Targeting Chronic and Neuropathic Pain: The N-type Calcium Channel Comes of Age

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Summary: The rapid entry of calcium into cells through activation of voltage-gated calcium channels directly affects membrane potential and contributes to electrical excitability, repetitive firing patterns, excitation-contraction coupling, and gene expression. At presynaptic nerve terminals, calcium entry is the initial trigger mediating the release of neurotransmitters via the calcium-dependent fusion of synaptic vesicles and involves interactions with the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor complex of synaptic release proteins. Physiological factors or drugs that affect either presynaptic calcium channel activity or the efficacy of calcium-dependent vesicle fusion have dramatic consequences on synaptic transmission, including that mediating pain signaling. The N-type calcium channel exhibits a number of characteristics that make it an attractive target for therapeutic intervention concerning chronic and neuropathic pain conditions. Within the past year, both U.S. and European regulatory agencies have approved the use of the cationic peptide Prialt for the treatment of intractable pain. Prialt is the first N-type calcium channel blocker approved for clinical use and represents the first new proven mechanism of action for chronic pain intervention in many years. The present review discusses the rationale behind targeting the N-type calcium channel, some of the limitations confronting the widespread clinical application of Prialt, and outlines possible strategies to improve upon Prialt's relatively narrow therapeutic window. **Key Words:** N-type calcium channel, Prialt, chronic pain, neuropathic pain, ω-conotoxin.

INTRODUCTION

Native calcium channels have been classified by both their electrophysiological and pharmacological properties and are generally divided into low-threshold (T-types) and high threshold (L-, N-, P/Q- and R-types). The L-, N-, P/Q- and R-type channels typically activate at membrane potentials near –30 mV and display diverse kinetic, voltage-dependent and pharmacological properties.[1] The availability of specific pharmacological agents targeting the high threshold channels has permitted elucidation of many of their physiological functions. The T-type calcium channels describe a class of molecules that transiently activate at relatively negative potentials (~−60 mV) and for which a general lack of high-affinity selective blockers has made their exact physiological contributions lag behind those of the high-voltage activated isoforms.

High-threshold neuronal calcium channels are heterotrimeric complexes consisting of a pore-forming α_1-subunit containing four conserved structural domains (domains I-IV) linked by hydrophilic linkers modeled to be cytoplasmic, a β-subunit that interacts cytoplasmically with the α_1-subunit in the domain I-II linker, and an $\alpha_2\delta$-subunit that contains a single transmembrane segment covalently linked to an extracellular heavily glycosylated component (Fig. 1A).[2] Although the existence of alternatively spliced variants can complicate attempts to correlate exactly native calcium currents with the biophysical properties of cloned calcium channels, it is generally accepted that the distinct α_1-subunits account for all known voltage-gated calcium currents; α_{1S} (Cav1.1), α_{1C} (Cav1.2), α_{1D} (Cav1.3) and α_{1D} (Cav1.4) all encode L-type channels, α_{1A} (Cav2.1) encodes the P/Q-type, α_{1B} (Cav2.2) the N-type, α_{1E} (Cav2.3) the R-type, whereas α_{1G} (Cav3.1), α_{1H} (Cav3.2) and α_{1I} (Cav3.3) all encode low-threshold T-type currents (Fig. 1B). In the mammalian genome, there are also four different β-subunit genes (β1–β4) and four $\alpha_2\delta$-subunit genes ($\alpha_2\delta$-1–$\alpha_2\delta$-4).[1]

Immunohistochemical studies show that most neurons express multiple types of α_1- and β-subunits, although differences in the relative levels of expression of indi-

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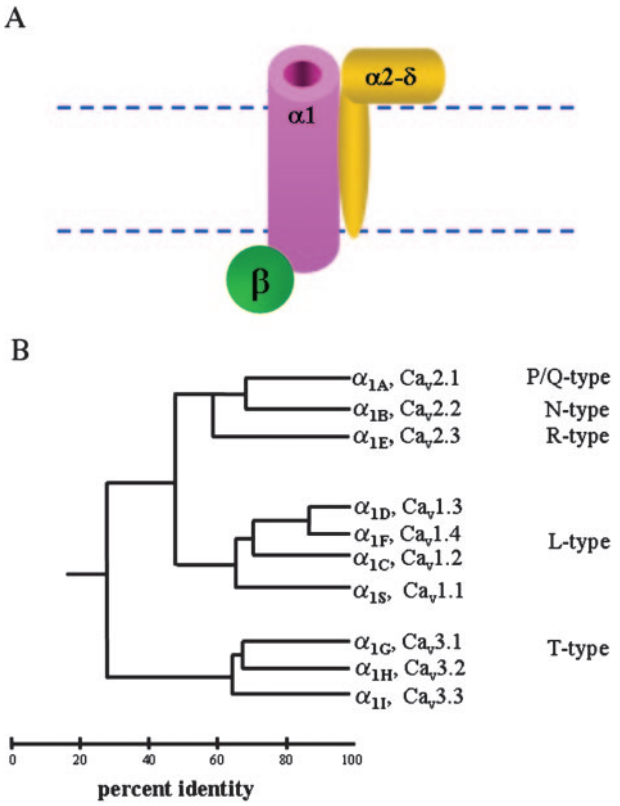

FIG. 1. Schematic showing the subunit composition of the N-type calcium channel. A: The channel complex consists of pore-forming α_1-subunit that also contains the voltage sensor and is the target of pharmacological agents, a β-subunit that modulates the biophysical and second-messenger-dependent properties of the α_1-subunit, and an $\alpha_2\delta$-subunit. B: Amino acid identity comparisons of the ten calcium channel α_1-subunit genes found in the mammalian genome.

vidual subtypes are found throughout the CNS. For example, the α_{1C} and α_{1D} L-types and α_{1E} R-type subunits are primarily localized on cell bodies and proximal dendrites, whereas the α_{1A} P/Q-type and α_{1B} N-type subunits are more highly distributed along the lengths of dendrites and at presynaptic terminals.[3] These distinctions are not absolute because α_{1E} R-type channels are also detected in cerebellar Purkinje cell dendritic branches and α_{1A} P/Q-type channels are abundantly expressed on Purkinje cell bodies. That each of the classes of calcium channels shows a distinct expression pattern likely reflects their specialized physiological roles. For example, that α_{1A} (Cav2.1) and α_{1B} (Cav2.2) subunits are concentrated at a large number of presynaptic terminals is consistent with a prominent role for N-type and P/Q-type channels in triggering neurotransmitter release.[4] That the α_{1C} (Cav1.2) and α_{1D} (Cav1.3) L-type channels appear to be largely restricted to cell bodies and proximal dendrites suggests that they likely serve to increase calcium influx at the base of major dendrites in response to the summation of synaptic inputs from dendritic trees and to also regulate somatic calcium-dependent signaling pathways (e.g., gene regulation).

In addition to their normal physiological functions, calcium channels are also implicated in a number of human pathophysiological conditions including congenital migraine, cerebellar ataxia, neuropathic/chronic pain, angina, epilepsy, hypertension, ischemia, and some arrhythmias. The clinical treatment of some of these disorders has been aided by the development of therapeutic calcium channel antagonists that selectively target those L-type channels largely localized to smooth muscle and the heart.[5]

N-TYPE CALCIUM CHANNELS

Biochemical purification of native N-type channels using cone snail peptide toxins as affinity ligands show the channel is a hetero-oligomeric complex consisting of α_{1B-} (Cav2.2), β-, and $\alpha_2\delta$-subunits.[2] There is no firm evidence that the N-type channel complex contains a γ-subunit similar to that found in the skeletal muscle L-type channel complex. The large α_{1B}-subunit (~2300 amino acid; ~260 kDa) contains the channel pore, selectivity filter, and voltage-sensing machinery and is the target of all known pharmacological agents. Exogenous expression studies show that the voltage-dependent and kinetic properties of the α_{1B} N-type channel can be differentially affected by co-expression with the distinct β-subunit isoforms.[6] The β-subunits also play a key role in N-type channel modulation via both G protein- and protein kinase C-dependent mechanisms.[7,8]

Whereas pharmacologically defined L-type and T-type currents are each encoded by multiple α_1-subunit genes (Fig. 1B), the α_{1B}-subunit is encoded by a single gene. The degree of similarity between N-type channels is significantly greater across species (e.g., rat α_{1B} N-type *vs* human α_{1B} N-type ~91% identity) than within the same species between N-type channels and the next closely related P/Q-type and R-type channels (rat N-type *vs* rat α_{1A} P/Q-type ~61% identity; rat N-type *vs* rat α_{1E} R-type ~55% identity). The higher degree of N-type channel similarity between species is consistent with the significant conservation of both biophysical and pharmacological properties between rat, rabbit, and human N-type channels.

RNA analyses show that α_{1B} transcripts are exclusively expressed in neurons and neuroendocrine cells such as chromaffin cells.[9,10] In the CNS, α_{1B} N-type channel mRNA is found in the cerebral cortex, hippocampus, forebrain, midbrain, cerebellum, brainstem, and spinal cord. Alternatively spliced variants have been identified that differ in primary sequence in domain IIIS3-S4, IVS3-S4, II-III linker, and the carboxyl tail region. In some instances, these variants alter channel biophysical properties and exhibit cell type-specific ex-

pression patterns (e.g., brain *vs* peripheral neurons), and there is some suggestion that primary afferents may exclusively express a particular N-type variant.[11,12] Although there have been no reports that specific alternatively spliced N-type channel variants alters pharmacological properties, splicing variants in homologous extracellular regions of the P/Q-type channel have been shown to affect the binding of certain peptide toxins.[13]

At the subcellular level, immunostaining shows α_{1B}-subunits to be predominantly but unevenly clustered along dendritic regions and more diffusely and less frequently on cell bodies throughout the CNS.[3] These results are largely in agreement with autoradiographic analyses using radiolabeled ω-conotoxin-GVIA, although they suggest a more specific distribution compared with that reported for a monoclonal antibody against ω-conotoxin-GVIA itself.[14] In agreement with pharmacological analyses showing that blockade of N-type channels disrupts a portion of central neurotransmission,[4] α_{1B}-subunits are found highly concentrated at a limited subset of presynaptic terminals in the central and peripheral nervous systems.[3]

N-type channel activity can be modulated by activation of a number of G protein-coupled receptors (GPCRs).[15] Of particular relevance, N-type currents are inhibited by GPCRs implicated in nociception including opioid,[8,16] cannabinoid,[17] neuropeptide Y,[18] and substance P.[19] In the spinal cord, opioids likely produce their antinociceptive actions act via a combination of two major mechanisms, both of which act via G protein-dependent pathways. In the one instance, activation of opioid receptors (both pre- and postsynaptic) releases $G_{\beta\gamma}$ from the trimeric G-$\alpha\beta\gamma$ complex and $G_{\beta\gamma}$ then physically interacts with potassium channels to up-regulate channel activity, hyperpolarizing the cell and decreasing synaptic excitability. In the second instance, the same opioid receptor G protein-dependent pathway results in $G_{\beta\gamma}$ molecules directly binding to presynaptic N-type calcium channels (in 1:1 stoichiometry) stabilizing the closed state and resulting in a ten-fold increase in the first latency of channel opening.[20–22] A direct consequence of decreasing presynaptic N-type channel activity is likely to significantly attenuate neurotransmitter release in response to subsequent incoming action potentials.

Evidence suggests that the opioid receptor-mediated regulation of N-type currents by $G_{\beta\gamma}$ is, however, significantly more complicated. For example, the inhibition by $G_{\beta\gamma}$ is strongly voltage dependent, can be relieved by rapid trains of action potentials, is affected by the nature of the calcium channel β-subunit associated with the N-type channel complex (e.g., β1 *vs* β2a), and the $G_{\beta\gamma}$ interaction can be itself inhibited by the PKC-dependent phosphorylation of the N-type channel.[8,20] Additionally, presynaptic N-type channels are also physically associated with the neurotransmitter release machinery, most specifically with the soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor (SNARE) proteins, syntaxin-1A and SNAP-25, through a binding site in the N-type α_{1B}-subunit domain II-II linker.[23] Disruption of this α_{1B}-subunit-synaptic protein interaction inhibits neurotransmitter release in cultured neurons. In the absence of exogenous G protein activation, syntaxin-1A can cause tonic inhibition of N-type channel activity, and syntaxin-1A itself also binds $G_{\beta\gamma}$.[24,25] Finally, a class of molecules called regulator of G protein signaling proteins can both alter the kinetics of $G_{\beta\gamma}$-mediated responses and also interfere with G_{β} actions on effectors including N-type calcium channels. The overlapping nature and complexity of these modulatory pathways, combined with the fact that multiple types of GPCRs are implicated in both nociception and N-type channel regulation, makes it difficult to make definitive conclusions concerning their individual roles in any particular nociceptive behavior.

N-TYPE CALCIUM CHANNELS AS A PRIMARY THERAPEUTIC TARGET FOR PAIN INTERVENTION

To a large degree, the case for involvement of N-type channels in various pathophysiological conditions rests with studies using high-affinity, selective peptides targeting N-type channels, as well as more recent work examining mice in which the N-type channel has been genetically deleted.

Two cone snail peptides, ω-conotoxin-GVIA and ω-conotoxin-MVIIA (also called SNX-111, Ziconotide and Prialt), have been the workhorses of numerous biochemical, pharmacological, and physiological studies examining the properties and roles of N-type channels.[26,27] The 27-amino acid ω-conotoxin-GVIA peptide isolated from *Conus geographus* is a potent, selective, and irreversible inhibitor of N-type channels. Similarly, ω-conotoxin-MVIIA (called Prialt hereafter), a 25-amino acid peptide from the venom of *Conus magnus* is a potent blocker of N-type channels although acts in a reversible manner. Due to their peptidic nature, the cone snail toxins are not orally available and they must be delivered directly into the CNS via intrathecal administration.

N-type channels are highly concentrated in both dorsal root ganglia cell bodies and also in the synaptic terminals they make in dorsal horn of the spinal cord (laminae I and II).[14,28] These primary afferents (mainly C-fibers and A-δ fibers) are implicated in the sensation of a variety of noxious painful stimuli including thermal, mechanical, and inflammatory. Critically, block of N-type currents inhibits the release of neuropeptides substance P and

calcitonin gene-related peptide (CGRP) from sensory neurons.[29–31]

Evidence suggests that the N-type channels targeted by intrathecal ω-conotoxin-GVIA and Prialt are not directly implicated in most acute thermal pain sensation (e.g., the hot plate and tail flick assays in rats) but rather play a more significant role in inflammatory and chronic neuropathic conditions.[32] These include potent efficacy in both the early and late phase of the formalin model, antihyperalgesic and antiallodynic effects associated with the Chung and Bennett models, as well as antihyperalgesic actions in the capsaicin and carrageenan inflammatory models. There has been one report that Prialt alleviates the hyperalgesia and allodynia associated with a rat model of postoperative pain.[33]

In side-by-side comparisons in animal models of chronic inflammatory and neuropathic pain Prialt is at least 10 times more potent than intrathecally administered morphine. Interestingly, pretreatment with Prialt can also prevent the establishment of hyperalgesia and allodynia suggesting a possible role for N-type channels in the establishment of hypersensitive pain states. Although Prialt is opioid sparing, it does not bind opioid receptors or prevent the development morphine-induced tolerance.[34]

The N-type calcium channel target is also validated through the construction of mice strains in which the N-type gene has been genetically deleted.[35–37] Somewhat surprisingly given the generally wide CNS distribution of N-type channels and their demonstrated role in neurotransmission, other than a partial degree of lethality in one out of the three knockout strains reported, the N-type channel deficient mice display relatively few deleterious affects. Mating behavior and life span are normal and there are no reports of ataxias or other motor-related defects commonly associated with block of N-type channels by Prialt (see below). The only behavioral alteration that might be ascribed to CNS function were decreased anxiety-related behaviors associated with the elevated plus-maze, open-field, and acoustic startle tests in the homozygous N-type-deficient animals.[37]

All three N-type channel gene knockout strains exhibit abnormal responses to pain behaviors. Although not all labs examined the same animal models and there were some inconsistencies between labs regarding results for acute pain models (paw-flick, hot plate), the data clearly show that the N-type channel is required for the development of the hyperalgesia and allodynia associated with neuropathic pain, as well as for inflammatory pain.[35–38]

In humans, Prialt has been examined in case studies (e.g., brachial plexus avulsion, spinal cord pain) as well as in open-label and double-blinded, placebo-controlled clinical trials for both nonmalignant (e.g., HIV-mediated neuropathic pain) and morphine-refractory malignant pain. Intrathecal Prialt provides statistically significant decreases in Visual Analog Scale of Pain Intensity scores across many of the chronic and severe conditions examined including in some instances providing complete pain relief in patients previously unresponsive to intrathecal morphine.[39–41] Interestingly, Prialt also appears to control the severe spasticity associated with spinal cord injury.[42]

The administration of Prialt is not without adverse effects in either animals or humans. Intrathecal administration in rats consistently shows serpentine tail movements and whole body shaking suggestive of cerebellar-related motor defects. In humans, intrathecal Prialt administration can result in severe but reversible psychiatric symptoms and neurological impairment including cognitive impairment, hallucinations, and changes in mood and consciousness.[39–41] Systemic administration of Prialt results in severe orthostatic hypotension, presumably from interactions with N-type channels in the sympathetic nervous system.[43]

Interestingly, even though both direct N-type channel blockade and spinal opioid receptor activation likely exert their antinociceptive effects at least in part via a common inhibition of neurotransmitter release, none of the N-type adverse effects appear similar to those commonly associated with opioid administration.[44] Neither animals nor humans administered intrathecal Prialt show significant respiratory depression, constipation, sedation, loss of motor coordination, or the euphoria/stupor associated with opioids. Additionally, continuous spinal perfusion of Prialt over long periods of time appears to alleviate pain symptoms without either the requirement for increasing drug dosage over time (tolerance) or being accompanied by withdrawal symptoms upon abrupt termination of drug (addiction).[39–42] Conversely, morphine administration does not result in the observed ataxias associated with N-type channel blockade by Prialt.

That morphine works well in animal models examining acute nociception but that Prialt is only moderately effective suggests that both mechanistic and spatial mechanisms come into play. As noted, spinally administered opioids likely act by both activating potassium channels and also inhibiting presynaptic N-type channels, whereas Prialt only exerts its analgesic effects through inhibiting N-type channels. Additionally, it is highly likely that not all opioid receptors are specifically colocalized with presynaptic N-type channels and similarly that not all N-type channels are susceptible to opioid-dependent modulation.[45] Indeed, centrally opioids are known to modulate multiple subtypes of widely distributed high-threshold calcium currents (including P/Q-type)[16] and thus the potential for a wider range of opioid-related adverse effects is greater than that for the more restricted N-type calcium channel target.

DOES BLOCK OF N-TYPE CHANNELS IN THE DORSAL HORN AFFECT MOST OR ALL PAIN PROCESSING OCCURRING VIA PRIMARY AFFERENT STIMULATION?

The dorsal horn of the spinal cord is a critical integration site involved in processing signals resulting from a variety of noxious stimuli including thermal, mechanical, and inflammatory. The thinly myelinated A-δ and unmyelinated C fibers make their terminals onto interneurons in the superficial laminae I and II and both P/Q-type and N-type calcium channels are localized in the dorsal horn, suggesting that both channel subtypes contribute to afferent signaling. Interestingly, most individual terminals in the dorsal horn express N-type or P/Q-type channels but not both subtypes, suggesting that these channels are individually associated with distinct aspects of primary afferent signaling.[46] Block of P/Q-type by intrathecal application of ω-agatoxin-IVA has only a mild affect on the allodynia associated with spinal nerve ligation, whereas blockade of N-type channels with Prialt has a major antinociceptive effect. Colocalization studies show that terminals expressing P/Q-type channels largely do not contain substance P, whereas those terminals using N-type channels to trigger release contain substance P.[46] Taken together with data showing that ω-conotoxin GVIA inhibits the release of substance P, it appears that blockade of N-type channels in laminae I and II affects nociception particularly associated with substance P release (and likely also CGRP and glutamate that tend to colocalize with substance P containing terminals.[47]

It should be noted parenthetically that, as N-type channels are localized on both excitatory and inhibitory terminals in the spinal cord (including some interneurons), their inhibition could have quite variable physiological consequences dependent upon the nature of the specific signaling pathways affected. Also, it remains unknown whether there are distinct subtypes of spinal N-type channels with unique biophysical properties that might be differentially affected by drugs acting via state-dependent mechanisms.

IS THERE A ROLE FOR N-TYPE CHANNELS IN PAIN PERCEPTION/INDUCTION IN THE HIGHER CNS?

In addition their direct role in transmitting nociceptive stimuli into the CNS via primary afferent terminals in the dorsal horn of the spinal cord, N-type channels may also play a key role in the hypersensitivity associated with certain neuropathic pain conditions. For example, both P/Q-type and N-type channels in the rostral ventromedial medulla contribute to tactile allodynia through activation of descending facilitatory systems.[48,49]

CAN PRIALT BE IMPROVED UPON? THE CASE FOR IDENTIFYING ORALLY AVAILABLE, STATE-DEPENDENT N-TYPE CHANNEL BLOCKERS

Although Prialt has been shown to offer significant therapeutic benefit in a number of chronic, opioid-unresponsive conditions, a case can be made that the development of future N-type channel therapeutics should address several issues.

1) The therapeutic index (ratio of relative toxicity to relative efficacy) of intrathecally administered Prialt is quite narrow in both animals (ratios range from 1.5–2.1) and in humans.[39–41] Clinically, Prialt must be titrated slowly in each individual patient, and typically doses are reached whereby some adverse affects are observed even before the dose is fully efficacious. Initial data with other natural peptides (e.g., ω-conotoxin-CVID and huwentoxin-I; see below) suggest that it may be possible to maintain the potent antinociceptive effects associated with N-type channel blockade and have fewer, less severe adverse effects.

2) The requirement that Prialt (or other peptide N-type channel blockers) be administered intrathecally is extremely limiting. Identifying safe and efficacious orally available N-type channel blockers is relatively straightforward to rationalize from both the patient and marketing perspectives. The invasive surgery and high costs associated with implantable pumps clearly limits the numbers of patients both eligible and willing to use Prialt regardless of its potential as a potent antinociceptive.

3) Voltage-gated ion channels are well known to exist in a number of discrete biophysical states (e.g., open, closed/resting, and inactivated) that presumably reflect distinct time-dependent and voltage-dependent conformations.[50] It has also been well established that the interaction of various drugs (both antagonists and agonists) with voltage-gated ion channels are significantly different depending upon the particular channel state. Certain drugs preferentially interact with channel sites in specific conformations and in some instances drug-channel binding affinity can vary by orders of magnitude from state to state of the channel. This phenomenon is of particular relevance as it relates to both the physiological consequences of drug action but is equally important to the safety profile of most drugs interacting with ion channels.

The biophysical states of calcium channels, and hence drug interaction, can be affected by various parameters including both resting membrane potential and the frequency of stimulation. In the case of the widely clinically used L-type channel antagonists, Bean provided the first evidence that the 1,4-dihydropyridine L-type channel blockers likely provide an excellent therapeutic window

due to their preferential binding and block of inactivated channels.[51]

In the case of N-type channels, an initial report found Prialt block to be reversed by strong membrane depolarization suggesting a state-dependent mechanism.[52] However, in a thorough analysis under more relevant physiological conditions, Prialt block was found to occur in all states—the resting, open and inactivated conformations—and there was little evidence of frequency or voltage dependence.[53] In this regard, the development of state-dependent N-type channel blockers would be predicted to significantly improve the therapeutic index over that for Prialt.

In consideration of blocking N-type channels for therapeutic intervention, there are some things that we either quite yet don't fully understand or have sufficient information to make definitive conclusions. For example, in the mammalian CNS, whereas N-type channels appear to be widely distributed (as evidenced by mRNA, immunohistochemical, and ω-conotoxin-GVIA binding studies), there seem to be relatively few adverse physiological consequences in knockout mice that completely lack all N-type channel activity. Similarly, there is good evidence for N-type channel expression in certain spinal motor neurons and neuroendocrine cells, and yet there is sparse evidence to indicate that animals lacking this channel suffer adverse neuroendocrine or motor affects. While it is possible that compensation by other calcium channel types overcomes any deleterious effects in the knockout animals, studies examining calcium current levels in the knockout mice do not find evidence for compensatory mechanisms.

Concerning a major direct involvement in motor transmission, whereas N-type channels have been described in spinal motor neurons and at mammalian neuromuscular junctions, they appear to account for a very small portion of neuromuscular terminals (<5%) compared with that for P/Q-type channels (~95%).[46] Even then, N-type channels are only found at a subset of specific neuromuscular terminals (e.g., tibialis anterior, gastrocnemius, and soleus muscles) and appear not to be localized at presynaptic junctions associated with other muscle such as the diaphragm.

Given the relative lack of CNS-related physiological consequences reported in N-type gene knockout mice (which essentially mimics complete blockade of all N-type channels), what then causes the significant cardiovascular and CNS adverse effects found in animals and humans treated with the clinical N-type channel blocker Prialt? This issue remains to be adequately addressed and whether Prialt adverse affects are specifically related to the N-type target itself, the biophysical mechanism of action of Prialt block on N-type channels, or to some as yet to-be-described nonspecific effects of Prialt on other targets remains to be fully elucidated.

Interestingly, other natural peptides with high affinity and selectivity for the N-type channel do not appear to have similar deleterious effects *in vivo*. For example, AM336 (ω-conotoxin-CVID), a 27-amino acid peptide from *Conus catus*, has been shown to be a potent blocker of N-type currents and to displace radiolabeled ω-conotoxin-GVIA binding at potencies similar to that for both ω-conotoxin-GVIA and ω-conotoxin-MVIIA.[54] In animal models of persistent inflammatory and neuropathic pain, AM336 results in potent dose-dependent antinociception. However, despite AM336's similar high potency block of N-type channels compared to that for ω-conotoxins-GVIA and -MVIIA, it appears to possess a remarkably better CNS safety profile in animals.[55,56] In rodents, intrathecally administered AM336 produces a five-fold better therapeutic index (ratio of relative toxicity to relative potency) concerning the incidence of the serpentine tail movements and whole body shaking observed for similarly administered Prialt.

In another example, intrathecal administration of huwentoxin-I, a 33-amino acid peptide from the venom of the Chinese bird spider *Ornithoctonus huwena* appears as effective as ω-conotoxin-MVIIA in both the early and late phases of the formalin model of inflammatory pain.[57] However, whereas huwentoxin-I blocks N-type channels with high affinity (EC_{50} = 100 nM) and selectivity, at high doses *in vivo* it exhibits a significantly lower degree of the motor dysfunction and ataxia compared with that for Prialt.

It is tempting to speculate that the significantly better therapeutic window in animals of AM336 compared with that for Prialt is due to the fact that, in radioligand binding assays, AM336 is approximately 100-fold more selective for N-type channels over P/Q-type channels compared to Prialt. P/Q-type channels are the major calcium channel subtypes implicated in triggering neurotransmission in the CNS in mammals, and even a moderate degree of blockade by intrathecally administered Prialt would be predicted have significant deleterious effects on central and motor neuron functions. It is also tempting to speculate that the fact that the N-type channel gene knockout mice do not exhibit the cerebellar ataxia or whole body shaking characteristic of Prialt administration reflects its action on central targets other than the N-type calcium channel.

N-type currents (along with other calcium channels subtypes) are described in both peripheral sensory neurons and neuroendocrine cells, and it remains unclear as to the specific consequences of N-type channel blockade on peripheral transmission and endocrine function. The ability to predict potential deleterious affects concerning N-type channel blockade in sympathetic neurons is far from clear. In some sympathetic neurons, neurotransmitter release mediate by N-type channels appears to be frequency dependent although not necessarily in the ex-

pected manner. In both carotid body and postganglionic sympathetic nerve terminals, for example, release triggered through N-type channel activation is actually higher at lower stimulation frequencies (0.4–2 Hz) than at higher frequencies (30 Hz).[58] In other sympathetic neurons (e.g., noradrenergic constrictions of the inferior vena cava and uterine artery), there is no affect of stimulation frequency on N-type channel-dependent release, whereas in still other cells sympathetic-mediated vasoconstriction does not appear to involve N-type channel-mediated release.[59]

In one homozygous N-type channel gene knockout strain, it was found that heart rate and blood pressure were unaffected by the complete lack of N-type channels, whereas in another independent strain both heart rate and blood pressure were elevated.[35,37] Affects on the carotid baroreflex were more definitive in that N-type gene knockout mice appeared to lack the normal response to carotid artery occlusion. Additionally, a contribution to sympathetic neurotransmission was suggested in that N-type-deficient mice showed an impaired cardiac inotropic response, although contradictorily it was noted that circulating norepinephrine levels were actually normal.[35]

In support of the notion for developing state-dependent N-type blockers for peripheral administration, N-type currents in adrenal chromaffin cells exhibit variable inactivation characteristics with a large fraction of the channels being resistant to inactivation even during prolonged depolarization.[6] Under such circumstances it could be envisioned that N-type blockers with significantly higher affinity for the inactivated states would have minimal affects on N-type channel-mediated endocrine functions.

Additional factors likely relevant to the discussion include the fact that it is well known that all neurotransmission is not created equal: different size synaptic vesicles exist, the different vesicles often contain distinct types of neurotransmitters and neuropeptides, and release can be differentially dependent upon the nature of stimulation (e.g., in some cases release from small vesicles can occur in response to single or low frequency stimuli, whereas large vesicle release can require stronger stimuli and/or higher frequency stimulation). Release dependent upon these various factors has been predicted to involve both differential vesicle interactions with SNARE proteins and also the involvement distinct subtypes of presynaptic calcium channels. Overall, the data suggest that some portion of sympathetic function would likely not be affected by N-type channel blockade, whereas in other instances state-dependent N-type channel blockers aimed at selectively targeting channel states associated with higher frequency stimulation might have minimal affect on normal sympathetic functions such as those associated with carotid bodies.

CONCLUSIONS

The selective N-type calcium channel peptide antagonist Prialt has demonstrated significant antinociceptive action even in morphine-unresponsive clinical situations. Whereas Prialt has a narrow therapeutic window, the general lack of serious opioid-related side effects such as respiratory depression, addiction, and tolerance has made the N-type channel an attractive therapeutic target for a variety of chronic/neuropathic pain conditions. Future directions include improving on both Prialt's limited delivery options and its safety profile through the development of small molecule, orally available N-type channel blockers that act via state-dependent mechanisms. Issues that remain to be fully elucidated include reconciliation of conflicting data between the relatively significant adverse effects observed for Prialt compared with the more mild effects observed by other peptidic N-type channel blockers and in N-type channel gene knockout mice. Additionally, the physiological consequences of systemically blocking N-type channels in the peripheral nervous system still needs to be adequately addressed.

Acknowledgments: I thank Dr. Gerald Zamponi for helpful comments. The author is a Professor in the Michael Smith Laboratories, University of British Columbia, and is also the founder and Chief Scientific Officer of Neuromed Technologies, Inc. Work in the Michael Smith Laboratories is supported by the Canadian Institutes for Health Research and by a Canadian Research Tier 1 Chair in Biotechnology and Genomics.

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