Univerzita Karlova v Praze

Přírodovědecká fakulta

Studijní program: Speciální chemicko-biologické obory

Studijní obor: Molekulární biologie a biochemie organismů

Tomáš Pelant

Molekulární fyziologie kanálů T-typu aktivovaných nízkým napětím u neuropatických bolestí

Molecular physiology of low-voltage activated T-type channels in neuropathic pain

Bakalářská práce

Školitel: Norbert Weiss, Ph.D.

Praha, 2016

Acknowledgements

I would like to thank Norbert Weiss for giving me valuable advice and showing me a real world of science.

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 18.8.2016

Podpis

Abstract

Low-voltage activated T-type channels contribute significantly to signal transmission in ascending pain pathway. Their electrophysiological and biochemical properties allow them to modulate neuronal excitability and neurotransmitter release. Alterations of electric currents associated with a number of neuronal disorders, including neuropathic pain and epilepsy, have been linked to this subtype of calcium channel, suggesting its prominent role in modulation of neuronal response to various noxious stimuli. Multiple diseases, such as diabetes, cancer or chronic nerve injury, are accompanied by painful neuropathic conditions. Specific inhibitors of T-type channels have been demonstrated to alleviate symptoms of neuropathic pain in mouse models, showing their potential for development of novel type of drugs possibly more effective than traditional analgesics, which exhibit minor effect in neuropathic pain treatment.

Key words: calcium channels, T-type channels, neuropathic pain, nociception, neuronal excitability, analgesics, diabetes

Abstrakt

Nízkonapěťově aktivované iontové kanály se významně podílí na přenosu signálu ve vzestupné dráze bolesti. Jejich elektrofyziologické a biochemické vlastnosti jim umožňují regulovat neuronální excitabilitu a uvolňování neurotransmiterů. Změny v elektrických proudech spojovaných s nervovými poruchami včetně neuropatické bolesti a epilepsie, jsou spojovány s tímto subtypem vápenných kanálů, naznačujíc tak jejich zásadní roli v modulaci neuronální odpovědi na různé obtěžující stimuly. Povícero nemocí, jako třeba cukrovka, rakovina nebo chronický úraz nervu, jsou doprovázeny bolestivými neuropatickými stavy. Specifické inhibitory T-typu kanálů vykazují zlehčující účinky na neuropatickou bolest ve zvířecích modelech, ukazujíce tak jejich potenciál k vývoji nových typů léků možná učinnějších než tradiční analgetika, která vykazují malý efekt při léčbě neuropatické bolesti.

Klíčová slova: vápenné kanály, kanály T-typu, neuropatická bolest, nocicepce, neuronální excitabilita, analgetika, cukrovka

Molecular physiology of low-voltage activated T-type channels in neuropathic pain

Table of Contents

Introduction

The ability to experience pain has its important role in our lives. Pain warns us when a potentially harmful event occurs or is about to occur, and commences a coordinated reflex and behavioral responses which serve as an important protective mechanism. It is a result of a physiological activation of nociceptive cells in peripheral tissues such as skin or visceral organs. This nociceptive pain is usually responsive to traditional pain management drugs and its protective function further continues even in chronic pain, in which case hypersensitivity to pain in inflamed areas protects us from further damaging by promoting careful behavior concerning the affected tissue.

However, pain may also arise without adequate stimulation of peripheral nociceptive pathways, generated by untypical activity of nociceptive neurons. This type of pain is referred to as neuropathic pain and can be defined as "pain arising as a direct consequence of a lesion or disease affecting the somatosensory system." (Treede et al. 2008) This definition distinguishes it from physiological inflammatory pain or pain caused indirectly by other neurological disorders. Neuropathic pain is often connected with several medical conditions, for example a spinal cord injury, multiple sclerosis, autoimmune diseases, diabetes or cancer (Marchettini et al. 2006). Symptoms of neuropathic pain, such as dysesthesia (abnormal sense of touch), hyperalgesia (increased sensitivity to painful stimuli) and allodynia (painful response to normally not painful stimuli), often lead to poor sleep, depression, and anxiety of patients (Turk et al. 2010). Neuropathic pain is also a relatively largely spread disease, affecting up to 7% of the European population (Bouhassira et al. 2008). This, combined with the fact that traditional pain management drugs do not exhibit sufficient results when used to treat neuropathic pain, leads to the necessity of novel drugs development (Finnerup et al. 2015).

One of the possible targets for those drugs are low-voltage activated calcium channels known as T-type calcium channels. Electric currents associated with these channels have been demonstrated to appear in mouse models of absent epilepsy and neuropathic pain. Further research followed, discovering that these channels indeed affect these two diseases.

This review focuses on T-type channels and how they affect physiology of neuropathic pain. Firstly, basic concepts behind pain signalling will be addressed and then the voltage-gated calcium channels will be focused on. Finally, the role of T-type channels in pain signalling will be demonstrated, followed by an overview of their involvement in neuropathic pain states.

Pain pathway

Anatomical review of pain pathway

The first step in ascending pain pathway is mediated by the population of neuronal cells called nociceptors. Their bodies are located in dorsal root ganglia (DRG) and in the trigeminal ganglion for the body innervation and for the face respectively, and by being pseudounipolar neurons they have two axons. The peripheral axon innervates different body parts such as skin, internal organs, joints or muscles, whereas the central axon conducts signal from the body of nociceptor to the second order neuron located in the dorsal horn (DH) of a spinal cord. These cells respond to a wide spectrum of noxious stimuli like heat, cold, inflammation and chemicals, and are usually excited when stimuli from their target location is strong enough to overcome their threshold of excitation, so that they can signalize a potentially harmful situation. Sensitivity of nociceptive fibers to different stimuli is represented by their modality (range of external signals that a specific population of cells in the fiber is sensitive to). Based on a distinctive type of fiber, nociceptors can be further divided into at least two distinct subpopulations which have different morpholigical, physiological and electrophysiological properties (Almeida et al. 2004).

The Aδ-nociceptors have thin myelinated axons and thus they provide a well-localised fast response to noxious stimuli (speed<30 m/s). They are sensitive to heat and mechanical inputs so they are sometimes referred to as AMHs (A-fiber mechano-heat). AMHs are further subdivided into the type I AMHs, which exhibit a high heat threshold and a lower mechanical threshold, and the type II AMHs, which have the opposite properties: a low heat threshold and a high mechanical threshold. The fast response to heat is mostly mediated by the type II AMHs. In contrast, the Type I fiber more likely mediates the first pain provoked by a pinprick and other intense mechanical stimuli.(R.D. Treede, R.A. Meyer 1998)

The C-type nociceptors are also a heterogenous population of neurons. Most of them are polymodal unmyelinated fibers responsive to heat and mechanical stimuli. As a result of unmyelinated axons, their conduction speed is much slower (1 m/s) and responsible for the “second pain” sensation appearing some time after painful stimulation (Van Hees & Gybels 1981)

Both types of these fibers also function as mechanoreceptors in a non-nociceptive way, mostly as receptors of light touch like light pressure of a blunt object or a touch of fur. Another group of neuronal fibers which is largely ignored when talking about pain is Aβ fibers. Most of Aβ fibers are considered Aβ-LTMs with a non-nociceptive function. The abbreviation LTM means low-threshold mechanoreceptor and is widely used. The LTMs' role in pain signalling is discussed as they are

generally viewed only as primary “light touch” receptors. However, certain transduction speeds attributed to Aδ signalling are, by some experts, considered much higher than expected and some other findings also show that nociceptive function of Aβ neurons could be more important than it was thought before. (Djouhri & Lawson 2004)

Different morphological properties of nociceptive cells in DRG are used as a tool to determine the neuron's function. Somas of Aδ- and C-type thin axon neurons associated with nociceptive function exhibit a smaller diameter (<31μm). On the other hand, Aβ-fiber neurons usually have a larger diameter (31-45μm) resulting in their medium-size classification, and their function can be both mechanoreceptive and nociceptive (Lee et al. 1986).

Central axons of primary afferent nociceptive fibers project to dorsal horn neurons in different laminae I, II and V. However, their central processes terminate mostly in superficial layers (lamina I and II). These laminae create major output of the dorsal horn to the CNS; mostly thalamus, but also medulla and brain stem, where they innervate specific locations. Thalamus then serves as a hub to connect different cortical areas which allow us to perceive pain. (Brooks & Tracey 2005)

Ion channels involved in pain pathway

Studies of the transient receptor potential (TRP) family of ion channels helped to establish their distinctive function and their role as nociceptors. More than 30 members of the family were identified and their importance has been indicated in many biological processes (Ramsey et al. 2006) However, the greatest attention is given to three types of these channels: TRPV1, TRPA1 and TRPM8, as they are closely related to pain signalling.

The most studied member of the family is the TRPV1 channel. This receptor is activated by vanilloids like capsaicin. When exposed to capsaicin or its agonists, and also at negative membrane potential, the channel opens, resulting in an influx of calcium and sodium, thereby depolarizing the cell. In this manner, the channel responds to chemical impulses (Hui et al. 2003). The TRPV1 channel also increases its activity when subjected to elevated extracellular proton concentrations, thereby increasing pain sensation (Jordt et al. 2000). Another way in which the channel is modulated is via heat. Experiments showed that when at holding potential of -60 mV and temperature above 43°C, the inward current abruptly increases and gives the channel a thermal-sensing modality (Tominaga et al. 1998).

TRPM8 is a ion channel responsible for cooling and menthol sensation. It induces cold stimuli below 26°C and at temperature of 15°C, it generates noxious stimuli peaking at 8°C. An interesting

property of this channel is that it is probably never co-expressed with the heat-activated TRP channel TRPV1, calcitonin gene-related peptide (CGPR) or isolectin B4 binding (Peier et al. 2002)

The TRPA1 channel responds to a wide range of stimuli in apparently unrelated modalities such as thermal, mechanical and chemical, which in turn activate nociceptive neurons. Research shows that TRPA1 affects mechanotransduction and pain signalling, but many aspects of this topic remain unclear (Garrison & Stucky 2011).

ATP is known to play a crucial role in almost all intracellular processes, however, it has been demonstrated that it can also contribute to pain signalling. ATP is released from damaged cells and can evoke nociception via P2X ionotropic receptors which are selectively expressed in small and medium DRG cells (Gu & MacDermott 1997).

Acid-gated ion channels, also known as proton-gated ion channels or ASICs (acid-sensing ion channels), are gated by pH reductions and probably responsible for pain sensation in inflamed tissues, as acidification is one of the results of inflammatory processes. They are expressed in small DRG cells, indicating their connection to the nociception (Chen et al. 1998).

After the primary signal transduction by the thermal, chemical or mechanical stimuli, signal conduction is carried by a variety of ion channels. The most important channels involved in the conduction of the signal come from the family of sodium channels. The primary role of voltage activated sodium channels is to generate high threshold spikes that convey nociceptive signals to the dorsal horn. There are ten variants identified, each encoded by a different gene with a highly conserved α-subunit and named accordingly $Na_v1.x$. Some of these, namely $Na_v1.7$ and $Na_v1.8$, play a major role in the conduction of the pain signal (Waxman et al. 1999).

Tetrodoxin (TTX)-resistant Na_v 1.8 is implicated as crucial for the pain conduction as the knockout exhibits selective disorders in pain pathways (Akopian et al. 1996). An abnormal activity of (TTX)-sensitive Nav1.7 leads to a number of human pain disorders. Gain-of-function mutations of Nav1.7 result in primary erythermalgia and a paroxysmal extreme pain disorder, whereas people without functional Nav1.7 channel exhibit a rare disorder of total insensivity to physical pain. Studies also show that Nav1.7 is up-regulated in a variety of animal models of inflammatory pain (Drenth et al. 2007). As there are 10 types of sodium channels of different distributions and functional properties, it can be safely assumed that their influence on overall pain management is substantial, making them a desirable target for analgesics (Wood et al. 2004).

Multiple voltage-gated potassium channels are widely expressed in DRG, some of them preferentially located in small DRG nociceptive cells and cells responding to the capsaicin. It also

appears that at least some of them occur in nerve endings, as potassium channel inhibitors administered to nerve endings, not axons, induced continuous discharges in most Aα, Aδ, and C-fibers (Gold et al. 1996).

Voltage-gated calcium channels

Contribution to signal transmission

Different types of Ca^{2+} channels are widely expressed through many different cell types and serve multiple purposes in signalling and cell regulation. The Ca^{2+} influx into the cell causes membrane depolarization at threshold and subthreshold levels and it participates in an electrical signal conduction. Additionally, an increased concentration of calcium ions in cytoplasm leads to a variety of cellular responses as the Ca^{2+} ions act as an important second messenger, causing a cellular response depending on a specific cell type. For example, in cardiac and smooth muscles an increased activity of voltage-gated Ca^{2+} channels (VGCCs) initiates a muscle contraction. Ca^{2+} ions activate ryanodine-sensitive receptors in the membrane of sarcoplasmic reticulum, which causes a Ca^{2+} efflux to the cytoplasm. (Reuter 1979)

In transverse tubules of skeletal muscle, VGCCs directly interact with ryanodine-sensitive Ca^{2+} channels of sarcoplasmic reticulum, causing muscle contraction. When impulses activating the muscle contraction come in a close succession, a cytoplasmic concentration of Ca^{2+} further increases and regulates the force of contraction, eventually leading to tetanus. (Catterall et al. 1991) VGCCs also mediate secretion in endocrine cells (Yang & Berggren 2006) and generally regulate gene expression, enzyme activity and biochemical processes in many cell types. (Flavell & Greenberg 2008) Because of their influence on the presynaptic neurotransmitter release and calcium currents affecting polarization of the membrane, activity of these channels is crucial for the signal conduction in neuronal signalling.

Voltage-gated Ca^{2+} channels are traditionally divided into several categories based on their physiological and pharmacological properties. We distinguish N-, L-, P/Q-, R- and T- types of channels, each blocked by a different specific blocker and with a specific function and expression. (Table 1) These can be further divided into two main classes based on their activation threshold. N-, L-, P/Q- and R- belong to the family of high-voltage activated (HVA) calcium channels and typically exhibit higher activation thresholds, larger conductances and variable inactivation kinetics. On the other hand, T- type channels have a much lower activation threshold, close to the resting potential of the cell, and thus called low-voltage activated channels (LVA). They also exhibit lower conductance and fast inactivation kinetics. (Tsien et al. 1991)

Channel	*Current*	*Localization*	*Specific antagonists*	*Cellular functions*
Cav 1.1	L	Skeletal muscle; transverse tubules	Dihydropyridines; phenylalkylamines; benzothiazepines	Excitation-contraction coupling
Cav 1.2	L	Cardiac myocytes; smooth muscle; myocytes; endocrine cells; neuronal cell bodies; proximal dendrites	Dihydropyridines; phenylalkylamines; benzothiazepines	Excitation-contraction coupling; hormone release; regulation of transcription; synaptic integration
Cav 1.3	L	Endocrine cells; neuronal cell bodies and dendrites; cardiac atrial myocytes and pacemaker cells; cochlear hair cells	Dihydropyridines; phenylalkylamines; benzothiazepines	Hormone release; regulation of transcription; synaptic regulation; cardiac pacemaking; hearing; neurotransmitter release from sensory cells
Cav 1.4	L	Retinal rod and bipolar cells; spinal cord; adrenal gland; mast cells	Dihydropyridines; phenylalkylamines; benzothiazepines	Neurotransmitter release from photoreceptors
Cav 2.1	P/Q	Nerve terminals and dendrites; neuroendocrine cells	ω -Agatoxin IVA	Neurotransmitter release; dendritic Ca transients; hormone release
Cav 2.2	N	Nerve terminals and dendrites; neuroendocrine cells	ω -Conotoxin-GVIA	Neurotransmitter release; dendritic Ca transients; hormone release
Cav 2.3	R	Neuronal cell bodies and dendrites	SNX-482	Repetitive firing; dendritic calcium transients
Cav 3.1	T	Neuronal cell bodies and dendrites; cardiac and smooth muscle myocytes	None	Pacemaking; repetitive firing
Cav 3.2	T	Neuronal cell bodies and dendrites; cardiac and smooth muscle myocytes	None	Pacemaking; repetitive firing
Cav 3.3	T	Neuronal cell bodies and dendrites	None	Pacemaking; repetitive firing

Table 1. Physiological function and pharmacology of voltage-gated calcium channels. Table from (Catterall et al. 2005)

The N-type channels were found to be highly expressed in presynaptic terminals of nociceptive DRG cells. This led to an assumption that their activity is somehow connected to pain signalling (Chaplan et al. 1994). Channels open in response to action potential, thereby causing a calcium influx and a rise of the intracellular concentration of Ca^{2+} ions. The concentration of Ca^{2+} ions heavily affects the rate of neurotransmitter release and this was proved by the following study. Short-term synaptic depression occurs during high synaptic activity, meaning that a number of readily-releasable vesicles is getting lower, thus attenuating neurotransmitter release. The Ca^{2+} modulates vesicle budding and filling only in a close vicinity of Ca^{2+} channels (Wang & Kaczmarek 1998). This spatial requirement suggested an interaction between a calcium channel and synaptic proteins. Studies indeed showed that certain types of N-type HVA channels share a synaptic protein interaction (synprint) site in a linker between domains I and II, which allows such interaction between the channel and SNARE proteins like syntaxin-1A, SNAP-25 and synaptotagmin (Sheng et al. 1994) (Zamponi 2003). This turned out to be a cross-talk, since these synaptic proteins reciprocally inhibit an activity of N-type calcium channels (Catterall et al. 1999), making the regulation of nociceptive signaling a far more complex process.

T-type channels were also described as modulating nociceptive signalling, but to further expand on their function, the structure and characteristics of VGCCs have to be introduced.

Structure of voltage-gated calcium channels

The analysis of VGCCs isolated from rabbit sceletal muscle transversal tubules showed that L-type channels consist of five subunits designated as α1, α2, γ, and δ. This analysis led to a model where 190kDa α1 subunit associates with a 170 kDa disulfide-linked α2δ dimer, a 55kDa intracellular phosphorylated β subunit, and a 33 kDa transmembrane γ subunit.(Takahashi et al. 1987)

The α1 was identified as a pore-forming unit of the channel. The subunit itself has around 2000 aminoacids in length and a structure similar to the pore-forming unit of previously characterized α subunit of the potassium channel. Each of four repeated domains I-IV consists of six membrane α-helical segments S1-S6. The domains are connected together by cytoplasmic regions and also N- and C- termini of protein are located in cytoplasm. (Tanabe et al. 1987) (Figure 1)

The γ subunit is only present in L-type VGCCs, other HVA channels do not contain this subunit and only consist of α1, α2, and δ subunits. T-type channels are specific for the absence of any other regulatory domain and are formed by a single α1 subunit. However, it seems that the α1 subunit is responsible for most of the pharmacological and electrophysiological diversity. (Hofmann et al. 1994)

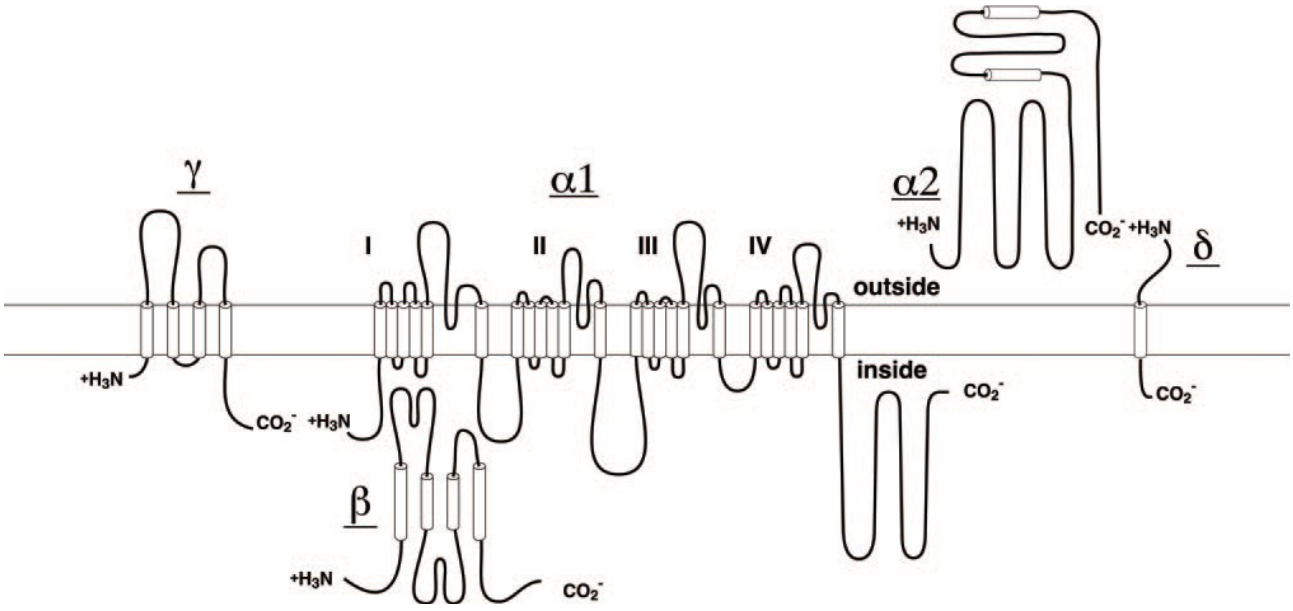


Figure 1 Subunit structure of Cav1 channels. Image taken from (Catterall et al. 2005)

As the identification and description of genes coding α1 subunit continued, new terminology based on a sequential identity between them was adopted. Nowadays, ten genes associated with VGCC are known and they are named $Ca_vX.Y$, where X represents the number of a subfamily and Y the subtype of a channel in an order of their discovery. This nomenclature, common in voltage-gated ion channels, arises from the nomenclature defined for more explored sodium channels. (Table 1) (Goldin et al. 2000)

T-type calcium channels

Molecular Physiology of T-type channels

There are three known members of the T-type subfamily with distinct functional properties, $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$. As mentioned above, they consist of a single α1 which than determines their functional properties. Subtypes of the T-type calcium channels are widely expressed in particular neural tissues in a specific pattern, suggesting that each of them serves a slightly different purpose (Talley et al. 1999).

Precise measurements of their activation kinetics and conductances were made possible by a wider spread of cloning methods as the researchers were able to express defined channels of a single type. DNA of $Ca_v3.1$ was isolated from a rat brain and expressed (and the current associated with the channel consequently measured) for the first time in *Xaenopus* oocytes (Perez-Reyes et al. 1998), $Ca_v3.2$ DNA originated from a human and it was expressed in HEK-293 cells (Cribbs et al. 1998), and finally, $Ca_v3.3$ channel DNA was isolated from a rat brain and expressed in *Xaenopus* oocytes (Lee et al. 1999).

The T-type channels activate in a low membrane potentials, so small positive changes in the membrane potential can open them. At the certain membrane potentials there is also an overlap between open, closed and inactivated state. Under these specific conditions a “window current” appears. A certain fraction of channels is opened, whereby basal influx of calcium ions can occur (Fig 2) (Stephen R. et al. 1997). These currents are associated with a number of thalamic functions, for example during NREM phase of sleep (Crunelli et al. 2006) . Another important attribute of T-type channels is their ability to burst-fire, which occurs during generation of low-threshold spikes (LTS). For LTS patterns to appear, the membrane has to be hyperpolarizated below -70 mV. At this potencial, the T-type channels start to de-inactivate. De-inactivation of LTS takes about 200ms (half of the channels recover after 100ms) and thus T-type channels may function as pacemaker(Perez-Reyes 2003). The LTSs have been also connected to epilepsy in mouse models(Tsakiridou et al. 1995)

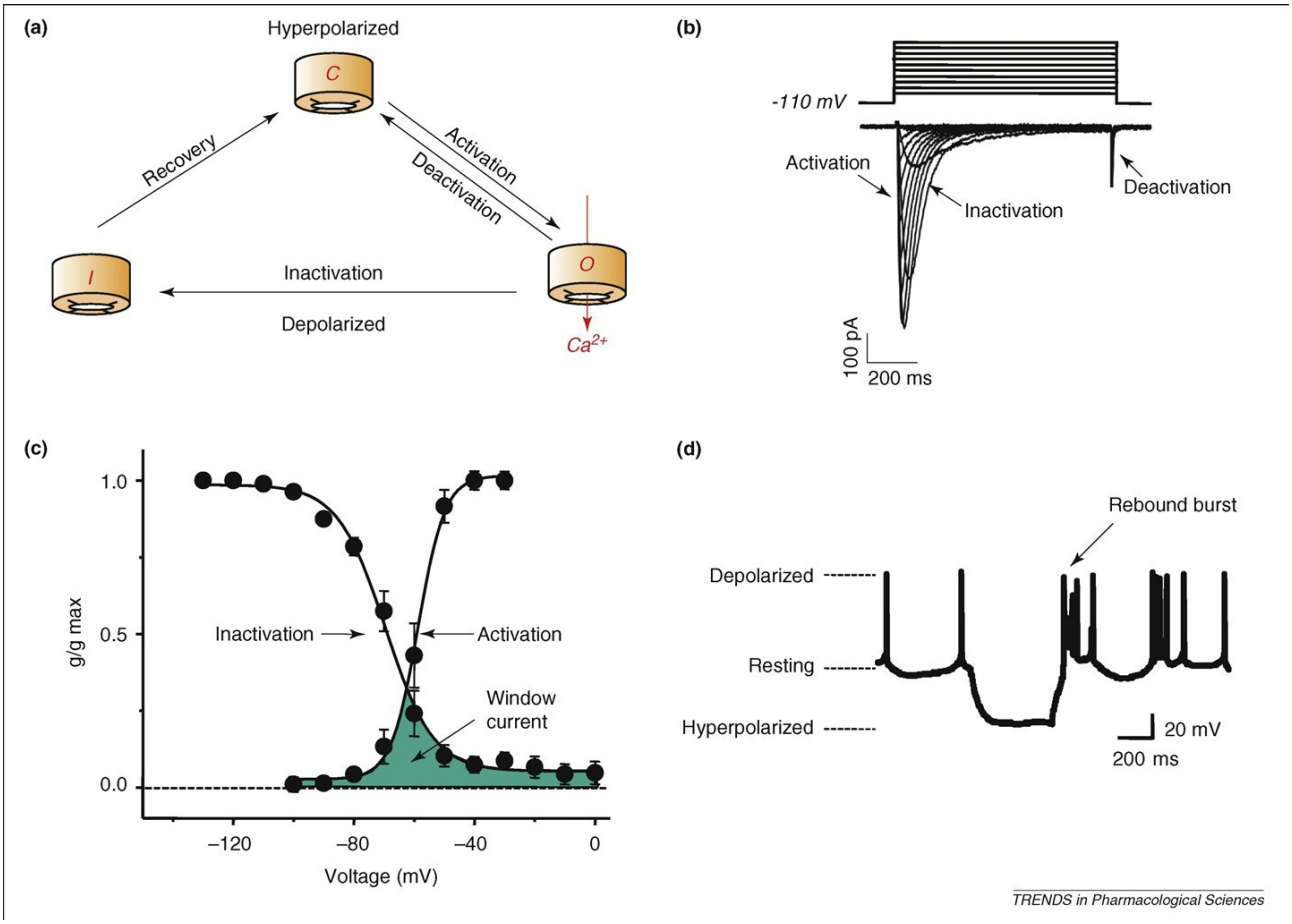
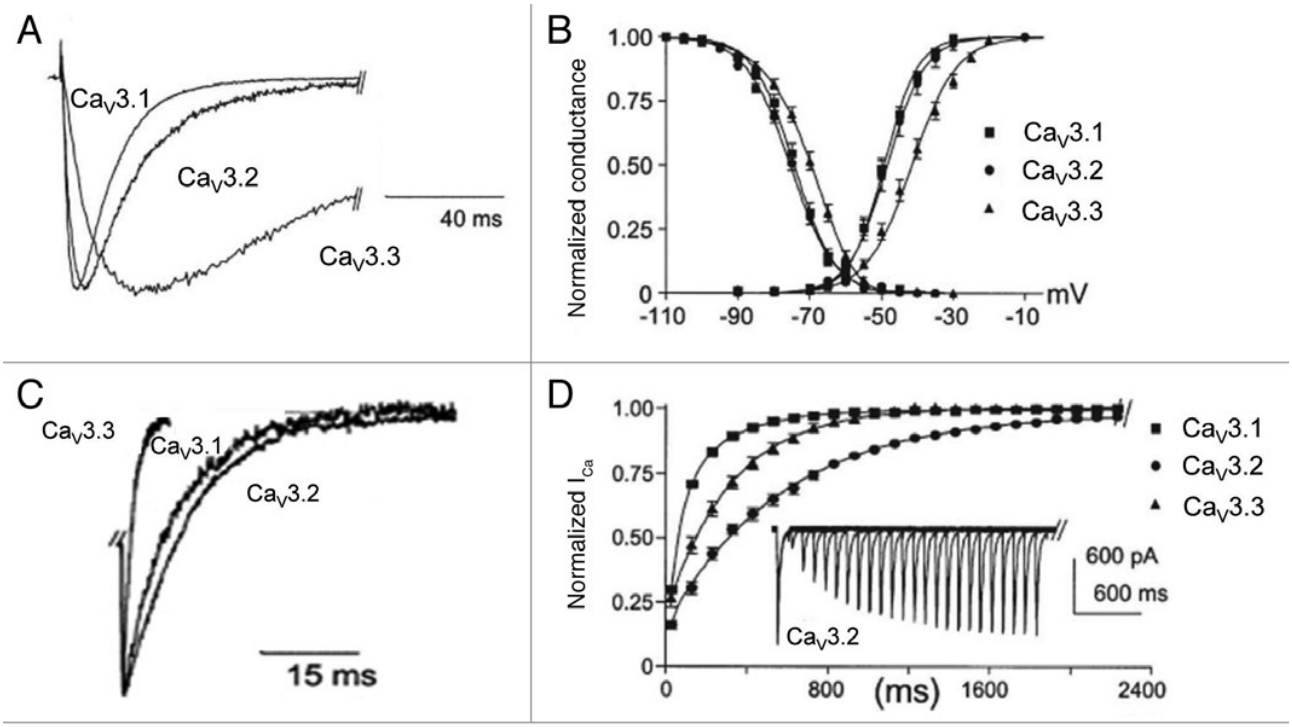


Figure 2. Key functional properties of T-type calcium channels. (a) Simplified scheme of T-type channel gating. At hyperpolarized voltages, channels are in the closed state(C). Membrane depolarization triggers a transition towards the open state (O), which allows calcium entry. Prolonged depolarization causes channels to enter theinactivated state (I), from which channels can be recovered by hyperpolarization. (b) Typical family of whole cell T-type calcium currents, elicited by stepping from a holding potential of -110 mV to a series of incremental depolarizations. (c) Voltage dependence of activation and inactivation. Note that at a typical neuronal resting membranepotential (-75 mV) a significant fraction of the channels are inactivated. Also note the overlap of the activation and inactivation curves, which gives rise to a window current(i.e. a voltage range where T-type channels can be tonically active). (d) Rebound bursting illustrated by the tonic firing of a habenular neuron. In response to amembrane hyperpolarization, T-type channels are recovered from inactivation, and their subsequent activation in response to a return to the resting membrane potential triggers an action potential burst. Image taken from (Iftinca & Zamponi 2009)

The voltage dependencies and rates of channel activation, deactivation, inactivation and recovery from inactivation, define an ability of an individual channel subtype to conduct calcium. The $Ca_v3.1$ and $Ca_v3.2$ both have a slightly different attributes compared to $Ca_v3.3$ isoform: both open at the membrane potential around -10 mV lower and both also inactivate at the membrane potential around -5mV lower, which result in the requirement of more hyperpolarized membrane to reach de-inactivation potential needed for the activation of those channel isoforms(Chemin et al. 2002). The same goes for the activation and deactivation of the channel, $Ca_v3.3$ kinetics in these apects are also much slower. On the contrary, the speed of deactivation is the fastest in $Ca_v3.3$. When the depolarization event occurs, $Ca_v3.1$ and $Ca_v3.2$ activate first, followed by $Ca_v3.3$, then inactivate.

During repolarization, the $Ca_v3.3$ channels close the quickest thanks to their deactivation kinetics. Recovery kinetics are different entirely, $Ca_v3.1$ being the fastest and $Ca_v3.2$ being the slowest (Figure 3) (Kozlov et al. 1999).



E

	Ca$_V$3.1	Ca$_V$3.2	Ca$_V$3.3
Tau Act (ms)	0.8 ± 0.1	1.34 ± 0.1	7.2 ± 0.8
Tau Inact (ms)	18.8 ± 1.6	23.4 ± 0.3	122 ± 5
Tau Deact (ms)	2.6 ± 0.2	3.6 ± 0.4	1.12 ± 0.1
Tau Recovery (ms)	137 ± 5	448 ± 36	260 ± 30

Figure 3. Basic biophysical properties of T-type calcium channels. (A) Representative currents, (B) conductance and inactivation profiles, (C) representative deactivating currents and (D) recovery from inactivation properties of cloned CaV3.1, CaV3.2 and CaV3.3 expressed in HEK293 cells (E) Table summarizing mean kinetic properties of the three T-subtypes at representative voltages. Image taken from (Cain & Snutch 2010)

Role of T-type channels in pain signalling

Low-voltage activated T-type channels have been considered significant for the excitation of DRG neurons for a long time as their presence in the DRG was demonstrated more than 30 years ago (Carbone & Lux 1984). As these channels open thanks to small depolarizations of a membrane, calcium influx into the cell can trigger activation of other voltage-gated channels activated at the slightly higher membrane potentials. In situations like that, T-type channels serve as an amplifier: originally a tiny change in the membrane polarization can cause cascade reaction resulting in a formation of action potential. This subthreshold excitations connected to the T-type channels were observed in a medium-size DRG cells (associated with either nociceptive or mechanosensory

function) (White et al. 1989). Another study showed that small DRG cells expressing the T-type currents are also sensitive to capsaicin, implicating a role of T-types in nociception (Cardenas et al. 1995). The after-depolarizing potential, which is important for setting a threshold for neurotransmitter release, also occurs in T-current rich cells. This, together with a subthreshold-depolarization potency of the T-type calcium channels, implies that T-types are at least partly responsible for setting the sensitivity to nociceptive stimuli (Nelson et al. 2007). These results showed that T-types are likely to contribute to the excitability of DRG neurons tightly connected to the pain signalling.

The T-type calcium channels also generate Ca^{2+} influx into the cell, which may in turn lead to the vesicle exocytosis. Since currents transduced by T-type channels are much lower than usual for other types of calcium channels, significance of T-type contribution was long disputed. However, T-type channels were associated with the low-threshold exocytosis in dorsal horn laminae I and II, where they generate "spontaneous" small excitation potentials (Bao et al. 1998). Since the T-types lack synprint motif present in the $Ca_v2.1$ and $Ca_v2.2$ N-type channels, a distinctive mechanism for the low-threshold exocytosis had to be proposed because, as mentioned earlier, close vicinity between the channel and a budding vesicle is required. A recent study shows that syntaxin-1A is recruited to the $Ca_v3.2$, therefore promoting needed interaction. As opposed to the N-type channels, this interaction is facilitated by carboxyl-terminal domain, suggesting a different interaction principle. Moreover, this interaction decreases channel availability by shifting the steady-state inactivation towards the more hyperpolarized potentials, resulting in a similar effect on the channel as it has on $Ca_v2.1$ and $Ca_v2.2$ N-type channels. (Weiss et al. 2012).

As the DRG nociceptive neurons are primarily connected to dorsal horn neurons in laminae I and II, research of the possible modulation of nociception by T-type channels seemed prospective. Indeed, a study mentioned earlier registered miniature excitatory postsynaptic currents (mEPSC) in these layers, which were caused by the T-type channels and resulted in the neurotransmitter release (Bao et al. 1998). However, a later study examining these currents in the DH discovered that besides mEPSCs, there are miniature excitatory postsynaptic currents (mIPSC) present as well. The authors demonstrated that specific pharmacological inhibition of the $Ca_v3.2$ T-channels by a novel drug TTA-P2 reduces mEPSC frequency in nociceptive projection neurons in the superficial laminae of the DH, while mIPSCs are not affected. This discovery indicates that activation of the presynaptic $Ca_v3.2$ channels may result in a selective increase of excitatory neurotransmitter release at the nociceptive spinal synapse and consequently fine tune the postsynaptic excitability. Although there is not much known about the precise principles behind this kind of regulation, because multiple

HVA channels also contribute to the calcium influx and many other variables are in play, this study opened new prospective research field for the T-type channels involvement in pain signaling (Jacus et al. 2012).

However, it was hard to prove a role of T-types in nociception for a long time due to the absence of selective T-type channel inhibitor. A study showed that injection of reducing agents L-cysteine or DTT (dithiothreitol) up-regulated the activity of DRG neurons and resulted in hyperalgesia. On the other hand, an oxidizing agent, 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) reversed up-regulation and restored the normal phenotype. Hyperalgesia caused by reducing agents was also reversed by the T-type channel blocker mibefradil, thus proving the involvement of the channel. Interestingly, this T-type channel blocker also caused a decreased pain sensation in non-hyperalgaic rats (Todorovic et al. 2001). Results of this study were expanded on by a discovery of "T-rich" cells. When subjected to the L-cystein, this subpopulation of small DRG nociceptive cells manifested lowered threshold of excitability and also started to burst fire. Electrophysiological recordings showed that reason for this different excitability lied in a shift of the T-type channels gating, thus proving their direct involvement in the nociception for the first time (Nelson et al. 2005).

Another challenge was to prove an essential role of the $Ca_v3.2$ subtype in nociception. This channel was known to be expressed more than the other two subtypes in DRG (Talley et al. 1999). Final proof of the prominent $Ca_v3.2$ subtype involvement in pain signalling was laid down by in vivo silencing of mRNA. Rat conditional knockdowns were induced by administering anti-sense oligodeoxynucleotides of each Ca_v3 subtype. Antisense silencing of $Ca_v3.2$ showed a great reduction of corresponding mRNA, protein expression and T-type currents in DRG, and also resulted in major anti-nociceptive, anti-hyperalgesic and anti-allodynic effects. This study gave a first direct evidence of $Ca_v3.2$ involvement in nociception (Bourinet et al. 2005). Results were further supported by a study done on mice lacking the $Ca_v3.2$ gene altogether. Effects similar to those of the previous study were observed. (Choi et al. 2007)

Evidence also shows that T-type channels are regulated by G-protein βγ subunits functionally connected with various G-protein-coupled receptors. The G-protein activated kinases interact with a linker between domain II and III, which serves them as a target site. This linker is therefore subject to the modulation by different hormones, further expanding the ways of T-type current regulation, possibly leading to the development of new drugs targeting T-type channel.

Protein kinase A (PKA) has been suggested to up-regulate a function of the Ca_v3 channels via known deregulations of T-type currents generated by serotonin, cAMP and PKA inhibitors in the rat

adrenal glomerulosa cells (Lenglet et al. 2002), later proven by (Chemin et al. 2007). Serotonin caused enhanced activity of the $Ca_v3.2$ in *Xaenopus* oocytes when coexpressed with 5-HT_7 serotonin receptor, which has its downstream effector in PKA. A target site for the PKA and other kinases was established by a chimeric construct: instead of the original $Ca_v3.2$ II-III linker, construct contained PKA-insensitive $Na_v1.4$ linker. The protein product of the construct was not sesitive to the PKA, indicating the II-III linker as the target site (Kim et al. 2006). On the contrary, dopamine inhibits the T-type currents in the bass retinal horizontal cells (Pfeiffer-Linn & Lasater 1993).

Same as for PKA, protein kinase C (PKC) up- and down-regulates the function of T-type channels. Angiotensin II (Rossier et al. 1995)and endothelin-1 (Furukawa et al. 1992) inhibit T-type channels, cobrotoxin (Zhang et al. 2011) and neurokinin 1 (Rangel et al. 2010) rather enhance function of T-types through different modification of the PKC. Most of these experiments were done on a mouse model neurons from different neuronal tissues, strongly suggesting that PKA and PKC regulatory effects on the T-type currents vary through different cell types, protein interactions and G-proteins involved in the regulatory pathways.

The lysophospatidic acid (LPA) acts through the activation of LPA GPCRs connected with Rho kinase pathway. Rho kinase reversibly inhibits transiently expressed Cav3.1 and Cav3.3 channels and shifts activation and inactivation profiles of Cav3.2 to more depolarized levels. When subjected to the either Rho pathway inhibitors, Rho-associated kinase (ROCK), or RhoA (Ras homolog family GTPase acting on ROCK), the LPA modulation disappeared, promoting the Rho pathway involvement (Iftinca et al. 2007). It should be noted that the LPA acts through at least two receptors, LPA_1 and LPA_3, both of which regulate T-type channels in different manner. LPA_1 signal causes demyelination and sprouting of the dorsal root fibers, induction of the synaptic reorganisation, allodynia, and also up-regulates $Ca_v2.1$ in DRG and PKCγ in dorsal horn. These effects can be used to increase our knowledge of the mechanisms characteristic for neuropathic hyperalgesia in the myelinated sensory A-type fiber. The LPA_3 mediates microglia activation at the early stages of nerve injury and LPA-induced LPA biosynthesis. Although it has been proven that this feed-forward system is associated with the initiation of neuropathic pain, it is not well known how this system functions in the later stages of a pain development (Ueda 2011).

T-type channels in neuropathic pain

Neuropathic pain resulting from mechanical nerve injury

Prominent role of the small DRG cells in neuropathic pain caused by chronic constriction injury (CCI) of sciatic nerve, was established by a study showing that the T-type current density significantly increased in layer 4 and 5 of DRG ipsilateral to CCI. This study also showed that the voltage dependency and activation kinetics of T-type channels were unaffected (Jagodic et al. 2008). Later study, done on a mice suffering from a spinal nerve injury, confirmed these results and indicated a significant rise of mRNA and $Ca_v3.2$ channel expression in rats suffering from CCI (Yue et al. 2013).

Initial studies, concentrated on the function of T-type channels in medium-size DRG cells, showed a mixed results. T-type currents observed in the rats with CCI of peripheral axons showed overall decrease of T-type currents compared to the sham-operated rats (McCallum et al. 2003). These results are in contrast with the data collected by (Yue et al. 2013)and (Jagodic et al. 2008), as they also examined T-type currents in medium-size DRG cells and found no significant change of currents in either way. Possible explanation for this discrepancy lies in the fact that distinctive subtypes of medium-size DRG cells express different amount of T-type currents, and these studies examined different populations. This hypothesis is in agreement with other results showing that the T-type currents appear in both nociceptive (Jagodic et al. 2007) and non-nociceptive (Shin et al. 2003) medium size DRG cells.

Research focused on effects of reducing agent L-cysteine in rats suffering from CCI described dose-dependent increase of hyperalgesia in both CCI and scam-operated rats. However, this increase of hyperalgesia was much smaller and last for the shorter time in CCI rats. DNTB (oxidizing agent) decreased L-cysteine induced hyperalgesia in both groups, although this effect was much more prominent in CCI rats, suggesting that CCI promotes reduction of the T-type channels and upregulate them in this way causing higher excitability of nociceptive neurons. Their data also showed that the mibefradil, T-type calcium channel blocker, abolished hyperalgesia in both CCI and control group of rats (Todorovic et al. 2004). These results were further expanded on by the study mentioned above. In vivo silencing of $Ca_v3.2$ led to the major antinociceptive, anti-hyperalgesic, and anti-allodynic effects in normal, sham-operated and also CCI rats, altogeter putting another data on the top of all these results strongly suggesting T-type prominent involvement in neuropathic pain signalling (Bourinet et al. 2005).

Diabetic neuropathic pain

Diabetes mellitus type 1 is a chronic, multifactorial disease caused by an autoimmune destruction of insulin producing B-cells in pancreas. Even though the insulin treatment shows better results in hyperglycemia management than before, diabetic patients are at a high risk of development of peripheral diabetic neuropathy (PDN), promoting hyperalgesia and allodynia (Alberti & Zimmet 1998). Evidence presented above shows that there is a strong case for the connection between neuropathic pain and the T-type channels. One of the mouse models of diabetes mellitus type 1, Bio Bred/Worchester rat, develops an immunologically mediated diabetes similar to that of the human with symptoms of hyperalgesia. BB/W rat was used for the study exploring relations between the T-type currents and diabetes. The results showed that the T-type currents increased threefold in mixed population of small and medium-size DRG neurons of the BB/W rat (Hall et al. 1995).

The DRG neurons of rats suffering from streptozotocin (STZ)-induced diabetes express lowered threshold for nociceptor activation, increased spontaneous neuronal activity and increased frequency of firing in response to a suprathreshold stimulus. Studies showed that different factors possibly contribute to the elevated pain sensation, namely up-regulation of HVA Ca2+ channel (Hall et al. 2001), increased activity of the voltage-gated Na+ channel (Hong et al. 2004), altered cell-specific expression and increased activity of the TRPV1 (Hong & Wiley 2005). However, later study showed that effects of PDN hyperalgesia in STZ-treated rats can be reversed almost completely by the in vivo silencing of the $Ca_v3.2$ channel (Messinger et al. 2009). Although not that impressive, healthy rats treated with the antisense $Ca_v3.2$ also showed significant decrease in pain sensation. Interesting thing about this study is that the T-type currents increased in small DRG neurons of diabetic rats with no apparent kinetic alterations of the channel. On the contrary, in the study focused on medium-size DRG neurons (also showing an up-regulation of the T-type currents in diabetic rats), depolarizing shift in the steady-state inactivation appeared (Jagodic et al. 2007). There is not much known about this discrepancy, but probable explanation is that a different regulation process of the T-type currents takes place in distinctive cell types. Although there is not much known about these regulatory processes, this topic is even more complex when we take the dorsal horn regulation facilitated by the T-type channels into account. Recent study showed that regulatory currents in DH caused by the T-type channels vary depending on their excitatory or inhibitory function in the DH of STZ-treated rats. However, all of them are subjected to the modulation by T-type channel inhibition (Jacus et al. 2012).

Diabetes mellitus type 2, most common type of diabetes, arises from the insulin resistance and is associated most often with the obesity. Therefore leptin-deficient ob/ob mouse model suffering from

obesity should be a good subject for testing an influence of the T-type channel blockers on PDN symptoms. The study made on this mouse model revealed that in hyperalgaetic obese mice, the mRNA expression of $Ca_v3.2$ channel rised threefold with no effect on the expression of other two T-type channels. The T-type currents were also increased and after treatment with the ECN (novel T-type channel inhibior), hyperalgesia was significantly alleviated, thus showing connection between morbid obesity diabetes, T-type currents and PDN. The observed increase of mRNA expression cannot account for the different T-type activation kinetics described by the authors of this study. This difference could be probably explained by a different glycosylation of Ca_v channels in hyperglycemic environment,which discussed below (Latham et al. 2009).

Recent studies addressed possible involvement of the T-type calcium channels glycosylation in activity and trafficking, both related to the glucosis level of the environment in which the cells were cultivated. Four conserved asparagin-linked N-glycosylation sites are present in the human Cav3.2 channel: on N192, N271, N1466, and N1710. The HEK cells transfected with Cav3.2 channel, exhibited almost complete loss of the T-type current and decreased expression of the channel when treated with N-glycosylation inhibitor. This happened probably due to the decrease in a surface trafficking, as the glycosylation is crucial for channel folding and also important for the membrane targeting. Results also showed that an enzymatic removal of N-glycans from the surface-expressed channels led to the decrease of T-type currents. Altering the glycosylation sites by substituting asparagin with glutamin in the construct breaks the site and, when expressed in the HEK-293 cells, causes different N-glycosylation patterns and channel behavior. Asparagine N192 was identified as responsible for the steady-state surface expression whereas asparagine N1466 reduced channel activity while retaining surface expression levels(Weiss et al. 2013).

These results appear to be particularly interesting in research of diabetic neuropathic pain. Increase of glucose concentration showed a positive effect on channel activity in HEK cells. Further, effects of the channel de-glycosylation facilitated by neuraminidase are more pronounced in small DRG cells expressing T-type channels of diabetic mice than in the same cells of WT mice. Treatment of diabetic mice with neuraminidase also resulted in decrease of hyperalgia, whereas same treatment in WT mice had no effect. From this it can be concluded that modulation of T-type activity by N-glycosylation is physiologicaly important and probably plays a significant role in diabetic neuropathic pain(Orestes et al. 2013).

Neuropathic pain in cancer treatment

Chemotherapy-evoked neuropathic pain is a major cause of discontinuation of otherwise successful anti-cancer chemotherapy. Paclitaxel is an effective anti-tumor suppresor, but with a strong

neuropathic side effects caused by its peripheral neurotoxicity. The symptoms of paclitaxel-induced neuropathy are mostly sensory and peripheral in their nature, consisting of mechanical allodynia, cold allodynia, ongoing burning pain, tingling and numbness and those symptoms often last for months or even years after finishing the treatment (Forsyth et al. 1997). These severe side effects make the paclitaxel-treated mice important experimental model for testing of analgesics decreasing the pain felt by cancer patients. Standard analgesics does not work well on paclitaxel-induced neuropathic pain, but results show that the ethosuximide, relatively selective T-type calcium channel antagonist, almost completely reverses the pain induced by paclitaxel treatment. Also, no tolerance to this analgesic appeared even after prolonged treatment (Flatters & Bennett 2004).

In another study, the researchers demonstrated that the mibefranil, more specific inhibitor of the T-type channels than ethosuximid, also showed an inhibitory effect on the neuropathic pain in paclitaxel-treated mice. On top of that, authors described a similar inhibitory effect of the cystathionine-γ-lyase (CSE) blocker (Okubo et al. 2011). This could be explained by the fact that CSE creates the H_2S from the L-cysteine(Kamoun 2004), well known up-regulator of the $Ca_v3.2$ channel activity. The H_2S preferably upregulates $Ca_v3.2$, not the other two T-types. This fact can be explained by looking at the Histidine 191 in the channel. It is well conserved in the $Ca_v3.2$, but not in the $Ca_v3.1$ nor $Ca_v3.3$, suggesting that His serves as a site for redox modulations, in which the H_2S participates. There is also a good possibility that the H_2S gasotransmitter is responsible for the up-regulation of $Ca_v3.2$ rather than an endogenous L-cystein, because the His191-containing linker is located extracellulary (Nelson et al. 2007). During experiments done on the paclitaxel-treated rats, total expression of CSE and $Ca_v3.2$ channels did not change, suggesting that the paclitaxel possibly induces a higher surface trafficking of the $Ca_v3.2$ channel (Okubo et al. 2011).

Conclusion

Recent results presented here strongly suggest the T-type channel involvement in the pain signalling. Although a lot of aspects and principles, such as lowering the threshold for neuronal excitability, neurotransmitter release, interactions with other ion channels and intracellular signalling pathways, regulation of the thalamic T-type burst-firing (Kim et al. 2003), remain to be further explored, it is probably safe to assume that the T-type channels can significantly alter nociceptive perception. However, further research needs to be done to identify the exact principles of how these channels relate to the overall pain signalling.

The data collected from animal models of pain are also pointing out that the T-type channel blockers may serve as a powerful potential analgesics in neuropathic states, because classical analgesics did

not show great success in a neuropathic pain management. However, these channel inhibitors exhibit different levels of specifity. To achieve analgesic effect, concentration of the ethosuximide, the T-channel blocker used in some of the studies presented above and also in a treatment of epilepsy (Huguenard 2002), have to be high, and at these levels it also blocks other types of ion channels (Leresche et al. 1998). Another popular and potent inhibitor was even approved for hypertension treatment in the US, but soon retracted as the other ion channel-blockers and cytochrom interactions appeared (Mullins et al. 1998).

Two T-type channel blockers, with similar effects as the TTA-P2 used in animal models of neuropathic pain, are currently being tested on humans for safety and pain reduction effects. The Z994 T-type blocker exhibited positive effects in reducing the pain inflicted by capsaicin and UV irritation of the skin and continues to the second phase of clinical testing (Lee 2014). The ABT-639 was used in a study performed on the patients suffering from diabetic neuropathic pain with mixed results. Although a short-term pain relief was observed, long-term effects did not significantly differ from the patients treated with placebo (Ziegler et al. 2015).

In conclusion, the T-type channels seem to be a good potential target for the treatment of neuropathic pain. Because of the prominent $Ca_v3.2$ expression in the peripheral neurons associated with nociceptive function, a future inhibitor of this T-type channel subtype not penetrating bloodstream-brain barrier could be a viable drug for the treatment of painful symptoms of peripheral neuropathy.

References

Akopian, A.N., Sivilotti, L. & Wood, J.N., 1996. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. Nature, 379(6562), pp.257–262.

Alberti, K.G.M.M. & Zimmet, P.Z., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. Diabetic Medicine, 15(7), pp.539–553.

Almeida, T.F., Roizenblatt, S. & Tufik, S., 2004. REVIEW: Afferent pain pathways: a neuroanatomical review. Brain Research, 1000(1), pp.40–56.

Bao, J., Li, J.J. & Perl, E.R., 1998. Differences in Ca2+ channels governing generation of miniature and evoked excitatory synaptic currents in spinal laminae I and II. The Journal of neuroscience : the official journal of the Society for Neuroscience, 18(21), pp.8740–50.

Bouhassira, D. et al., 2008. Prevalence of chronic pain with neuropathic characteristics in the general population. PAIN, 136(3), pp.380–387.

Bourinet, E. et al., 2005. Silencing of the Cav3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception. The EMBO journal, 24(2), pp.315–24.

Brooks, J. & Tracey, I., 2005. From nociception to pain perception: imaging the spinal and supraspinal pathways. Journal of Anatomy, 207(1), pp.19–33.

Cain, S.M. & Snutch, T.P., 2010. Contributions of T-type calcium channel isoforms to neuronal firing. Channels, 4(6), pp.475–482.

Carbone, E. & Lux, H.D., 1984. A low voltage-activated, fully inactivating Ca channel in vertebrate sensory neurones. Nature, 310(5977), pp.501–502.

Cardenas, C.G., Del Mar, L.P. & Scroggs, R.S., 1995. Variation in serotonergic inhibition of calcium channel currents in four types of rat sensory neurons differentiated by membrane properties. Journal of neurophysiology, 74(5), pp.1870–9.

Catterall, W.A. et al., 1991. Excitation-contraction coupling in vertebrate skeletal muscle: a tale of two calcium channels. Cell, 64(5), pp.871–4.

Catterall, W.A. et al., 2005. International Union of Pharmacology. XLVIII. Nomenclature and Structure-Function Relationships of Voltage-Gated Calcium Channels. Pharmacological Reviews, 57(4), pp.411–425.

Catterall, W.A. et al., 1999. Reciprocal regulation of P/Q-type Ca2+ channels by SNAP-25,syntaxin and synaptotagmin. Nature Neuroscience, 2(11), pp.939–941.

Cribbs, L.L. et al., 1998. Cloning and Characterization of 1H From Human Heart, a Member of the T-Type Ca2+ Channel Gene Family. Circulation Research, 83(1), pp.103–109.

Crunelli, V., Cope, D.W. & Hughes, S.W., 2006. Thalamic T-type Ca2+ channels and NREM sleep. Cell Calcium, 40(2), pp.175–190.

Djouhri, L. & Lawson, S.N., 2004. REVIEW:Aβ-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. Brain Research Reviews, 46(2), pp.131–145.

Drenth, J.P.H. et al., 2007. Mutations in sodium-channel gene SCN9A cause a spectrum of human genetic pain disorders. Journal of Clinical Investigation, 117(12), pp.3603–3609.

Finnerup, N.B. et al., 2015. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. The Lancet Neurology, 14(2), pp.162–173.

Flatters, S.J.. & Bennett, G.J., 2004. Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. Pain, 109(1), pp.150–161.

Flavell, S.W. & Greenberg, M.E., 2008. REVIEW: Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annual review of neuroscience, 31, pp.563–90.

Forsyth, P. a. et al., 1997. Prospective study of paclitaxel-induced peripheral neuropathy with quantitative sensory testing. Journal of Neuro-Oncology, 35(1), pp.47–53.

Furukawa, T. et al., 1992. Endothelin-1 enhances calcium entry through T-type calcium channels in cultured neonatal rat ventricular myocytes. Circulation Research, 71(5), pp.1242–1253.

Garrison, S.R. & Stucky, C.L., 2011. The dynamic TRPA1 channel: a suitable pharmacological pain target? Current pharmaceutical biotechnology, 12(10), pp.1689–97.

Gold, M.S., Shuster, M.J. & Levine, J.D., 1996. Characterization of six voltage-gated K+ currents in adult rat sensory neurons. Journal of neurophysiology, 75(6), pp.2629–46.

Goldin, A.L. et al., 2000. Nomenclature of Voltage-Gated Sodium Channels. Neuron, 28(2), pp.365–368.

Gu, J.G. & MacDermott, A.B., 1997. Activation of ATP P2X receptors elicits glutamate release from sensory neuron synapses. Nature, 389(6652), pp.749–753.

Hall, K.E. et al., 2001. Impaired inhibitory G-protein function contributes to increased calcium currents in rats with diabetic neuropathy. Journal of neurophysiology, 86(2), pp.760–70.

Hall, K.E., Sima, A.A. & Wiley, J.W., 1995. Voltage-dependent calcium currents are enhanced in dorsal root ganglion neurones from the Bio Bred/Worchester diabetic rat. The Journal of physiology, (Pt 2), pp.313–22.

Van Hees, J. & Gybels, J., 1981. C nociceptor activity in human nerve during painful and non painful skin stimulation. Journal of neurology, neurosurgery, and psychiatry, 44(7), pp.600–7.

Hofmann, F. et al., 1994. REVIEW: Annual review of neuroscience 4i contents. JA, 17.

Hong, S. et al., 2004. Early painful diabetic neuropathy is associated with differential changes in tetrodotoxin-sensitive and -resistant sodium channels in dorsal root ganglion neurons in the rat. The Journal of biological chemistry, 279(28), pp.29341–50.

Hong, S. & Wiley, J.W., 2005. Early Painful Diabetic Neuropathy Is Associated with Differential Changes in the Expression and Function of Vanilloid Receptor 1. Journal of Biological Chemistry, 280(1), pp.618–627.

Huguenard, J.R., 2002. Block of T -Type Ca(2+) Channels Is an Important Action of Succinimide Antiabsence Drugs. Epilepsy currents / American Epilepsy Society, 2(2), pp.49–52.

Hui, K., Liu, B. & Qin, F., 2003. Capsaicin activation of the pain receptor, VR1: multiple open states from both partial and full binding. J Gen Physiol., 84, pp.2957–2968.

Chaplan, S.R., Pogrel, J.W. & Yaksh, T.L., 1994. Role of voltage-dependent calcium channel subtypes in experimental tactile allodynia. The Journal of pharmacology and experimental therapeutics, 269(3), pp.1117–23.

Chemin, J. et al., 2002. Specific contribution of human T-type calcium channel isotypes (alpha(1G), alpha(1H) and alpha(1I)) to neuronal excitability. The Journal of physiology, 540(Pt 1), pp.3– 14.

Chemin, J. et al., 2007. Temperature-dependent Modulation of CaV3 T-type Calcium Channels by Protein Kinases C and A in Mammalian Cells. Journal of Biological Chemistry, 282(45), pp.32710–32718.

Chen, C.-C. et al., 1998. A sensory neuron-specific, proton-gated ion channel. Proceedings of the National Academy of Sciences, 95(17), pp.10240–10245.

Choi, S. et al., 2007. Attenuated pain responses in mice lacking Ca(V)3.2 T-type channels. Genes, brain, and behavior, 6(5), pp.425–31.

Iftinca, M. et al., 2007. Regulation of T-type calcium channels by Rho-associated kinase. Nature Neuroscience, 10(7), pp.854–860.

Jacus, M.O. et al., 2012. Presynaptic Cav3.2 channels regulate excitatory neurotransmission in nociceptive dorsal horn neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience, 32(27), pp.9374–82.

Jagodic, M.M. et al., 2007. Cell-specific alterations of T-type calcium current in painful diabetic neuropathy enhance excitability of sensory neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience, 27(12), pp.3305–16.

Jagodic, M.M. et al., 2008. Upregulation of the T-type calcium current in small rat sensory neurons after chronic constrictive injury of the sciatic nerve. Journal of neurophysiology, 99(6), pp.3151–6.

Jordt, S.-E., Tominaga, M. & Julius, D., 2000. Acid potentiation of the capsaicin receptor determined by a key extracellular site. Proceedings of the National Academy of Sciences, 97(14), pp.8134–8139.

Kamoun, P., 2004.REVIEW: Endogenous production of hydrogen sulfide in mammals. Amino Acids, 26(3), pp.243–254.

Kim, D. et al., 2003. Thalamic control of visceral nociception mediated by T-type Ca2+ channels. Science (New York, N.Y.), 302(5642), pp.117–9.

Kim, J.-A. et al., 2006. Augmentation of Cav3.2 T-type calcium channel activity by cAMPdependent protein kinase A. The Journal of pharmacology and experimental therapeutics, 318(1), pp.230–7.

Kozlov, A.S. et al., 1999. Distinct kinetics of cloned T-type Ca2 + channels lead to differential Ca2 + entry and frequency-dependence during mock action potentials. The European journal of neuroscience, 11(12), pp.4149–58.

Latham, J.R. et al., 2009. Selective T-type calcium channel blockade alleviates hyperalgesia in ob/ob mice. Diabetes, 58(11), pp.2656–65.

Lee, J.H. et al., 1999. Cloning and expression of a novel member of the low voltage-activated Ttype calcium channel family. The Journal of neuroscience : the official journal of the Society for Neuroscience, 19(6), pp.1912–21.

Lee, K.H. et al., 1986. Correlation of cell body size, axon size, and signal conduction velocity for individually labelled dorsal root ganglion cells in the cat. The Journal of comparative neurology, 243(3), pp.335–46.

Lee, M., 2014. Z944: A first in class T-type calcium channel modulator for the treatment of pain. Journal of the Peripheral Nervous System, 19(S2), pp.S11–S12.

Lenglet, S. et al., 2002. Activation of 5-HT(7) receptor in rat glomerulosa cells is associated with an increase in adenylyl cyclase activity and calcium influx through T-type calcium channels. Endocrinology, 143(5), pp.1748–60.

Leresche, N. et al., 1998. On the action of the anti-absence drug ethosuximide in the rat and cat thalamus. The Journal of neuroscience : the official journal of the Society for Neuroscience, 18(13), pp.4842–53.

Marchettini, P. et al., 2006. REVIEW: Painful Peripheral Neuropathies. Current Neuropharmacology, 4(3), pp.175–181.

McCallum, J.B. et al., 2003. Loss of T-type calcium current in sensory neurons of rats with neuropathic pain. Anesthesiology, 98(1), pp.209–16.

Messinger, R.B. et al., 2009. In vivo silencing of the Ca(V)3.2 T-type calcium channels in sensory neurons alleviates hyperalgesia in rats with streptozocin-induced diabetic neuropathy. Pain, 145(1-2), pp.184–95.

Mullins, M.E. et al., 1998. Life-threatening interaction of mibefradil and beta-blockers with dihydropyridine calcium channel blockers. JAMA, 280(2), pp.157–8.

Nelson, M.T. et al., 2007. Reducing agents sensitize C-type nociceptors by relieving high-affinity zinc inhibition of T-type calcium channels. The Journal of neuroscience : the official journal of the Society for Neuroscience, 27(31), pp.8250–60.

Nelson, M.T. et al., 2005. The endogenous redox agent L-cysteine induces T-type Ca2+ channeldependent sensitization of a novel subpopulation of rat peripheral nociceptors. The Journal of neuroscience : the official journal of the Society for Neuroscience, 25(38), pp.8766–75.

Okubo, K. et al., 2011. Inhibition of T-type calcium channels and hydrogen sulfide-forming enzyme reverses paclitaxel-evoked neuropathic hyperalgesia in rats. Neuroscience, 188, pp.148–156.

Orestes, P. et al., 2013. Reversal of neuropathic pain in diabetes by targeting glycosylation of Ca(V)3.2 T-type calcium channels. Diabetes, 62(11), pp.3828–38.

Peier, A.M. et al., 2002. A TRP Channel that Senses Cold Stimuli and Menthol. Cell, 108(5), pp.705–715.

Perez-Reyes, E. et al., 1998. Molecular characterization of a neuronal low-voltage-activated T-type calcium channel. Nature, 391(6670), pp.896–900.

Perez-Reyes, E., 2003. REVIEW: Molecular physiology of low-voltage-activated t-type calcium channels. Physiological reviews, 83(1), pp.117–61.

Pfeiffer-Linn, C. & Lasater, E.M., 1993. Dopamine modulates in a differential fashion T- and L-type calcium currents in bass retinal horizontal cells. The Journal of General Physiology, 102(2), pp.277–294. R.D.

Treede, R.A. Meyer, J.N.C., 1998. No Ti, Myelinated mechanically insensitive afferents from monkey hairy skin: heat-response propertiestle. J. Neurophysiol., (80), pp.1082–1093.

Ramsey, I.S., Delling, M. & Clapham, D.E., 2006. REVIEW:An introduction to TRP channels. Annual Review of Physiology, 68(1), pp.619–647.

Rangel, A., Sanchez-Armass, S. & Meza, U., 2010. Protein Kinase C-Mediated Inhibition of Recombinant T-Type CaV3.2 Channels by Neurokinin 1 Receptors. Molecular Pharmacology, 77(2), pp.202–210.

Reuter, H., 1979. Properties of Two Inward Membrane Currents in the Heart. Annual Review of Physiology, 41(1), pp.413–424.

Rossier, M.F. et al., 1995. Inhibition of low threshold calcium channels by angiotensin II in adrenal glomerulosa cells through activation of protein kinase C. The Journal of biological chemistry, 270(25), pp.15137–42.

Sheng, Z.-H. et al., 1994. Identification of a syntaxin-binding site on N-Type calcium channels. Neuron, 13(6), pp.1303–1313.

Shin, J.-B. et al., 2003. A T-type calcium channel required for normal function of a mammalian mechanoreceptor. Nature neuroscience, 6(7), pp.724–30.

Stephen R., W. et al., 1997. The “window” component of the low threshold Ca2+ current produces input signal amplification and bistability in cat and rat thalamocortical neurones. Journal of Physiology, 505, pp.689–705.

Takahashi, M. et al., 1987. Subunit structure of dihydropyridine-sensitive calcium channels from skeletal muscle. Proceedings of the National Academy of Sciences of the United States of America, 84(15), pp.5478–82.

Takahashi, T. et al., 2010. Upregulation of Cav3.2 T-type calcium channels targeted by endogenous hydrogen sulfide contributes to maintenance of neuropathic pain. PAIN, 150(1), pp.183–191.

Talley, E.M. et al., 1999. Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels. The Journal of neuroscience : the official journal of the Society for Neuroscience, 19(6), pp.1895–911.

Tanabe, T. et al., 1987. Primary structure of the receptor for calcium channel blockers from skeletal muscle. Nature, 328(6128), pp.313–318.

Todorovic, S.M. et al., 2001. Redox Modulation of T-Type Calcium Channels in Rat Peripheral Nociceptors. Neuron, 31(1), pp.75–85.

Todorovic, S.M., Meyenburg, A. & Jevtovic-Todorovic, V., 2004. Redox modulation of peripheral T-type Ca2+ channels in vivo: alteration of nerve injury-induced thermal hyperalgesia. Pain, 109(3), pp.328–339.

Tominaga, M. et al., 1998. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron, 21(3), pp.531–43.

Treede, R.-D. et al., 2008. Neuropathic pain: Redefinition and a grading system for clinical and research purposes. Neurology, 70(18), pp.1630–1635.

Tsakiridou, E. et al., 1995. Selective increase in T-type calcium conductance of reticular thalamic neurons in a rat model of absence epilepsy. The Journal of neuroscience : the official journal of the Society for Neuroscience, 15(4), pp.3110–7.

Tsien, R.W., Ellinor, P.T. & Horne, W.A., 1991. REVIEW: Molecular diversity of voltagedependent Ca2+ channels. Trends in Pharmacological Sciences, 12, pp.349–354.

Turk, D.C. et al., 2010. Assessment and treatment of psychosocial comorbidities in patients with neuropathic pain. Mayo Clinic proceedings, 85(3 Suppl), pp.S42–50.

Ueda, H., 2011. Lysophosphatidic acid as the initiator of neuropathic pain. Biological & pharmaceutical bulletin, 34(8), pp.1154–8.

Wang, L.-Y. & Kaczmarek, L.K., 1998. High-frequency firing helps replenish the readily releasable pool of synaptic vesicles. Nature, 394(6691), pp.384–388.

Waxman, S.G. et al., 1999.REVIEW: Sodium channels and pain. Proceedings of the National Academy of Sciences, 96(14), pp.7635–7639.

Weiss, N. et al., 2012. A Cav3.2/Syntaxin-1A Signaling Complex Controls T-type Channel Activity and Low-threshold Exocytosis. Journal of Biological Chemistry, 287(4), pp.2810–2818.

Weiss, N. et al., 2013. Surface expression and function of Cav3.2 T-type calcium channels are controlled by asparagine-linked glycosylation. Pflügers Archiv - European Journal of Physiology, 465(8), pp.1159–1170.

White, G., Lovinger, D.M. & Weight, F.F., 1989. Transient low-threshold Ca2+ current triggers burst firing through an afterdepolarizing potential in an adult mammalian neuron. Proceedings of the National Academy of Sciences of the United States of America, 86(17), pp.6802–6.

Wood, J.N. et al., 2004. REVIEW: Voltage-gated sodium channels and pain pathways. Journal of Neurobiology, 61(1), pp.55–71.

Yang, S.-N. & Berggren, P.-O., 2006. REVIEW: The Role of Voltage-Gated Calcium Channels in Pancreatic β-Cell Physiology and Pathophysiology. Endocrine Reviews, 27(6), pp.621–676.

Yue, J. et al., 2013. Upregulation of T-type Ca2+ channels in primary sensory neurons in spinal nerve injury. Spine, 38(6), pp.463–70.

Zamponi, G.W., 2003. Regulation of Presynaptic Calcium Channels by Synaptic Proteins. Journal of Pharmacological Sciences, 92(2), pp.79–83.

Zhang, Y. et al., 2011. Activation of M3 muscarinic receptors inhibits T-type Ca2+ channel currents via pertussis toxin-sensitive novel protein kinase C pathway in small dorsal root ganglion neurons. Cellular Signalling, 23(6), pp.1057–1067.

Ziegler, D. et al., 2015. A randomized double-blind, placebo-, and active-controlled study of Ttype calcium channel blocker ABT-639 in patients with diabetic peripheral neuropathic pain. Pain, 156(10), pp.2013–20.