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Targeting N-type and T-type calcium channels for the treatment of pain

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Severe chronic pain afflicts a large number of people worldwide but satisfactory relief from such pain is difficult to achieve with drugs that are currently available, and so there is a great need for the development of new, efficacious and safe analgesics. Voltage-gated calcium-permeable ion channels are multi-subunit complexes that regulate neuronal excitability, action-potential firing patterns and neurotransmission in nociceptive pathways. Although multiple subtypes of voltage-gated calcium channels exist, pharmacological and ion-channel gene knockdown approaches in animals have revealed N-type and T-type calcium channels to be particularly attractive molecular targets for the discovery and development of new analgesic drugs. The recent approval of Prialt® (Elan Pharmaceuticals) provides the ultimate target validation for N-type calcium channels, namely proof that they are key regulators of nociceptive signaling in humans.

Basic aspects of pain

Pain is an unpleasant sensory and emotional experience that is associated with actual or potential tissue damage (definition promoted by the International Association for the Study of Pain®). Each year millions of patients visit their doctors seeking medication to relieve their pain and the annual costs in terms of healthcare and lost productivity, in the USA alone, have been estimated to be >US$60 billion [1]. Because pain is a highly subjective and personal experience, the manner in which it impacts an individual's quality of life and work productivity will depend on a variety of factors including previous experience, personal history and individual tolerance.

Neuronal pathways involved in pain sensation

The process of pain sensation is initiated when nociceptive nerve endings in skin, bone, joints, muscle or visceral organs are stimulated mechanically, thermally, chemically or electrically. Following stimulation, sodium-dependent action potentials propagate away from the nerve endings towards the spinal cord along the small-diameter unmyelinated (C-fiber) or myelinated (Aδ-fiber) axons of primary afferent nociceptors whose cell bodies are located in the dorsal root ganglia (DRG). When the action potentials reach the central terminals of sensory neurons in the spinal cord, calcium influx through presynaptic voltage-gated calcium channels triggers the release of pronociceptive neurotransmitters and neuromodulators such as substance P, calcitonin gene-related peptide (CGRP) and glutamate [2–4]. These substances bind to specific postsynaptic receptors to excite or sensitize secondary sensory neurons that are located in superficial laminae of the dorsal horn. It is believed that the pain signals are then transmitted along the axons of the secondary sensory neurons via two parallel polysynaptic pathways (i.e. the lateral and medial pain systems). The lateral pain system is thought to encode the sensory-discriminative properties of painful stimuli. In this system, signals pass through the lateral thalamic nuclei to brain regions, including the primary and secondary somatosensory cortices (SI and SII), where they are perceived as pain and localized to a particular body region. The medial pain system is thought to encode the motivational-affective properties of painful stimuli. In this system, signals pass through medial thalamic nuclei to brain areas including the prefrontal and anterior cingulate cortices where they are perceived as unpleasant, prompting the initiation of appropriate defense responses.

Acute pain

Pain can be classified clinically on the basis of its etiology, underlying mechanisms, anatomical location, duration, disease association

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and drug sensitivity. In terms of time-course, pain can be acute or chronic and in terms of intensity, it can be mild, moderate or severe. Acute pain is a normal sensation that can be evoked by any surgery, accidental injury or trauma that excites nociceptive neurons. Acute pain serves a protective function and in its mild forms can usually be relieved with over-the-counter drugs containing active ingredients such as acetaminophen (e.g. Tylenol®, McNeil Consumer and Specialty Pharmaceuticals). Although too much pain can be extremely debilitating and is more problematic to treat, some therapies are available on prescription for the alleviation of moderate-to-severe acute pain. These include opioid drugs such as oxycodone (Oxycontin®, Purdue Pharma) and morphine (e.g. MS-Contin®, Purdue Pharma), which act as agonists of opioid receptors in the central nervous system (CNS). However, these drugs can cause serious adverse side-effects, are prone to addiction and can promote the development of tolerance with repeated use. Hence, moderate-to-severe acute pain remains a considerable medical problem.

Chronic inflammatory pain

In contrast to acute pain, chronic pain is an abnormal condition. By definition, it does not resolve over time and unfortunately it can be partially or totally unresponsive to treatment. Chronic pain can be subcategorized as inflammatory or neuropathic, although more-complex pain syndromes can include components of both categories. Chronic inflammatory pain occurs in response to tissue injury or the presence of an invading foreign substance. Proinflammatory mediators, such as prostaglandins, adenosine, histamine and bradykinin, that are released by damaged tissue and/or immune cells can sensitize or activate nociceptors and, as a result, the individual experiences hypersensitivity as well as spontaneous and persistent pain. Inflammatory pain can be treated with nonsteroidal anti-inflammatory drugs (NSAID's) such as ibuprofen (e.g. Advil®, Wyeth) and naproxen (e.g. Aleve®, Bayer). These NSAID's reduce prostaglandin synthesis by inhibiting the activity of the cyclooxygenase enzymes (COX-1 and COX-2) in the spinal cord. Unfortunately, inhibition of COX-1 is associated with reduced gastric secretions and can cause ulcers. The COX-2-selective anti-inflammatory drugs such as rofecoxib (Vioxx®, Merck), valdecoxib (Bextra®, Pfizer) and celecoxib (Celebrex®, Pfizer) were developed as safer alternatives to the nonselective COX inhibitors, but recently they have been the subject of negative publicity following reports of adverse cardiovascular events, including heart attack and stroke, in patients taking these drugs in high doses for long periods of time. Following these controversial revelations, Vioxx® and Bextra® were withdrawn from the market and Celebrex® now carries a strict warning about its potential dangers. Even the nonselective NSAID's did not escape the controversy and these drugs also carry warnings. Not surprisingly, there is now a renewed need for safe medicines to treat pain associated with chronic inflammatory diseases such as arthritis. There are several animal models of inflammatory pain that are available to assist researchers in their attempts to discover new drugs [5–7]. Many of these standardized models employ biochemical agents to sensitize or activate primary afferent neurons, causing spontaneous pain as well as hyper-responsiveness to noxious stimuli (called hyperalgesia) and innocuous stimuli (called allodynia). Often, the experimental protocols involve administration of specific proinflammatory mediators that act on their respective, pharmacologically characterized receptors or nonspecific agents such as carrageenan. Despite the abundance of proinflammatory mediators that can be released in unknown quantities and proportions following tissue injury, animal models involving biochemical challenge are often predictive of analgesic drug efficacy in humans.

Chronic neuropathic pain

Neuropathic pain is initiated or caused by damage, lesion or dysfunction in the nervous system and can result from a broad variety of underlying conditions (e.g. trauma, metabolic disorder, viral infection, drug-induced or cancer-related). Nerve injury can be associated with abnormal sensory phenomena including spontaneous pain, dysesthesias and paresthesias, as well as hyperalgesia and allodynia. Peripheral and central mechanisms contribute to the sensory deficits. Peripheral mechanisms include sensitization of Aδ- and C-fibers, phenotypic switching of Aβ-fibers and awakening of silent nociceptors. Central mechanisms include sensitization of secondary and tertiary sensory neurons, as well as spinal and cortical circuit reorganization. In peripheral and central neurons, these events are associated with altered ion-channel expression [8,9] and increased membrane excitability [10], all of which can contribute to abnormal ectopic discharging in affected neurons. Because of the complexity and diversity of the pathophysiological mechanisms involved in neuropathic pain, successful therapeutic treatment remains difficult to predict and achieve. Currently, only a few drugs have been approved by the FDA for the treatment of neuropathic pain syndromes. These drugs include carbamazepine (Tegretol®, Novartis), gabapentin (Neurontin®, Pfizer), pregabalin (Lyrica®, Pfizer) and duloxetine (Cymbalta®, Eli Lilly). In recent years there has been a great surge of interest in re-evaluating various classes of drugs for the treatment of neuropathic pain. As a result of successful clinical trials in patients with diabetic peripheral neuropathy or postherpetic neuralgia, several other drugs are now commonly used 'off-label' for the symptomatic relief of neuropathic pain. These drugs include tricyclic antidepressants (e.g. amitriptyline, Elavil®, AstraZeneca), antiepileptics (e.g. lamotrigine, Lamictal®, GlaxoSmithKline), and antiarrhythmics (e.g. mexiletine, Mexitil®, Boehringer Ingelheim) [11], many of which block voltage-gated sodium and calcium channels and are capable of suppressing abnormal firing in injured nerves. However, these drugs often require high doses, have a high incidence of nonresponders, deliver suboptimal efficacy and can be associated with serious side-effect profiles. Given these limitations, their continued use only highlights the paucity of satisfactory medicines. It is probable that future treatments for neuropathic pain will come from a better understanding of the underlying mechanisms of disease and from the targeting of specific neurotransmitters, receptors or ion channels involved in the initiation or maintenance of these pain states. There are several animal models of nerve-injury-evoked pain that can be used in the laboratory to test new compounds for their potential to treat symptoms of neuropathic pain [12–14]. These surgical models can be used to produce animals that display symptoms that mimic some of the sensory abnormalities that are reported by neuropathic pain patients. However, because of the complexities of the underlying mechanisms involved in initiation and maintenance of neuropathic pain, the relevance of these animal models to human conditions is unclear. Nevertheless, the models are predictive of the ability of certain classes of drugs to produce analgesic efficacy in humans.

BOX 1

Calcium channel structure

Most voltage-gated calcium channels are multi-subunit complexes that permit calcium entry into excitable and nonexcitable cells under conditions of membrane depolarization. The subunits that comprise calcium channels include the large pore-forming α_1 subunit, which is essential for creating the native channel. In addition to determining the permeation properties of the calcium channel, the α_1 subunit also contains the binding sites for most pharmacologically relevant ligands, including peptide toxins, the classical dihydropyridine (e.g. nicardipine, Cardene®, Roche), phenylalkylamine (e.g. verapamil, Calan®, Pfizer) and benzothiazepine (e.g. diltiazem, Cardizem®, Biovail) channel blockers, as well as newer molecules such as the tetralol, mibefradil (Posicor®, Roche). There are ten subtypes of calcium channel α_1 subunit and these correspond to three broad families of currents that are described in native cells [129] (see Table 1). The high-voltage-activated L-type calcium channels are represented by the α_{1C}, α_{1D}, α_{1F} and α_{1S} ($Ca_V1.1$–1.4) subunits. The high-voltage-activated P- and Q-type, N-type and R-type calcium channels are represented by α_{1A} ($Ca_V2.1$), α_{1B} ($Ca_V2.2$) and α_{1E} ($Ca_V2.3$), respectively. The family of low-voltage-activated T-type calcium channels is formed by α_{1G}, α_{1H} and α_{1I} ($Ca_V3.1$–3.3, respectively). Often, the α_1 subunit will associate with one or more auxiliary subunit(s) such as the $\alpha_2\delta$, β or γ subunits, which serve to modulate not only membrane insertion of the α_1 subunit, but also the voltage-dependence and kinetic properties of channel gating [59] (Figure I). For all calcium channel subunits, multiple subtypes are encoded by separate genes and, for each gene product, multiple splice variants exist, sometimes displaying tissue-specific localization.

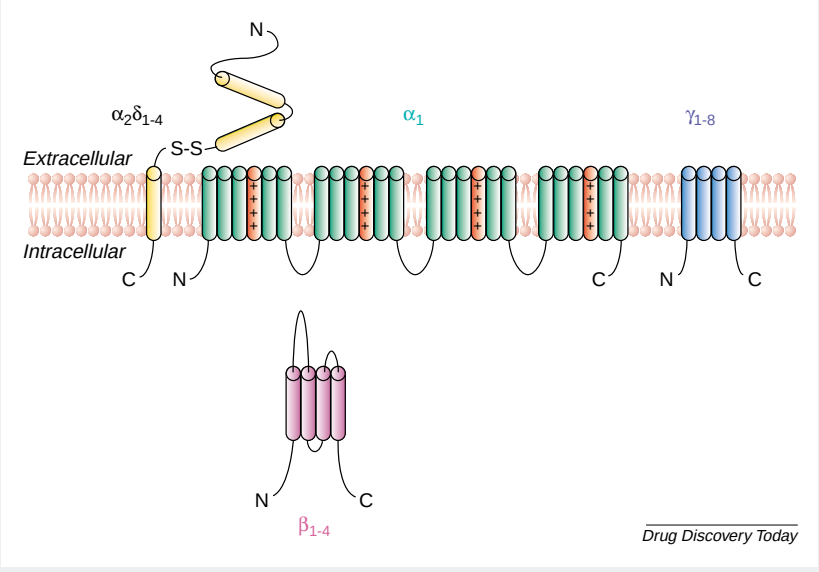

FIGURE I

Proposed structural domains and membrane topology of calcium channel subunits. The large pore-forming α_1 subunit has four homologous domains (I–IV), each with six transmembrane segments [shown in green, except for the 4th transmembrane segments (i.e. the voltage-sensor regions), which are shown in red]. The $\alpha_2\delta$ subunit (in yellow) is the product of a single gene, but the α_2 and δ portions are post-translationally cleaved and then linked by disulphide bonds. The α_2 portion is located extracellularly and is essential for modifying the function of the α_1 subunit. The δ portion contains a transmembrane segment that serves to anchor the subunit in the cell membrane. The γ subunit (in blue) has four transmembrane segments. The β subunit (in magenta) is located in the cytosol where it interacts with the intracellular loop connecting domain I to domain II in the α_1 subunit.

Ion channels and pain

Voltage-gated ion channels control sensory neuron excitability, action potential generation and propagation, as well as neurotransmitter release. All of the ion channels and neurotransmitter systems involved in pain-signal transmission, whether they are located peripherally or centrally, are considered viable targets for the discovery of new analgesic drugs. In terms of specific ion channels that are implicated in the initiation and/or maintenance of chronic pain states, the N-type and T-type voltage-gated calcium channels are relevant [15]. In particular, the N-type channels have received much attention because of their key role in controlling painful neurotransmission in the spinal cord and as a result of the recent approval of Prialt®, an analgesic drug that works by specifically targeting N-type calcium channels [16]. The T-type calcium channels have been the subject of less investigative research but recently published data suggest that they also contribute to nociceptive information processing [17]. Relevant data implicating both subtypes of calcium channel in pain sensation will now be reviewed.

N-type calcium channels and pain

Validation of N-type calcium channels as targets for the treatment of pain

N-type calcium channels exist as a complex comprising the pore-forming α_{1B} subunit (also known as $Ca_V2.2$; see Box 1 and Table 1) along with auxiliary $\alpha_2\delta$ and β subunits. N-type calcium channels are found almost exclusively on neurons and immunohistochemical studies with α_{1B}-subunit-selective antibodies have demonstrated that these channels are present at high levels in the presynaptic terminals of neurons [18], where they are complexed with a variety of proteins that are involved in neurotransmitter secretion, including syntaxin, synaptotagmin and SNAP-25 [19]. In the spinal cord, the presynaptic distribution of N-type calcium channels supports a role in controlling the transmission of nociceptive information [20]. Interestingly, α_{1B} and $\alpha_2\delta$ subunits are upregulated in DRG neurons following nerve injury or tissue inflammation [21–25] suggesting that N-type calcium channels continue to play a dominant role in controlling nociceptive signal transmission under pathological conditions.

Peptide ω-conotoxins, including GVIA (27 amino acids, *Conus geographus*) [26], MVIIA (25 amino acids, *Conus magus*) [27,28] and CVID (27 amino acids, *Conus catus*) [29] bind selectively and with high affinity to N-type calcium channels (Table 1). They inhibit ion permeation and can attenuate the rise in intracellular calcium concentration that is necessary to trigger exocytotic vesicle fusion [30]. In the dorsal horn of the spinal cord, ω-conotoxins inhibit the release of pronociceptive neurotransmitters and neuromodulators from the central nerve terminals of primary afferent neurons [31–33]. Consistent with this action, spinal administration of ω-conotoxin reduces noxious stimulus-evoked electrical activity in dorsal horn neurons of normal (no nerve injury) or nerve-injured

TABLE 1

Calcium channel subunits and known pharmacology

Class of calcium channel	α_1 subunit gene	Pharmacological sensitivity
L-type	$Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$ and $Ca_V1.4$ (α_{1C}, α_{1D}, α_{1S}, α_{1F})	Dihydropyridines (e.g. nifedipine, **μM**), phenylalkylamines (e.g. verapamil, **μM**) and benzothiazepines (e.g. diltiazem, **μM**) Calcicludine (**nM**, from snake, *Dendroaspis angusticeps*)
P- and Q-type	$Ca_V2.1$ (α_{1A})	ω-agatoxin IVA (**nM**, from spider, *Agelenopsis aperta*) ω-conotoxin MVIIC (**nM**, from marine cone snail, *Conus magus*)
N-type	$Ca_V2.2$ (α_{1B})	ω-conotoxin GVIA (**nM**, from marine cone snail, *Conus geographus*) ω-conotoxin MVIIA (**nM**, from marine cone snail, *Conus magus*) ω-conotoxin CVID (**nM**, from marine cone snail, *Conus catus*)
R-type	$Ca_V2.3$ (α_{1E})	SNX-482 (**nM**, from tarantula, *Hysterocrates gigas*)
T-type	$Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ (α_{1G}, α_{1H}, α_{1I})	Nickel (**μM**) Ethosuximide (**mM**), zonisamide (**μM**) and mibefradil (**μM**) Kurtoxin (**nM**, from scorpions, *Parabuthus transvaalicus* and *Parabuthus granulatus*)

Note: concentration units shown in bold type are intended only to indicate approximate concentrations that block the corresponding native and/or recombinant channels.

rats [34]. Furthermore, in behavioral models of nerve injury or inflammatory pain, spinal administration of ω-conotoxins reduces hyperalgesic and allodynic responses to mechanical, chemical or thermal stimulation of the paw [35–37]. Three groups of researchers have reported on the phenotypic behavior of α_{1B} knockout mice in models of pain [38–40]. Compared with the wild-type mice, homozygous α_{1B} knockout mice display significant resistance to the development of hyperalgesia and allodynia following either nerve or tissue injury, suggesting a crucial role of N-type calcium channels in the development of chronic pain.

Prialt®: a great leap forward

N-type calcium channels are unique among the calcium channel family because of unequivocal evidence linking them to pain transmission in humans. The recent approval of Prialt®, by several government regulatory bodies worldwide, for the treatment of severe chronic pain associated with cancer, AIDS and neuropathies represents a significant advancement for pain therapy. Prialt® is a synthetic version of ω-conotoxin MVIIA, which is also known as SNX-111 and ziconotide. Clinical trials with Prialt® demonstrated potent efficacy in a postsurgical setting [41] and in patients suffering from a variety of chronic, and otherwise intractable, severe pain conditions [42,43] (see Table 2). Importantly, Prialt® does not induce tolerance and, furthermore, it works in patients who no longer respond to opioid drugs. However, to deliver its efficacy and remain safe, Prialt® must be administered intrathecally. The most serious adverse side-effect of Prialt® is orthostatic hypotension [44], which probably results from a block of N-type calcium channels on neurons that regulate blood pressure [45]. Synthetic ω-conotoxin CVID, also known as AM336, is currently in Phase II clinical trials for the treatment of severe chronic pain in cancer patients (see Table 2). CVID holds promise because it appears to be more efficacious than MVIIA at reducing pain-related behaviors in animals [33] and is less prone to causing cardiovascular side effects [46]. Compared with MVIIA, CVID has a different profile of blocking effects at N-type calcium channel splice variants and is more selective over P- and Q-type calcium channels [29]. In addition, CVID has a more rapid onset and offset of action *in vitro*. All of these features could contribute to its improved efficacy and the reduced postural hypotension in animal models.

Future prospects for N-type calcium channel blockers

Given the potent efficacy of Prialt® in treating the symptoms of severe pain, there is hope that small-molecule drugs targeting N-type calcium channels will eventually replace analgesic opioids in the clinic. Desirable properties of these future drugs will be high efficacy, superior safety and ease of administration, as well as better tolerability profiles relative to Prialt® and the opioids. The fact that ziconotide's mechanism of action and limitations are known has stimulated intense research activity to identify novel drugs with specificity for the N-type calcium channels that are expressed in pain-sensing neurons. In the search for nociceptor-specific N-type calcium channel blockers, better selectivity could arise from a greater binding affinity for N-type calcium channel variants in DRG neurons or from improved functional selectivity that results from a use-dependent mechanism of action.

Multiple splice variants of the α_{1B} subunit exist. The most interesting of these contains a DRG-specific exon (37a) and is expressed mainly in capsaicin-sensitive sensory neurons [47]. Swapping between exon 37a and exon 37b results in 14 amino acid changes in an intracellular region of the channel near the C-terminus. Compared with channels containing exon 37b, inclusion of exon 37a appears to produce larger calcium currents in the *Xenopus laevis* expression system. If replicated in sensory neurons, this mechanism could serve to facilitate calcium influx and pronociceptive neurotransmitter release from afferent nerve endings in the spinal cord. Although inhibition of N-type calcium currents by ω-conotoxin GVIA appears to be exon-37-independent, it might be possible to identify small molecules that target exon 37a and that could modulate or inhibit N-type calcium channel function through an allosteric mechanism. However, exon 37a was identified in rat DRG neurons and it remains to be determined whether it is also expressed in human DRG neurons. Regardless, it is possible that human N-type calcium channels might be subject to their own alternative splicing that could be leveraged in the search for new drugs that treat severe pain in patients.

Neuromed Technologies is pioneering efforts to discover and develop small-molecule use-dependent blockers of N-type calcium channels for the treatment of severe chronic pain. Their experimental stage drug NMED-160 is an orally available blocker of N-type calcium channels that is now in Phase II clinical trials for

TABLE 2

Calcium channel modulators (marketed and experimental stage drugs) for treating pain in humans

Drug	Aliases	Company	Molecular target	Types of pain in humans
[1]Prialt®	Ziconotide, SNX-111, ω-conotoxin MVIIA	Elan Pharmaceuticals	N-type calcium channel α_1 subunit	Severe chronic pain associated with cancer, AIDS and neuropathies
[2]Lyrica®	Pregabalin, *S*-(+)-3-isobutylgaba	Pfizer	Calcium channel $\alpha_2\delta_1$ subunit	Diabetic peripheral neuropathy, fibromyalgia and postherpetic neuralgia
[2]Neurontin®	Gabapentin	Pfizer	Calcium channel $\alpha_2\delta_1$ subunit	Postherpetic neuralgia, various types of neuropathic pain
[2]Zarontin®	Ethosuximide	Pfizer	T-type calcium channel α_1 subunit	Refractory migraine
[2]Zonegran®	Zonisamide	Eisai	T-type calcium channel α_1 subunit	Central poststroke pain and refractory migraine
[1]AM336	ω-conotoxin CVID	CNSBio	N-type calcium channel α_1 subunit	Under Phase II clinical investigation for treatment of severe chronic pain in cancer patients
[2]NMED-160		Neuromed Technologies	N-type calcium channel α_1 subunit	Currently in Phase II clinical trials for a variety of pain conditions

[1]Prialt® is a peptide drug that must be administered intrathecally using either a programmable implanted variable-rate microinfusion device or an external microinfusion device and catheter. The risks associated with use of spinal injection systems include infection, trauma and bleeding. If approved, AM336 will probably be administered using similar systems.
[2]Small-molecule drugs that are (or are expected to be) administered orally.

a variety of pain conditions (see Table 2). In preclinical testing, NMED-160 displayed a broad efficacy profile in animal models of neuropathic and inflammatory pain and also had a good safety profile [48,49]. However, it still remains to be ascertained whether this drug is analgesic in patients with severe chronic pain.

Gabapentin, pregabalin and the calcium channel $\alpha_2\delta$ subunit

Gabapentin is approved in the USA for the management of postherpetic neuralgia [50] and is often prescribed for the treatment of other neuropathic pain syndromes including diabetic peripheral neuropathy [51], neuropathic pain related to spinal cord injury [52] and cancer-related neuropathic pain [53] (see Table 2). Gabapentin dosing must often be titrated to high levels in search of adequate pain control and, although it is a safe drug, pain relief is often suboptimal. Pregabalin is a newer drug that has improved-potency and superior bioavailability compared with gabapentin. In a variety of clinical trials, pregabalin was effective against pain associated with diabetic peripheral neuropathy [54–56], fibromyalgia [57] and postherpetic neuralgia [58]. Pregabalin was approved recently by the FDA for the treatment of diabetic peripheral neuropathy and postherpetic neuralgia (see Table 2).

Calcium channel auxiliary subunits associate directly with the α_1 subunits, serving to modulate channel insertion in the cell membrane as well as the kinetics and voltage-dependence of channel gating [59]. Although most calcium channel modulating drugs bind directly to pore-forming α_1 subunits, pregabalin and gabapentin are unique in that they bind with high affinity to the $\alpha_2\delta$ auxiliary subunits [60,61]. Although the $\alpha_2\delta_1$ subtype is essential for the analgesic actions of pregabalin [62], no conclusive mechanism of action has been presented. Gabapentin is reported to have inhibitory effects on high-voltage-activated calcium currents in peripheral and central neurons [63–67] and it could act presynaptically to reduce excitatory and inhibitory synaptic transmission in superficial layers of the dorsal horn in the spinal cord [68]. By contrast, a significant number of studies have found no effect of gabapentin on calcium currents and synaptic transmission in brain [69–73]. A possible explanation for the discrepancies could be the subunit composition of the native calcium channels in different neuronal populations [74]. Consequently, it has been postulated that the binding of gabapentin and pregabalin to the $\alpha_2\delta$ subunit of N-type and/or P- and Q-type calcium channels reduces the release of pronociceptive neurotransmitters from sensory nerve terminals [75–77] and inhibits the phenomenon of central sensitization [78,79]. Consistent with such a mechanism of action, both gabapentin and pregabalin are efficacious in animal models of inflammatory [80] and neuropathic pain [23,81,82].

T-type calcium channels and pain

Despite their presence in peripheral and central neurons of the pain pathway [83–86], low-voltage-activated T-type calcium channels were not seriously considered as potential targets for treating pain until recently. The primary reason for the lack of consideration is the relative paucity of potent and selective pharmacological agents with which to test hypotheses about the role of T-type calcium channels as regulators of nociceptive information processing. However, recent studies with Ca_V3-selective antisense oligonucleotides have provided a level of validation that was previously nonexistent [17].

T-type calcium channels and neuronal function

Three α_1 subunits (i.e. $Ca_V3.1$–3.3) represent the family of calcium channels that gives rise to T-type calcium currents in excitable and nonexcitable cells [87–89] (see Box 1 and Table 1). These subtypes differ in their tissue distribution (e.g. $Ca_V3.3$ is exclusive to neurons) [90], in their sensitivity to divalent cations such as nickel ($Ca_V3.2$ is most sensitive) [91], in their sensitivity to kurtoxin ($Ca_V3.3$ is least sensitive) [92] and in their kinetics of activation and inactivation ($Ca_V3.3$ is slowest) [89]. Although heterologous coexpression of auxiliary subunits can affect the functional properties of Ca_V3 α_1-subunit-mediated calcium currents in mammalian cells [93,94], it is unclear at present whether these subunits are actually complexed in native T-type calcium channels.

In neurons, T-type calcium channels are localized mainly in somatic and dendritic sites and are involved in determining responsiveness to excitatory input [95,96]. In particular, T-type calcium channels underlie the switch from tonic to phasic firing mode in peripheral and central neurons [97,98]. In tonic mode, a neuron will respond to graded excitatory input with unitary action potentials at increasing frequencies. In phasic mode, a neuron will generate an 'all or nothing', T-type calcium-channel-dependent, low-threshold calcium spike (LTS) that is associated with a high frequency burst of action potentials. Phasic firing underlies rhythmic and oscillatory behavior in certain neuronal circuits, as observed normally during sleep, and can also be manifested in an uncontrolled manner in certain pathological conditions [99].

T-type calcium channels in peripheral neurons involved in nociception

T-type calcium currents have been detected in small and medium-sized neurons of the DRG [85,86]. The relatively fast calcium-current kinetics and high sensitivity to nickel implicate $Ca_V3.2$ as the predominant subtype in these neurons [100] and, consistent with this, $Ca_V3.2$ is the major subunit identified by *in situ* hybridization studies [90]. The expression level of T-type calcium currents in primary afferent neurons can change in response to nerve injury and the type of injury appears to influence the changes that take place. Following nerve transection, T-type calcium currents can either increase [101] or not change [102,103]. By contrast, following nerve ligation, T-type calcium currents appear to decrease [104,105]. Paradoxically, it has been postulated that the latter observation is actually associated with increased neuronal excitability as a consequence of reduced activity of calcium-activated potassium channels to which the T-type channels might be functionally coupled [106,107].

To date, perhaps the most convincing evidence in support of T-type calcium channels as potential therapeutic targets for the treatment of pain comes from studies with Ca_V3-specific antisense oligonucleotides in rats [17]. Intrathecal administration of pan-Ca_V3 or $Ca_V3.2$-specific, but not $Ca_V3.1$- or $Ca_V3.3$-specific, antisense oligonucleotides leads to antinociceptive, antiallodynic and antihyperalgesic effects in models of acute and neuropathic pain. These effects are correlated with the loss of Ca_V3 mRNA and a reduction in T-type calcium currents in neurons of the DRG. These data are important because they specifically implicate $Ca_V3.2$-containing calcium channels as important regulators of nociceptive processing in peripheral sensory neurons. Thus, there is a clear opportunity to discover and develop a peripherally restricted $Ca_V3.2$-selective blocker of neuronal T-type calcium channels for the treatment of chronic pain.

T-type calcium channels in central neurons involved in nociception

T-type calcium currents have also been recorded in many neurons of the CNS. In lamina I projection neurons of the spinal cord dorsal horn, T-type calcium channels appear to play a role in the development of long-term potentiation [108], which could be a correlate of central sensitization [109]. In the relay and reticular nuclei of the thalamus, kinetically distinct T-type calcium currents have been recorded [84]. Neurons of the relay nuclei display only fast T-type calcium currents, whereas neurons of the reticular nucleus display fast currents in the soma and slow currents in the dendrites [110]. These electrophysiological results have been confirmed by detection of $Ca_V3.1$ (fast kinetics), $Ca_V3.2$ (fast kinetics) and $Ca_V3.3$ (slow kinetics) mRNA and protein in the thalamus [90]. Thalamic T-type calcium channels are intimately involved in the generation of burst firing and oscillatory behavior in synaptically interconnected relay and reticular neurons. Of particular note, activity of inhibitory neurons in the reticular nucleus will tend to hyperpolarize neurons in the relay nuclei, placing them in a hyperpolarized state that supports burst firing. Interestingly, some patients with chronic neurogenic pain present with LTS and burst firing in specific thalamic nuclei [111]. Not only are these electrophysiological phenomena obliterated by targeted lesions in the thalamus, but the patients also experience pain relief. This highlights a possible link between LTS, thalamic dysrhythmias and sensory abnormalities [99].

Few studies have attempted to address the potential contribution of individual T-type calcium channel subtypes in the CNS to painful sensory processing. One study has reported the behavior of $Ca_V3.1$ knockout mice in various tests of nociception [112]. Surprisingly, the knockout mice presented with visceral hyperalgesia. Furthermore, injection of mibefradil into the ventroposterolateral nucleus enhanced pain responses in wild-type mice. Taken together, these data suggest that $Ca_V3.1$ channels serve an antinociceptive function in the CNS. However, until tissue-specific knockdown of individual Ca_V3 subtypes is achieved it will be difficult to unravel the mechanisms involved. Although it is unclear whether the $Ca_V3.3$ subtype has significant involvement in nociceptive processing, a thalamic mechanism can be postulated. It is possible that selective targeting of $Ca_V3.3$-expressing neurons in the reticular nucleus could inhibit γ-aminobutyric acid (GABA) release. In turn, this would lead to less hyperpolarization of neurons in the relay nuclei with decreased availability of their T-type calcium channels. The consequence could be a switch from phasic to tonic firing with interruption of pathological rhythmic and oscillatory electrical activity.

Pharmacological evidence for T-type calcium channels and pain

Recent pharmacological evidence from clinical and preclinical studies also supports a role for T-type calcium channels in pain perception. Ethosuximide (Zarontin®, Pfizer) is an antiepileptic drug that blocks, albeit with low affinity, native and recombinant T-type calcium channels [100,113,114], although additional mechanisms of action have been proposed [115]. Ethosuximide has similar affinities for each of the Ca_V3 subtypes but, as a result of open channel interactions, it appears to be a slightly more potent blocker of the slowly inactivating $Ca_V3.3$ [114]. In rat spinal cord, ethosuximide inhibits electrically, mechanically and thermally evoked neuronal responses in normal and neuropathic animals [116]. In addition, ethosuximide exerts antiallodynic and antihyperalgesic actions in animal models of neuropathic and inflammatory pain [117–120], although whether this occurs through a peripheral and/or central mechanism is unclear. In humans, one anecdotal report tells of patients (with valproate- or propanolol-resistant migraine) who received complete relief from pain upon dosing with ethosuximide [121] (see Table 2). However, no double-blinded, placebo-controlled trials of ethosuximide in migraineurs or patients with severe pain have been reported so the true potential of ethosuximide as an

analgesic drug remains unexplored. Zonisamide (Zonegran®, Eisai) is a newer antiepileptic drug that blocks T-type calcium channels [122,123]. Like ethosuximide, this drug is analgesic in the clinic [124,125] (see Table 2) and displays antihyperalgesic actions in rats with sciatic nerve injury [126]. Mibefradil is a relatively potent and somewhat selective blocker of T-type calcium channels that was marketed as a unique antihypertensive drug (Posicor®, Roche) until its withdrawal from the market as a result of several drug interactions that resulted from inhibition of the cytochrome P-450 isoforms CYP3A4 and CYP2D6. Nevertheless, mibefradil has remained in widespread use as a research tool to probe the functional role of T-type calcium channels in neuronal and non-neuronal tissues. Intraperitoneal or local, but not intrathecal, administration of mibefradil yields efficacy in animal models of pain [118,127]. These data are supportive of a role for peripheral T-type calcium channels in nociceptive information processing, however they must be interpreted with caution because of the additional ability of mibefradil to block voltage-gated sodium channels [128].

Final remarks

Severe pain is an undesirable symptom that is associated with a wide range of chronic diseases and disorders. Because of the unsatisfactory nature of currently available medications, there is extensive research ongoing (in academic and industrial laboratories) to understand the pathophysiological mechanisms involved in initiation and maintenance of severe chronic pain, as well as to identify new, efficacious and safe analgesic drugs. A lot of research into new analgesic drugs is focusing on novel mechanisms of action, such as selective inhibition of ion-channel function. Although this review focuses on the importance of N-type and T-type calcium channels, this is clearly not the whole story of pain perception. There are many other ion-channel targets that could have important functional roles in chronic pain states, for example TRPV1 (the capsaicin receptor), P_2X_3 and P_2X_4 (ionotropic ATP receptors), $Na_V1.8$ [a voltage-gated tetrodotoxin (TTX)-resistant sodium channel] and $Na_V1.3$ (a TTX-sensitive sodium channel), but validation of these channels either relies only on animal-model data or on scant clinical data. By contrast, the N-type calcium channel has the advantage of clinical validation with Prialt®. However, this drug will probably be superseded in the future by superior small-molecule drugs. Although NMED-160 represents the most advanced of the potential replacements, it is still undergoing Phase II trials and, therefore, its approval is several years away.

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