathy

Little is known about the function of VGCCs in the development of abnormal excitability of sensory neurons and NPP symptoms in animal models of diabetic neuropathy. In diabetic BB/W rats, a model for autoimmune-mediated type I diabetes, up-regulation of HVA Ca^{2+} current amplitude by about twofold occurred in small nociceptive DRG cells, consequently decreasing opioid-induced inhibition of HVA currents (Hall *et al.*, 1996; 2001). BB/W rats, after 8 months in a diabetic state, similarly up-regulated T-channels in small DRG cells (Hall *et al.*, 1995). Similar up-regulation of HVA Ca^{2+} channels in small DRG cells has been reported in rats with streptozotocin (STZ)-induced diabetic neuropathy, a model of type I diabetes (Hall *et al.*, 2001). In contrast, another study with STZ-induced diabetic neuropathy, which used electrophysiological recordings from acutely isolated IB_4-positive medium-size DRG cells, found that T-current amplitude was up-regulated about twofold, while HVA current was not significantly altered (Jagodic *et al.*, 2007; Messinger *et al.*, 2009).

Selective knock-down of DRG $Ca_V3.2$ currents *in vivo* has effectively reversed mechanical and thermal hyperalgesia in STZ-induced diabetic neuropathy in rats (Messinger *et al.*, 2009). Furthermore, significant up-regulation of $Ca_V3.2$ T-channel transcripts in DRG tissue homogenates and concomitant up-regulation of $Ca_V3.2$ T-currents in small nociceptive DRG cells has been reported in another model of painful diabetic neuropathy, leptin-deficient ob/ob mice (Latham *et al.*, 2009). In the same study, these authors found that selective pharmacological antagonism of T-channels by systemically administered ECN effectively reversed thermal and mechanical hyperalgesia in diabetic ob/ob mice, but was completely ineffective in diabetic $Ca_V3.2$ knock-out mice. Thus, by blocking $Ca_V3.2$ T-channels in DRG cells, it may be possible to alleviate intractable pain in patients with diabetic neuropathy.

Conclusions

Recent results strongly implicate T-channels in the processing of nociceptive signals in both the peripheral and CNS. Moreover, many drugs and natural substances at clinically relevant concentrations modulate the function these channels. The $Ca_V3.2$ isoform of these channels expressed in peripheral sensory neurons, spinal DH and thalamic neurons has a well-documented function in supporting cutaneous, muscular and visceral nociceptive transmission. $Ca_V3.2$ channels are also strongly implicated as contributing to the development of neuropathic pain in conditions following mechanical injury of peripheral nerves and peripheral diabetic neuropathy. In contrast, current evidence supports the idea that activation of thalamic $Ca_V3.1$ channels may suppress visceral pain transmission. The putative role of the $Ca_V3.3$ isoform of T-channels in pain processing

has not yet been validated. Because current treatments for both acute and chronic pain are insufficient and often associated with serious side effects, it would be highly useful to develop drug therapies that selectively target the $Ca_V3.2$ isoform of T-channels in pain pathways. Of particular interest would be selective voltage-dependent T-type channel blockers that have no effect in 10- to 100-fold higher concentrations on other classes of voltage-gated ion channels (e.g. Na^+, K^+, HVA-type Ca^{2+} channels). Many previous functional studies were done using non-selective Ca^{2+} channel blockers such as ethosuximide and mibefradil, and these data should be critically re-examined and interpreted using newly available molecular (*in vivo* knock-down) and/or genetic (knockout mice) tools (e.g. Latham *et al.*, 2009). Furthermore, more selective and potent blockers of T-channel are being developed (e.g. TTA compounds, neuroactive steroids) that can further help in deciphering the roles of T-channels in sensory transmission and nociception in particular. However, it is certainly true that T-channel isoforms are expressed in many areas of the nervous system including non-nociceptive peripheral sensory neurons (Table 1), as well as vascular and cardiac tissues that can all be affected by T-channel blockers *in vivo*. Currently, very few clinical and pre-clinical studies are available to assess possible side effects of systemic blockade of T-channels (e.g. sedation, motor weakness, cardiac arrhythmia). However, based on currently available data using rodents, it appears that peripherally acting, voltage-dependent, potent and selective blockers of $Ca_V3.2$ isoform could offer pain relief in acute and chronic pain conditions with minimal side effects.

Acknowledgements

Conflict of interest

None.

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