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DRUG DEVELOPMENT RESEARCH 67:404–415 (2006)

Research Overview

T-Type Calcium Channels: An Emerging Therapeutic Target for the Treatment of Pain

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT It has become generally accepted that presynaptic high voltage–activated N-type calcium channels located in the spinal dorsal horn are a validated clinical target for therapeutic interventions associated with severe intractable pain. Low voltage–activated (T-type) calcium channels play a number of critical roles in nervous system function, including controlling thalamocortical bursting behaviours and the generation of spike wave discharges associated with slow wave sleep patterns. There is a growing body of evidence that T-type calcium channels also contribute in several ways to both acute and neuropathic nociceptive behaviours. In the one instance, the Cav3.1 T-type channel isoform likely contributes an anti-nociceptive function in thalamocortical central signalling, possibly through the activation of inhibitory nRT neurons. In another instance, the Cav3.2 T-type calcium channel subtype acts at the level of primary afferents in a strongly pro-nociceptive manner in both acute and neuropathic models. While a number of classes of existing clinical agents non-selectively block T-type calcium channels, there are no subtype-specific drugs yet available. The development of agents selectively targeting peripheral Cav3.2 T-type calcium channels may represent an attractive new avenue for therapeutic intervention. Drug Dev. Res. 67:404–415, 2006.

Key words: calcium channel; T-type; Cav3.2; nociception; mibefradil; ethosuximide

INTRODUCTION

Twenty-five years ago, Llinas and Yarom [1981a,b] first described low-threshold calcium-dependent spikes in the mammalian inferior olive, a phenomenon that has since been demonstrated in many brain nuclei including those found in the hippocampus, hypothalamus, thalamus, habenula, cortex, and globus pallidus [reviewed in Perez-Reyes, 2003]. Low-threshold calcium spikes generally act as pacemakers, helping to trigger bursts of sodium-dependent action potentials after neuronal membrane hyperpolarization [Huguenard and Prince, 1992; McCormick and Huguenard, 1992]. They also contribute to oscillatory and rebound burst firing behaviors relevant to both normal physiological functions (e.g., thalamocortical processes such as deep sleep) and to pathophysiological states (e.g., spike-wave discharges associated with absence epilepsy [reviewed in Huguenard, 1996, 2002]. Underlying low-threshold calcium-dependent spiking activity is a physiologically and pharmacologically unique class of voltage-gated calcium channel called low-voltage-activated (LVA) or T-type calcium channels [Carbone and Lux, 1984; Nowycky et al., 1985].

Grant sponsor: Canadian Institutes for Health Research.

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Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ddr.20103

T-type calcium channels are generally distinct from high voltage–activated (HVA) calcium channels (the L-, N-, P/Q, and R-types) in their both negative and overlapping activation (initial activation ~−70 to −60 mV) and inactivation ($V50_{inact}$ ~−55 to −85 mV) ranges, fast kinetics of inactivation (τ_{inact} ~10 to 20 ms), rapid recovery from inactivation, slow deactivation (closing), and small single-channel conductance (~5 to 10 pS). The low-threshold calcium spikes first observed by Llinas can largely be attributed to the unique properties of T-type calcium channels, which become deinactivated after inhibitory synaptic inputs and subsequently trigger calcium-dependent bursting as a result of their negative activation properties.

T-type calcium channels are expressed in many central and peripheral neurons, as well as in other tissues including the heart, smooth muscle, kidney, embryonic skeletal muscle, pituitary, pancreas, adrenal, retina, and testes [reviewed in Perez-Reyes, 2003]. In addition to their pacemaker roles in neurons, these channels also contribute to secretatory processes such as hormone release, the regulation of muscle contraction, olfaction, and cellular differentiation and proliferation. The complete description of the physiological contributions of native T-type calcium channels has been complicated by several factors including (1) the co-expression in many cells of multiple types of HVA and LVA calcium currents with overlapping voltage-dependent and kinetic properties, and (2) a lack of specific, high-affinity T-type channel pharmacological tools. Additionally, even amongst native T-type currents there exits considerable heterogeneity in their activation, inactivation, permeation, and pharmacological properties. While historically referred to as a single class of ion channel, native T-type calcium channels are now known to be encoded by at least three distinct α_1 subunit genes (α_{1G}/Cav3.1, α_{1H}/Cav3.2, and α_{1I}/Cav3.3) and that considerable alternative splicing exists to generate further diversity (Fig. 1) [Cribbs et al., 1998; Lee et al., 1999; McRory et al., 2001; Mittman

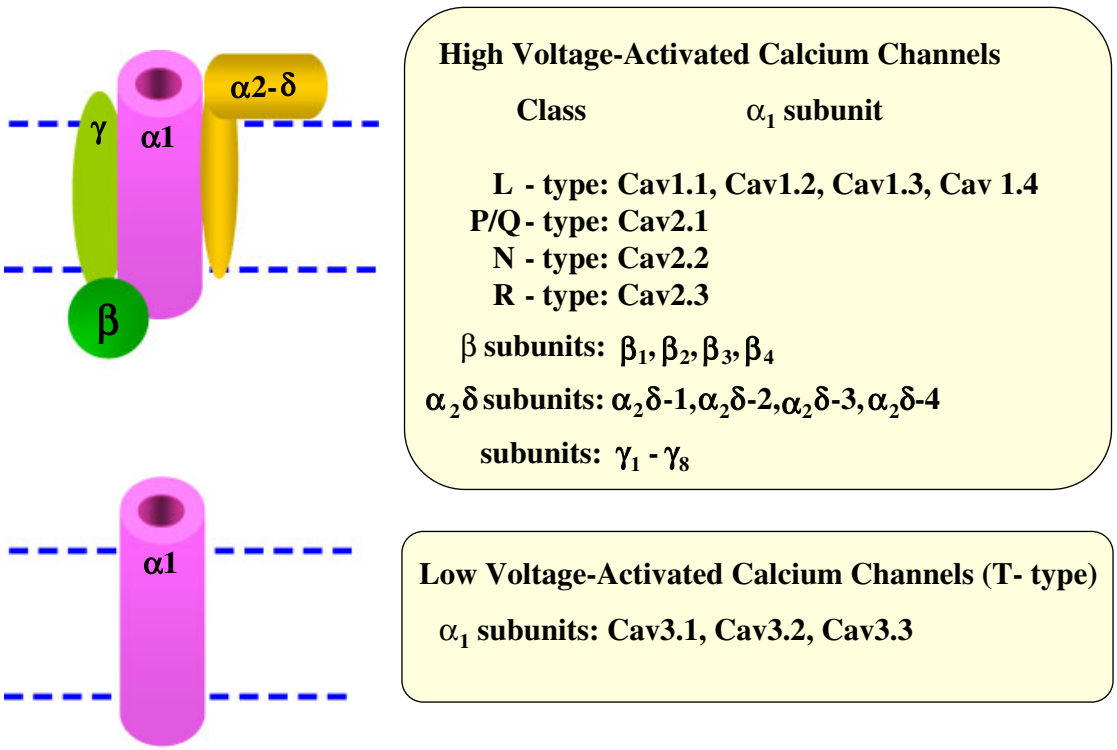

Fig. 1. Composition of neuronal voltage-gated calcium channels. High voltage-activated (HVA) calcium channels are a heteromeric complex consisting of a large (~200–260 kDa) pore-forming α_1 subunit that contains the voltage-sensor and pore region and is the target of known pharmacological agents. There are ten identified α_1 subunit genes in the mammalian genome. Neuronal HVA channels also contain an ancillary β subunit (four genes) and $\alpha_2\delta$ subunit (four genes) that contribute to modulating a number of channel functions including activation, inactivation and kinetic properties, second-messenger regulation, and channel complex intracellular processing. Biochemical purification of the skeletal muscle HVA L-type calcium channel (Cav1.1) shows that it contains a fourth subunit, γ, although reconstitution of neuronal HVA channel properties does not require a γ subunit and it remains to be determined whether native neuronal HVA calcium channel complexes contain this protein. Low voltage-activated (LVA or T-type) calcium channels have not yet been biochemically purified although known biophysical, pharmacological, and regulatory characteristics can be fully reconstituted with a Cav3 α_1 subunit alone.

et al., 1999; Monteil et al., 2000a,b; Perez-Reyes et al., 1998].

Pathophysiologically, both Cav3.1 and Cav3.2 T-type calcium channels may contribute to the genesis of absence seizures: (1) the genetic absence epilepsy inbred strain of rat (GAERS) exhibits spontaneous spike-wave discharges and absence seizures that are associated with an increased basal level of thalamic reticular T-type currents [Tsakiridou et al., 1995]; (2) gene knock-out of the Cav3.1 T-type channel gene in mice results in animals insensitive to $GABA_B$ receptor agonist-induced spike wave discharges [D. Kim et al., 2001]; and (3) a number of point mutations have been recently identified in the Cav3.2 T-type channel gene in patients with childhood absence epilepsy and generalized idiopathic epilepsy [Chen et al., 2003b; Heron et al., 2004]. Introduction of some of the epilepsy mutations into the wildtype Cav3.2 channel results in biophysical changes consistent with gain-of-function alterations to channel activity and are consistent with the notion that some clinical antiepileptics act mechanistically to inhibit T-type calcium channel activity [Coulter et al., 1989; 1990; Khosravani et al., 2004, 2005; Peloquin et al., 2006; Vitko et al., 2005].

CALCIUM CHANNELS AND PAIN

Nociceptive processes are known to be highly sensitive to intracellular calcium levels and to date there have been two distinct classes of pain therapeutics developed to target components of HVA calcium channels. In one instance, the N-type calcium channel blocking peptide, ziconitide, is a 25 amino acid synthetic peptide (ω-conotoxin MVIIA) derived from the marine hunting cone snail *Conus magus*, which has recently been approved (Prialt™) both in the United States and in Europe for the treatment of intractable pain [Snutch, 2005]. N-type calcium channels are highly concentrated in the cell bodies and synaptic terminals of a subset of primary afferents that terminate in the dorsal horn of the spinal cord (mainly C-fibers and A-δ fibers). In animals, block of N-type channels by the intrathecal administration of ziconitide inhibits the release of the nociceptive transmitters, substance P and CGRP, consistent mechanistically with the role of N-type channels in triggering neurotransmission at dorsal horn primary afferent terminals [Evans et al., 1996]. The activation μ-opioid receptors attenuates N-type channel activity through the direct binding of a single $G_{\beta\gamma}$ dimer to the N-type channel α_1 subunit consistent with the notion that opioids in part mediate their analgesic affects through inhibiting presynaptic calcium channel activity [Bourinet et al., 1996; Soldo and Moises, 1998; Zamponi et al., 1997; Zamponi and Snutch, 1998]. Knock-out of the N-type channel genetically in mice results in animals largely resistant to the induction of neuropathic and inflammatory pain although otherwise exhibiting normal sensory and motor functions [Ino et al., 2001; C. Kim et al., 2001; Saegusa et al., 2001]. Clinically, intrathecal ziconitide (Prialt™) is highly efficacious in the treatment of morphine-refractory neuropathic and malignant pain conditions, although it exhibits a narrow therapeutic index (ratio of relative toxicity to relative efficacy) and must be titrated carefully in each patient. Interestingly, while the N-type channel is downstream in the opioid receptor pathway, the direct N-type channel blockade by ziconitide does not result in opioid-type side effects such as tolerance and addiction [Brose et al., 1997; McGuire et al., 1997; Ridgeway et al., 2000; Staats et al., 2004].

In the second instance of approved pain therapeutics targeting HVA calcium channels, the orally administered small organic molecules gabapentin and pregabalin bind to the $\alpha_2\delta$ subunit associated with HVA calcium channel complexes. Gabapentin and pregabalin are clinically effective anticonvulsants that while synthetic analogs of the neurotransmitter γ-aminobutyric acid (GABA), do not exert their effects via interacting with GABA receptors or transporters but rather bind with high affinity to the HVA calcium channel ancillary $\alpha_2\delta$-1 and $\alpha_2\delta$-2 subunits [Gee et al., 1996; Marais et al., 2001]. Peripheral nerve injury upregulates $\alpha_2\delta$ expression in both the DRG and spinal dorsal horn, leading to the proposal that the $\alpha_2\delta$ subunit contributes to central sensitization [Li et al., 2004; Luo et al., 2002]. Numerous open label and double-blinded clinical trials show that gabapentin is efficacious in the treatment of neuropathic pain conditions including diabetic neuropathy, postherpetic neuralgia, trigeminal neuralgia, migraine, and pain associated with cancer and multiple sclerosis [Backonja et al., 1998; Caraceni et al., 1999; Di Trapani et al., 2000; Houtchens et al., 1997; Laird and Gidal, 2000; Rowbotham et al., 1998]. Interestingly, while the $\alpha_2\delta$ subunit is associated with all known HVA calcium channel α_1 subunits, including the L-type channels found in skeletal, smooth, and cardiac muscles, gabapentin and pregabalin exhibit relatively few motor or cardiovascular adverse effects even at high therapeutic doses. Along these lines, determination of the exact mechanism of action of gabapentin has proven elusive with reports both supporting and refuting direct inhibitory actions on HVA calcium channels [Bayer et al., 2004; Brown and Randall, 2005; Sutton and Snutch, 2002].

Is there a role for T-type calcium channels in pain processing? A significant component of neuropathic

pain related to peripheral nerve injury is thought to result from hypersensitivity and/or abnormal spontaneous firing along the primary afferent pathway. Wind-up is a frequency-dependent facilitation of spinal cord excitability mediated via afferent C-fibers and has been suggested to be linked to the central sensitization observed after peripheral nerve damage [for review see Herrero et al., 2000]. As T-type calcium channels activate at sub-threshold membrane potentials, one physiological route to altering the ectopic discharge of primary afferents may involve either the altered expression and/or modulation of T-type calcium channels. Of particular relevance, reducing agents such as L-cysteine modulate both thermal and mechanical nociception when injected into peripheral receptive fields [Todorovic et al., 2001]. Redox modulation appears to occur through a mechanism involving the selective up-regulation of T-type whole cell currents in a subset of DRGs [Nelson et al., 2005; Todorovic et al., 2001, 2004]. Interestingly, both in the higher CNS and spinal cord there also exists a number of similarities between the proposed physiological functions of T-type calcium channels in processes such as long-term potentiation and kindling, and those for the central sensitization associated with neuropathic pain wherein postsynaptic responses progressively increase [Ikeda et al., 2003].

Which of the three functionally distinct T-type calcium channel isoforms might be involved in nociceptive behaviors? In the periphery, a subset of small- and medium-size DRG neurons are known to express large whole cell T-type calcium currents [Schroeder et al., 1990; Scroggs and Fox, 1992]. In situ hybridization and reverse-transcription PCR studies show that of the three known T-type channel subtypes, Cav3.2 (α1 H) is most highly expressed in DRGs while these same cells express relatively low levels of Cav3.3 (α_{1I}) and little to none of Cav3.1 (α_{1G}). In D-hair cell mechanoreceptors (a subset of medium sized DRGs), the Cav3.2 T-type channel has also been shown to contribute to a slow after depolarizing potential that lowers the voltage-threshold for action potential generation. Pharmacological block of Cav3.2 in D-hair cells suggests that this T-type channel subtype is required for the normal transduction of slow-moving mechanical stimuli [Dubreuil et al., 2004; Shin et al., 2003].

Utilizing intrathecal injection of antisense oligonucleotides, Bourinet et al. [2005] found that selective Cav3.2 T-type channel knock-down affects both acute and neuropathic pain behaviors in rat. An approximate 50% reduction in Cav3.2 mRNA expression resulted in a 75 to 90% decrease in whole cell T-type current density in small- and medium-size DRGs, and a concomitant increase in both the vocalization threshold and tail withdrawal latency in response to noxious acute mechanical and thermal stimuli. Similarly, a complete reversal of mechanical allodynia in the Bennett neuropathic model was noted in Cav3.2 knock-down animals. In agreement with the low levels of detectable Cav3.1 and Cav3.3 in the DRG, the intrathecal injection of antisense oligonucleotides against Cav3.1 and Cav3.3 did not significantly affect nociceptive behavior in rats. Taken together, these data are strongly suggestive for the Cav3.2 T-type calcium channel selectively contributing both to normal acute nociception and to chronic pain hyperexcitable states.

While the low expression of the Cav3.1 T-type in DRG neurons suggests a minimal role for this calcium channel related to peripheral pain mechanisms, the Cav3.1 channel is highly expressed in the thalamus and appears to play a significant role in central pain processing at least as it relates to visceral pain. Kim and co-workers found that either knock-out of the Cav3.1 gene in mice or infusion of mibefradil directly into the ventroposterolateral (VPL) thalamus (to block Cav3.1 channels) act to enhance the pain response elicited by intraperitoneal administration of acetic acid or magnesium sulphate [Kim et al., 2003]. In response to visceral pain stimuli, wild type VPL neurons generate both increased single spikes and clustered bursts of action potentials. In Cav3.1 knockout mice, VPL neurons exhibit normal single spike activity but an almost total absence of burst spikes suggesting that Cav3.1-dependent bursting activity mediates a downstream inhibitory process likely involving nRT neurons. In contrast to that for the Cav3.2 channel, it therefore appears that central native Cav3.1 T-type channels act in an anti-nociceptive capacity. It remains to be determined whether the selective pharmacological blockade of this low-threshold calcium channel might have the unwanted effect of enhancing the central perception of noxious stimuli.

CLINICAL AGENTS WITH T-TYPE CALCIUM CHANNEL BLOCKING ACTIVITY

There appears a strong connection both mechanistically and pharmacologically between epilepsy, neuropathic pain, and migraine headache; thus targeting the T-type calcium channels that contribute to these pathophysiological processes is quite attractive. Although selectively targeting T-type calcium channels for therapeutic purposes has been of significant interest, to date there are no "pure" T-type channel blockers presently in clinical usage. In spite of this critical pharmacological limitation, there are a number of structurally distinct classes of drugs that more broadly interact with multiple ionic conductances

including T-type calcium channels. These agents may provide important clues concerning the validation of the T-type channel targets, and perhaps also suggest chemical backbones relevant towards future compound-based structure-activity development.

Antiepileptics

Zonisamide (Fig. 2) is a widely utilized broad-spectrum antiepileptic. Mechanistically, zonisamide is known to variously inhibit nitric oxide formation, to increase serotonergic transmission and basal acetylcholine and gamma-aminobutyric acid (GABA) release, and to block both voltage-gated sodium channels (Kd ~1 μM) and T-type calcium channels [Mimaki et al., 1990; Schauf, 1987; Zhu et al., 2002]. Zonisamide blocks T-type calcium currents in a concentration-dependent manner without altering either the voltage-dependence of activation or inactivation kinetics. In cultured neurons of rat cerebral cortex, the mean percentage reduction in T-type current is approximately 60% at 500 μM with no observed block of L-type currents [Suzuki et al., 1992]. In addition, 50 μM zonisamide also reduces T-type currents (by ~40%) in cultured neuroblastoma cells [Kito et al., 1996]. In the Bennett chronic constriction rat model, zonisamide relieves thermal hyperalgesia in a dose-dependent manner although it has little effect on mechanical allodynia [Hord et al., 2003]. Clinically, in a number of open-label case studies, zonisamide has been shown to be effective in a variety of treatment-refractory neuropathic pain conditions [Guay, 2003; Takahashi et al., 2004]. Additionally, in several open-label analyses of treatment-refractory migraine patients, zonisamide is also highly effective as a prophylactic agent [Drake et al., 2004]. Zonisamide is contraindicated in patients with sulfonamide allergies

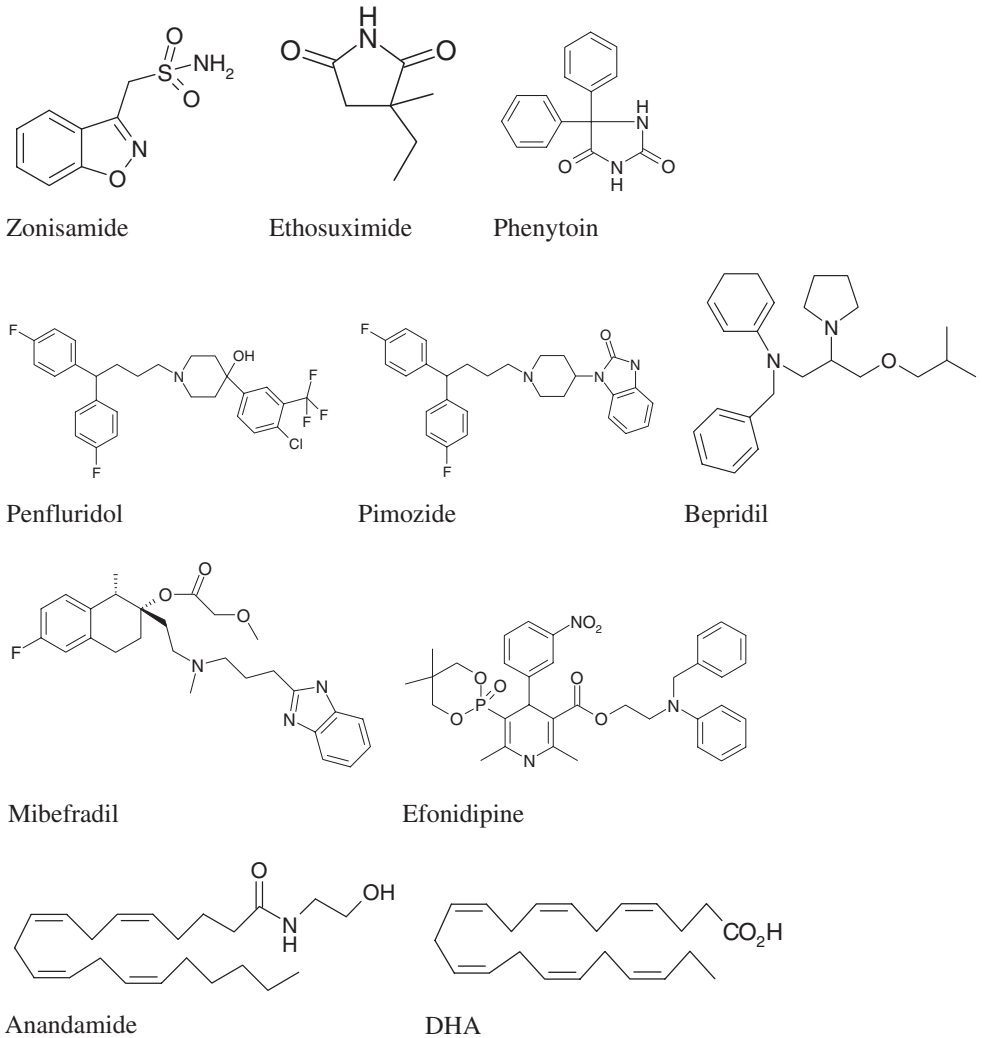

Fig. 2. Structures of compounds mentioned in the text.

and any future structure-activity relation (SAR) studies targeted at improving T-type affinity and/or selectivity might also address this limitation.

Ethosuximide

The succinimides methyl-phenyl-succinimide (MPS) and ethosuximide (Fig. 2) are widely utilized antiepileptics thought to in part act therapeutically via the inhibition of cortical-thalamic T-type calcium channels involved in mediating 3 Hz spike-wave discharges. MPS and ethosuximide inhibit cloned T-type calcium channels in a state-dependent manner and at concentrations considered to be clinically relevant (IC_{50}s; ~0.3 to 1 mM for Cav3.1, Cav3.2, and Cav3.3 subtypes vs. therapeutic plasma levels ~0.1 mM for MPS and ~0.3 to 0.7 mM for ethosuximide [Gomora et al., 2001]). In both nerve-injured and sham-operated animals, in vivo recordings show that ethosuximide applied directly to the spinal cord inhibits both mechanical and thermal-evoked responses in a dose-dependent manner [Matthews and Dickenson, 2001]. Direct spinal application of ethosuximide produces the greatest maximal inhibition on C-fibers and Aδ-fibers compared to Aβ-fibers, consistent with the notion both that T-type channels are differentially expressed amongst DRG neurons and are preferentially localized to C-fibers and Aδ-fibers that convey thermal and nociceptive information and not to Aβ-fibers that subserve proprioception and responses to tactile stimuli.

Examining L5/L6 nerve-injured animals, Dogrul and colleagues found that i.p. administration of ethosuximide produces a dose-dependent inhibition of both thermal hyperalgesia (A_{50} = 126 mg/kg) and mechanical allodynia (A_{50} = 174 mg/kg) [Dogrul et al., 2003]. Direct intrathecal (i.t.) administration of ethosuximide is without effect, perhaps suggesting a peripheral target site of action, although the direct injection of ethosuximide into the injured paw (intraplantar) is also without effect. Ethosuximide administered i.p. also completely reverses capsaicin-induced mechanical allodynia (ED_{50} = 108 mg/kg) and is antinociceptive in both the early and late phases of the formalin response as well as the acute tail flick assay [Barton et al., 2005]. Finally, i.p.-administered ethosuximide is highly efficacious in reversing paclitaxil- and vincristine-induced peripheral neuropathy [Flatters and Bennett, 2004]. In spite of these promising animal data, in the over 40 years that ethosuximide has been utilized clinically there are few if any reports of it being efficacious towards human neuropathies.

Phenytoin

Phenytoin (Fig. 2) is clinically utilized as both an anticonvulsant as well as an analgesic for neuropathic pain [McCleane, 1999]. The antinociceptive properties of phenytoin have been attributed to its ability to block both voltage-dependent sodium and calcium channels. Phenytoin blocks sodium channels from rat cortical synaptosomes (IC_{50} > 800 μM) and cloned sodium channels expressed in *Xenopus* oocytes [Anderson et al., 2003; Lingamaneni and Hemmings, 1999; Twombly et al., 1988]. In N1E-115 neuroblastoma cells, phenytoin at concentrations of between 3 and 100 μM inhibits T-type calcium currents without altering channel activation or kinetic properties. However, the steady-state inactivation profile is shifted more hyperpolarized. Phenytoin blocks cloned α_{1G} (Cav3.1) and α_{1H} (Cav3.2) T-type channels expressed in HEK 293 cells at IC_{50}s of 140 and 8.3 μM, respectively [Todorovic et al., 2000]. In addition, in cultured dorsal root ganglia neurons (DRGs) phenytoin blocks whole cell T-type calcium currents in a concentration-dependent manner (IC_{50} ~8.3 μM). In a bradykinin-induced pain model in rats, phenytoin produces dose-dependent analgesic affects at an ED_{50} of 3 mg/kg applied subcutaneously [Foong et al., 1982]. In a mouse acute pain model using plantar and tail pressure to evaluate acute thermal and mechanical nociception, phenytoin preferentially relieves thermal pain at dose between 2.5 to 25 mg/kg applied intraperitoneally [Sakaue et al., 2004]. Clinically, in a randomized, double-blinded, placebo-controlled, crossover study, phenytoin relieves flare-ups of chronic neuropathic pain and has also been shown to significantly enhance buprenorphine analgesia in cancer patients [McCleane, 1999; Yajnik et al., 1992].

Antipsychotics

The neuroleptics comprise a chemically diverse set of molecules that largely act clinically to inhibit dopamine D2 receptors although, interestingly, a subset of these agents also exhibit potent calcium channel blocking activity. In particular, the diphenyl-butylpiperidines, pimozide and penfluridol (Fig. 2), block T-type channels in a variety of cell types including from adrenal, heart, neural crest, and spermatogenic tissues [Enyeart et al., 1990a,b, 1992]. Examination of pimozide and penfluridol on cloned T-type channels showed that they block all three mammalian T-type channel isoforms (Cav3.1, Cav3.2, and Cav3.3) with a higher affinity than either ethosuximide or mibefradil (K_ds ranging from ~40 to 100 nM) [Santi et al., 2002]. Block is state dependent, shifting T-type channel steady-state inactivation profiles to more negative potentials, but does not affect T-type channel activation or kinetic parameters. Interestingly, from a structure-activity perspective, the highly structurally related diphenyldiperazine,

flunarizine, and the butyrophenone antipsychotic, haloperidol, show both significantly less potent T-type channel-blocking activities (K_ds ranging from ~500 to 3,500 nM for Cav3.1, Cav3.2, and Cav3.3) and also exhibit distinct clinical pharmacologies in patients [Opler and Feinberg, 1991].

In one study examining a mouse formalin model of inflammatory pain, relatively low doses of pimozide (0.05–0.25 mg/kg i.p.) were not shown to be highly efficacious [Saddi and Abbott, 2000]. Interestingly, however, although pimozide has been widely used clinically as a neuroleptic to treat conditions such as schizophrenia, Tourette's, and obsessive compulsive disorder, it has also proven efficacious in several neuropathic pain conditions. In particular, and while the pathophysiological mechanism underlying its therapeutic effects are unknown, pimozide appears to provide significant relief in the management of trigeminal neuralgia, a relatively uncommon but severe facial pain syndrome associated with repetitive action potentials [Green and Selman, 1991; Lechin et al., 1989].

Antiarrhythmics and Antihypertensives

A number of cardiovascular agents are thought to act in part mechanistically via inhibiting T-type calcium channels, either solely or as mixed T-type and L-type calcium channel blockers. None of these agents has yet to be shown efficacious in the clinical setting for pain management although given their pharmacological characteristics, there is compelling reason to examine some of these drugs in various neuropathies.

Bepridil (Fig. 2) is a widely utilized clinical antiarrhythmic agent with antianginal properties known to non-specifically inhibit a variety of ionic conductances including various sodium (IC_{50} ~30 μM) and potassium channels (IC_{50} from 1 to 30 μM) as well as the L-type calcium channel (IC_{50} from 0.5 to 30 μM) [Hollingshead et al., 1992; Li et al., 1999; Wang et al., 1999; Yatani et al., 1986]. More recently, bepridil has been shown to inhibit the Cav3.2 (α1 H) T-type calcium channel with an IC_{50} ~400 nM. Block is not affected by pulse frequency but is strongly dependent upon holding potential and also shifts steady-state inactivation and activation profiles to more hyperpolarized potentials [Uchino et al., 2005].

Mibefradil

Next to ethosuximide, mibefradil (Fig. 2) is probably the most widely recognized agent generally described as a selective T-type calcium channel blocker. In fact, while this tetralol derivative was originally developed by Roche and briefly brought onto the market as an effective antihypertensive and chronic stable angina pectoris agent targeting T-type channels, mibefradil has been shown to be a somewhat non-selective blocker of both HVA calcium channels (IC_{50} values in barium recording saline; P/Q-type ~0.3 μM, R-type ~0.4 μM, L-type ~10 to 20 μM) and T-type channels (IC_{50} values; ~1 μM for Cav3.1, Cav3.2, and Cav3.3) [Jimenez et al., 2000]. There are conflicting reports concerning the mechanism of mibefradil mediated T-type channel blockade with resting-, inactivated-, and open-state block all being suggested, and with some evidence that reducing channel availability can increase affinity by up to tenfold [Martin et al., 2000].

In L5/L6 nerve-injured rats, i.p.-administered mibefradil effectively inhibits both tactile allodynia (A_{50} = 7.4 mg/kg) and thermal hyperalgesia (A_{50} = 12 mg/kg) [Dogrul et al., 2003]. Interestingly, while the direct injection of mibefradil into the injured limb also produces a dose-dependent reversal of tactile allodynia (A_{50} = 92 μg) suggestive of a peripheral mechanism of action, a similar direct administration of ethosuximide (up to 500 μg) is without effect. Barton and colleagues report that while i.p.-administered mibefradil has no effect on capsaicin-induced allodynia, i.t.-administered mibefradil both potently reverses mechanical allodynia in a dose range similar to that for intrathecal morphine (ED_{50} = 9.2 and 4.1 μg for mibefradil and morphine, respectively) and is also antinociceptive in both the early and late phases of the formalin response [Barton et al., 2005]. In contrast to that for ethosuximide, mibefradil (up to 30 μg/rat i.t.) is without affect in the acute tail flick reflex. Dogrul and coworkers also observed no effect of i.t.-administered mibefradil in the rat acute tail-flick assay but found that mibefradil significantly potentiates the ability of low-dose i.t. morphine to prolong response latency (a 5-fold increase in ED_{50} for morphine) and that the response is specific for the μ-opioid receptor subtype (a 30-fold increase in the ED_{50} for DAMGO) [Dogrul et al., 2001]. While mibefradil was removed from the market for issues related to drug–drug interactions, it may yet represent an attractive chemical backbone for the further development of selective T-type calcium channel antagonists.

Efonidipine

Efonidipine (Fig. 2) is an orally active antihypertensive with inhibitory effects on both L- and T-type calcium channels [Masumiya et al., 2000]. In baby hamster kidney (BHK) cells and *Xenopus* oocytes, efonidipine (mixture of R(−) and S(+)-isomers) inhibits exogenously expressed HVA α_{1C} (L-type) calcium currents with IC_{50} values ranging from 0.5 to 2 μM (BHK cells) to 8 to 20 μM (oocytes). It also blocks the cloned Cav3.1 T-type calcium channel with similar affinities in both cell types [Furukawa et al.,

2004]. Interestingly, the R(−)-efonidipine isomer selectively blocks Cav3.1 T-type channels. Inhibition is frequency-dependent, with an increasing potency at higher stimulation frequencies. In fact, in myocardial cells, efonidipine was shown to inhibit native T-type calcium currents in a frequency-dependent manner with IC_{50} values of 13 nM, 2 μM, and 6.3 μM with stimulation frequencies of 1, 0.2, and 0.05 Hz, respectively [Masumiya et al., 2000]. Clinically, efonidipine decreases heart rate and has favourable effects on the nervous system supporting its significance in improving the prognosis in patients with hypertension and its protective influence on the heart and other organs [Harada et al., 2003].

ω-3 fatty acids

The cis-polyunsaturated ω-3 fatty acids are essential dietary agents that exhibit a range of physiological effects including possessing both cardioprotective and neuroprotective activities. At least in part, their protective effects may result from their blockade of voltage-gated sodium channels and HVA L-type calcium channels resulting in reduced electrical excitability in cardiac muscle and neurons [for review, see van der Stelt and Di Marzo, 2005]. More recently, Enyeart and colleagues found that the ω-3 fatty acids docosahexaenoic acid (DHA; Fig 2), eicosapentaenoic acid, and α-linolenic acid also inhibit native T-type calcium channels at potencies significantly higher than that for the clinically utilized succinimides [Danthi et al., 2005]. Block of whole cell T-type currents by the ω-3 fatty acids in bovine adrenal zona fasiculata cells occurs with IC_{50}s ranging from 2.5 to 14 μM and is accompanied by changes in T-type channel voltage-dependent and kinetic parameters. DHA in particular shows significant use-dependent inhibition, suggestive of a preferential interaction with T-type channel open or inactivated states, and a characteristic of most clinical ion channel blocking agents that exhibit good therapeutic ratios. The major T-type channel isoform expressed in zona glomerulosa cells is reported to be Cav3.2 [Schrier et al., 2001], the same subtype implicated in primary afferent nociceptive behaviour and it will, therefore, be interesting to examine the affects of DHA on both acute and neuropathic pain states. A significant number of ω-3 fatty acid derivatives have already been synthesized around this backbone and, given the abundance of DHA in the human diet, both DHA and its metabolites should prove relatively safe [Itoh et al., 2006]

Anandamide

Endocannabinoids are highly lipophilic molecules thought to act as retrograde messengers and to protect, in part, against excitotoxicity by modulating neuronal excitability. Anandamide (N-arachidonyl-ethanolamine; Fig. 2) is an endogenous CB1 cannabinoid receptor ligand that mimics many of the psychoactive effects of delta^9-tetrahydrocannabinol, the most widely recognized active component of marijuana [Lambert and Fowler, 2005]. Anandamide has also been shown to activate TRPV1 vanilloid and α7-nicotinic acetylcholine receptors, to inhibit Kv1.2 and TASK-1 potassium channels, and to bind to the 1,4-dihydropyridine site of L-type calcium channels, although the exact physiological consequences of these interactions remain unknown.

Independent of CB1 receptors, at sub-micromolar concentrations anandamide has also been shown to block the Cav3.2 T-type calcium channel (IC_{50} ~300 nM for Cav3.2) and at micromolar concentrations to inhibit Cav3.1 and Cav3.3 T-type channels (IC_{50} ~4 μM for Cav3.1 and 1 μM for Cav3.3) [Chemin et al., 2001]. Anandamide does not affect T-type activation properties but blockade is strongly dependent upon the channel inactivation state and would therefore result in a significant decrease in the available window current. In the case of Cav3.3 channels, the potency of a block by anandamide could be increased ~ tenfold (IC_{50} ~100 nM) under depolarizing waveforms that mimic thalamocortical firing activity. Of particular relevance, unlike that for the effects of cannabinoids on the high threshold N-type and P/Q-type calcium channels [Mackie and Hille, 1992], anandamide blockade of the T-type channels appears to be a result of direct binding to the channel and is independent of G-proteins, phospholipases, and protein kinases. Similar to that for DHA, it will be interesting to examine the effects of both peripherally and centrally administered anandamide on acute and neuropathic pain states. While the psychoactive effects of anandamide likely precluded the use of this agent for the treatment of pain (at least centrally), there exists considerable room for the development of structurally related derivatives.

POTENTIAL ADVERSE AFFECTS OF CAV3.2 T-TYPE CHANNEL BLOCKADE?

Implication of the Cav3.2 T-type calcium channel in pain mechanisms raises a whole new series of clinically relevant issues that may require addressing. For example, gene knockout of the Cav3.2 T-type channel gene in mice has been shown to result in abnormal cardiovascular function including constitutively constricted coronary arterioles and focal myocardial fibrosis [Chen et al., 2003a]. T-type calcium channels are known to be critically involved in early development and neuritogenesis; thus, there also may be developmentally related issues of concern [Chemin

et al., 2002; McCobb et al., 1989]. T-type calcium channels are also implicated in the calcium-dependent secretion of a variety of hormones from endocrine tissues and the Cav3.2 channel appears selectively expressed in the adrenal cortex and implicated in aldosterone secretion [Schrier et al., 2001]. What might be the physiological consequences of long-term blockade of this channel aimed at treating chronic/neuropathic pain conditions? In isolation, these issues may seem to raise a significant barrier to targeting the Cav3.2 T-type calcium channel. In fact, as described above a number of clinical agents that non-selectively target T-type calcium channels have been long used clinically, many with few apparent serious adverse affects. Additionally, as per the reality of many other clinical agents targeting voltage-gated ion channels (e.g., L-type calcium, sodium, and potassium channels), while many of the apparent physiological barriers in isolation might suggest the potential for serious clinical obstacles, these can often be overcome by the development of selective, state-dependent drugs that block a subset of pathophysiologically relevant target molecules.

ACKNOWLEDGMENTS

We thank Ms. Cynthia Chow and Dr. Hassan Pajouhesh for help with the figures and Dr. Emmanuel Bourinet for comments on the manuscript. The authors' work at the Michael Smith Laboratories is supported by an operating grant from the Canadian Institutes for Health Research and by a Canadian Research Tier 1 Chair in Neurobiology-Genomics. L.S.D. is supported by a fellowship from the Heart and Stroke Foundation of Canada.

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