

Voltage-dependent calcium channels – beyond dihydropyridine antagonists

Terrance P Snutch*, Kathy G Sutton† and Gerald W Zamponi‡

The blockade of L-type calcium channels by dihydropyridines, phenylalkylamines and benzothiazepines has been well described and forms the basis of a multibillion dollar market for the treatment of cardiovascular disease and migraine. More recently, neuron-specific calcium channels have become the subject of intense interest regarding their potential as therapeutic targets for the treatment of chronic and neuropathic pain. A number of recently described agents that selectively target neuronal calcium channels have been described and appear promising for a variety of pain conditions.

Addresses

*Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia, Canada

†Pfizer Global R & D, Cambridge University Forvie Site, Cambridge, UK

‡Department of Physiology and Biophysics, University of Calgary, Calgary, Alberta, Canada

Correspondence: Terrance P Snutch; e-mail: snutch@zoology.ubc.ca

Current Opinion in Pharmacology 2001, 1:11–16

1471-4892/01/\$ – see front matter

© 2001 Elsevier Science Ltd. All rights reserved.

Abbreviations

Ca²⁺ calcium
DRG dorsal root ganglia
GABA γ -aminobutyric acid

Introduction

Calcium (Ca²⁺) influx through voltage-gated Ca²⁺ channels mediates a range of cytoplasmic responses, including muscle contraction, release of neurotransmitters, Ca²⁺-dependent gene transcription and the regulation of neuronal excitability [1,2]. In addition to their normal physiological functions, Ca²⁺ channels are also implicated in a number of human disorders, including congenital migraine, cerebellar ataxia, angina, epilepsy, hypertension, ischemia and some arrhythmias. The clinical treatment of some of these disorders has been aided by the development of therapeutic Ca²⁺ channel antagonists [3]. The clinical contribution of high-threshold Ca²⁺ channels towards nociception, hyperalgesia and allodynia has also recently been the subject of considerable interest.

Ca²⁺ channels as therapeutic targets for pain intervention

A number of Ca²⁺ channel subtypes have been identified and are classified by their distinct electrophysiological and pharmacological properties into T-, N-, L-, P/Q- and R-types (see [4*] for review). The low voltage-activated T-type channels generally activate at potentials more negative than –50 mV, whereas the N-, L-, P/Q- and R-type channels activate at more positive potentials (hence the term high-voltage activated). Although the high threshold

channels exhibit overlapping electrophysiological profiles, they can be distinguished by their pharmacological characteristics: that N-type channels are specifically blocked by the marine snail peptide, ω -conotoxin GVIA; P- and Q-type channels are differentially blocked by ω -agatoxin IVA; L-type channels can be both inhibited and activated by dihydropyridines; and R-type channels are defined by their resistance to these blockers, but can be blocked by SNX482, a peptide toxin isolated from tarantula venom.

High-threshold neuronal Ca²⁺ channels are formed through association of multiple subunits, α_1 , α_2 - δ , β and possibly γ (reviewed in [4*,5]). The α_1 subunit determines the major functional properties of the channel and defines the Ca²⁺ channel subtype, whereas the other subunits modulate various properties of the α_1 subunit. Expression studies have revealed that alternative splicing of the α_{1A} gene generates both P- and Q-type channels, α_{1B} encodes N-type channels, α_{1C} , α_{1D} and α_{1F} are L-type channels, and α_{1G} , α_{1H} and α_{1I} form distinct T-type channels. Although it has been suggested that α_{1E} encodes R-type currents, this has not been completely accepted and recent evidence indicates that mice null for the α_{1E} gene still express significant R-type currents [6].

All of the high-threshold Ca²⁺ channel types are expressed in the spinal cord and there are reports both supporting and refuting the contributions of L-, N- and P/Q-types in short-term nociception (reviewed in [7**]). In contrast, examination of the functional roles of these channels in more long-term pain conditions strongly indicates a pathophysiological role for the N-type channel. In a variety of animal models, the selective block of N-type channels via intrathecal administration of ω -conotoxin GVIA or ω -conotoxin MVIIA significantly depresses the formalin phase 2 response, thermal hyperalgesia, mechanical allodynia and post-surgical pain [7**]. Although block of N-type channels does not appear to prevent the development of opiate tolerance there is strong evidence for synergistic effects between N-type blockers and morphine, suggesting the possibility of fewer side effects by co-administration. P/Q-type channels do not appear to be significantly involved in acute pain sensation, although there is some evidence for their involvement in hyperalgesia and allodynia induced by capsaicin or inflammation [8,9].

Whereas most work has focused on the N-type channel as a target for affecting pain transmission, a recent paper by Saegusa *et al.* [10*] suggests that the α_{1E} channel also contributes to both spinal and supraspinal pain mechanisms. The authors show that the α_{1E} Ca²⁺ channel is highly expressed in the superficial laminae I and II of the spinal

cord dorsal horn as well as in the primary afferent neurons of the dorsal root ganglion. In mice lacking α_{1E} gene expression by embryonic stem cell knockout, no detectable affect on pain transmission related to acute mechanical or noxious stimuli or in the formalin phase 1 response was observed. In contrast, α_{1E} mutant mice displayed lowered formalin phase 2 response, suggesting a role for this Ca^{2+} channel in transmitting inflammatory pain sensation. Interestingly, whereas $+/- \alpha_{1E}$ mutant mice exhibited a reduced response to visceral inflammatory pain, $-/- \alpha_{1E}$ mice showed a normal response, leading the authors to propose either that a compensatory pathway is present in the $-/- \alpha_{1E}$ mice and/or that the $\alpha_{1E} \text{Ca}^{2+}$ channel contributes to descending antinociception by controlling excitatory transmission.

Classes of Ca^{2+} channel blockers

The Ca^{2+} channel α_1 subunit is the primary target for most Ca^{2+} channel blockers, which can be subdivided into three general classes: inorganic blockers; peptide toxins; and small organic blockers. Inorganic Ca^{2+} channel blockers include divalent and trivalent metal ions such as nickel, cadmium and holmium. Although useful research tools, they are of little practical use from a clinical standpoint and their actions at the molecular level are reviewed in detail elsewhere [10•]. Peptide toxins isolated from hunting spiders and marine snails include both pore blockers (e.g. ω -conotoxin GVIA and ω -conotoxins MVIIA and MVIIC) and gating inhibitors (e.g. ω -agatoxin IVA, ω -grammotoxin and SNX-482). Much of the recent research in the area of novel Ca^{2+} channel therapeutics to treat neuropathic pain has centered around Ziconotide (SNX-111), a synthetic derivative of ω -conotoxin MVIIA (see below). Small organic blockers include the dihydropyridines, phenylalkylamines and benzothiazepines that target the L-type Ca^{2+} channel and are widely prescribed for the treatment of cardiovascular disease and migraine. In addition to distinct receptor sites for these small organic blockers, the L-type α_1 subunit contains unique receptor sites for pyrazines, piperidines, indolizinsulfones and benzothiazinones [11,12]. In contrast to L-type channels, comparatively little is known concerning receptor sites specific for small organic molecules relevant to the neuronal Ca^{2+} channels implicated in pain transmission. In the following sections we review more recent results concerning Ca^{2+} channel blocking molecules with unique and diverse structural features relevant to pain.

Ziconotide

Ziconotide is a synthetic form of the 25 amino acid cationic *Conus magus* peptide toxin, MVIIA. A selective antagonist of the N-type Ca^{2+} channel, Ziconotide has been shown to be antinociceptive in animal models of persistent, post-operative and neuropathic pain (reviewed in [13•]). Ziconotide is up to several orders of magnitude more potent than morphine on intrathecal administration and does not appear to exhibit tolerance or addiction characteristics. Its probable site of action is the high concentration

of N-type Ca^{2+} channels found in the superficial laminae of the spinal cord dorsal horn, although there is also some evidence for efficacy on injured peripheral nerves upon subcutaneous administration.

In a recent comparative study of Ziconotide and morphine, Wang *et al.* [14•] showed that intrathecal bolus injection of Ziconotide is significantly more potent and long-lasting for the inhibition of heat hyperalgesia and mechanical allodynia in a rat model of post-operative pain. In addition, Ziconotide appears to be more specific in its action compared to morphine. Pretreatment with intrathecal Ziconotide was also shown to prevent the establishment of allodynia and hyperalgesia. There are conflicting reports regarding the efficacy of Ziconotide in acute models of pain [7••].

Ziconotide has been evaluated in a number of clinical trials via intrathecal administration for the treatment of a variety of conditions including post herpetic neuralgia, phantom limb syndrome, HIV-related neuropathic pain and intractable cancer pain. In phase II and III clinical trials with patients unresponsive to intrathecal opiates, Ziconotide was shown to significantly reduce pain scores and in a number of specific cases result in relief after many years of continuous pain. Ziconotide is also being examined for the management of severe post-operative pain as well as for brain damage following stroke and severe head trauma [13•]. In two case studies, Ziconotide has been further examined for application in the management of intractable spasticity following spinal cord injury in patients unresponsive to baclofen and morphine [15]. In one example, Ziconotide decreased the spasticity from the severe range to the mild-to-none range with few side effects. In another patient, Ziconotide also reduced spasticity to the mild range, although at the required dosage significant side effects such as memory loss, confusion and sedation prevented continuation of the therapy.

Although generally well tolerated, it has been known for some time that intravenous administration of Ziconotide can produce a range of side effects, including orthostatic hypotension, that are probably caused by inhibition of peripheral sympathetic transmission. The issue of significant adverse effects associated with intrathecal administration of Ziconotide has also recently been reported. Penn and Paice [16•] describe three case studies in which patients experienced a variety of side effects that included nystagmus, sedation, confusion, auditory and visual hallucinations, severe agitation and unruly behavior. In two of the cases, the patients also suffered severe periods of disorientation and unresponsiveness, one case lasting three weeks after discontinuation of treatment with Ziconotide. The drug has recently received priority review status from the US Food and Drug Administration for the treatment of severe chronic pain via intrathecal administration, however, it appears that, at least in certain patients, the administration of Ziconotide may have to be carefully titrated and closely monitored.

Dipeptidyl-amine and piperidine blockers of N-type Ca²⁺ channels

Over the past two years, several interesting N-type Ca²⁺ channel blocking molecules have been identified through the high-throughput screening of compound libraries. Hu *et al.* [17••] identified a novel class of compounds, N,N-dialkyl-dipeptidyl-amines, as potent blockers of N-type Ca²⁺ channels expressed in IMR32 cells (Figure 1, PD 173212). These compounds are formed through coupling of N,N-disubstituted leucine acid with a tyrosine amine, and are structurally distinct from previously identified classes of Ca²⁺ channel blockers. Certain derivatives have been shown to display blocking affinities as high as 40 nM, making these types of compounds some of the highest affinity N-type Ca²⁺ channel blockers identified to date [18]. Whole animal work suggests that these compounds are efficacious in the treatment of audiogenic seizures in DBA/2 mice [19].

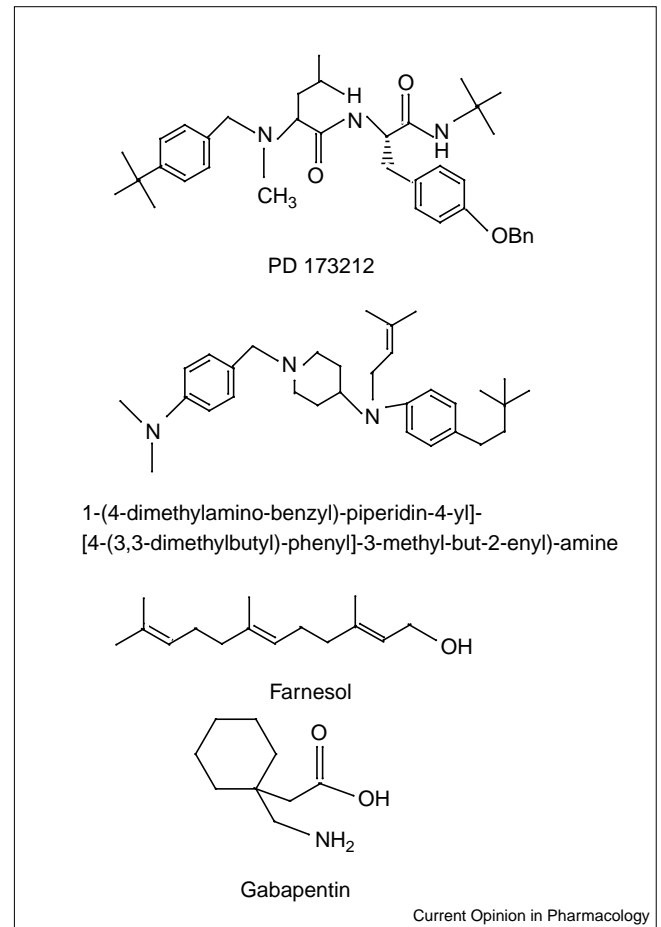
The identification of N,N, dialkyl-peptidyl-amine blockers has also stimulated the discovery of a number of non-peptidyl derivatives. Hu and coworkers [20] have also reported that certain 4-benzyloxyaniline analogs block N-type Ca²⁺ channels at sub to low micromolar concentrations and are effective as anticonvulsants and analgesics in animals. Additional derivatives of this series yielded 1-[4-dimethylamino-benzyl]-piperidin-4-yl]-[4-(3,3-dimethylbutyl)-phenyl]-3-methyl-but-2-enyl)-amine, a compound that inhibits N-type Ca²⁺ channels with 700 nM affinity and is an effective oral analgesic [21]. Interestingly, this compound contains a piperidine structure, suggesting a possible action at the piperidine receptor site of the channel.

Despite the promising advances that have occurred in the discovery of these N-type Ca²⁺ channel blockers, specificity profiles are lacking and at this point it is still too early to rule out an action of these compounds on other types of Ca²⁺ channel, or more generally, on other types of voltage-dependent ion channels.

Block of N-type Ca²⁺ channels by long carbon chain molecules

It is tempting to speculate about the existence of natural high-affinity endogenous ligands that target voltage-dependent ion channels and that modulate protein function *in vivo*. Despite considerable efforts, the identification of such endogenous compounds has been largely unsuccessful. Nonetheless, Fraser *et al.* [22] suggested that arachidonic acid might act as an endogenous ligand for the local anaesthetic receptor on voltage-dependent sodium channels, indicating that fatty acids could directly modulate ion channel function. Consistent with this notion, Xiao *et al.* [23] demonstrated the block of sodium channels by polyunsaturated fatty acids and it has been reported that fatty acids also directly modulate cardiac L-type Ca²⁺ channels (reviewed in [24]). Subsequently, Roulet *et al.* [25] showed that micromolar concentrations of farnesol (Figure 1), an intermediate of

Figure 1



Structures of several new classes of neuronal calcium channel blockers.

the mammalian mevalonate pathway, block smooth muscle L-type Ca²⁺ channels.

A more pronounced effect of farnesol has recently been described for block of N-type Ca²⁺ channels. Roulet *et al.* [26•] reported that farnesol exerted dual effects on N-type Ca²⁺ channel function. In the micromolar range, farnesol mediated open and resting channel block of N-type Ca²⁺ channels as well as other types of high-threshold Ca²⁺ channels. In contrast, at nanomolar concentrations farnesol mediated an N-type channel-selective inactivated channel block. This type of block, occurring at physiological farnesol concentrations, suggests that farnesol could be a high affinity endogenous ligand for N-type Ca²⁺ channels. It may be possible to take clinical advantage of this pathway either through modulating farnesol production via the mevalonate pathway or by the development of farnesol derivatives (for example, see [27]). Whether farnesol exhibits any antinociceptive activity in animal models for pain, however, remains to be determined. Nonetheless, long carbon chain molecules, such as farnesol and its derivatives, may offer a novel approach towards the development of high affinity N-type Ca²⁺ channel blockers.

Gabapentin

Gabapentin, 1-(aminomethyl) cyclohexaneacetic acid (Neurontin®), is a novel anticonvulsant drug found to be active in a variety of animal seizure models (Figure 1; [28]). It has proven effective in the clinic against partial seizures, anxiety and has more recently been accepted as an effective analgesic for the treatment of a wide range of neuropathic conditions. The efficacy of gabapentin in preventing hyperalgesia has been demonstrated in a number of different animal models of neuropathic pain [29•]. In humans, numerous case studies and three large clinical studies now provide supporting evidence for gabapentin as a useful alternative in the treatment of a number of different neuropathic pain syndromes, including diabetic neuropathy, postherpetic neuralgia, trigeminal neuralgia and migraine [29•,30]. Gabapentin as a treatment is particularly attractive in that it offers reasonable efficacy, yet is associated with a well-documented and favorable side effect profile.

Despite its effectiveness, the mechanism of action of gabapentin remains unclear. It is an amino acid originally designed as a structural analog of the inhibitory neurotransmitter γ -aminobutyric acid (GABA). Nevertheless, gabapentin does not bind to either GABA_A or GABA_B receptors, is not converted metabolically into GABA and is neither a substrate nor an inhibitor of GABA transport [28,31]. The gabapentin binding site was purified from pig brain and gabapentin was identified as the first ligand to interact with the $\alpha_2\delta$ subunit of high-threshold Ca²⁺ channels [32]. Both the $\alpha_2\delta$ subunit protein and [³H]+gabapentin binding sites are up-regulated in the dorsal horn following sciatic nerve chronic constriction injury [33]. In addition, the differing $\alpha_2\delta$ binding affinities of stereoselective analogs of gabapentin correlate well with their structure–activity relationships in animal models of pain [34••]. Taken together, this data indicates that $\alpha_2\delta$ may play an important role in mediating the action of gabapentin in reducing chronic pain.

The association of $\alpha_2\delta$ with the pore-forming α_1 subunit of the Ca²⁺ channel has been shown to modulate channel function [35], providing a means for gabapentin to mediate neuronal excitability indirectly. Voltage-gated Ca²⁺ channels play vital roles in mediating neurotransmitter release, gene expression and neuronal outgrowth throughout the nervous system, and yet gabapentin exerts relatively specific actions with only minor side effects. It is possible that the ubiquitous distribution of Ca²⁺ channels may require distinct subunit combinations coupled with unique modulatory conditions in order to permit inhibition by gabapentin under specific circumstances. The recent identification of three different $\alpha_2\delta$ subtypes provides the potential for subunit-specific interaction with gabapentin, and may contribute to its specificity of action [36].

Experimental evidence supports the notion that different high-threshold Ca²⁺ channels are differentially susceptible to the action of gabapentin. Electrophysiological

studies on cortical neurons by Stefani *et al.* [37] were the first to demonstrate inhibition of Ca²⁺ channel current by gabapentin, although the reduction was shown to vary between different cell types. Early studies indicated that gabapentin had no effect on freshly dissociated dorsal root ganglia (DRG; [38]). More recent work has shown, however, that gabapentin reduces Ca²⁺ channel influx in cultured DRG neurons, which can be detected both electrophysiologically and by using Ca²⁺ imaging techniques [31,39,40]. Recordings from dorsal horn neurons in spinal slices from adult hyperalgesic rats or neonatal control slices have demonstrated a presynaptic (DRG) site of action for gabapentin [41••,42]. Gabapentin also reduces P/Q-type Ca²⁺ influx into synaptosomes [43] and inhibits the release of excitatory amino acids in neocortical slices [44••]. Nevertheless, effects on spinal glutamatergic synaptic transmission are not consistently observed in the literature. Studies undertaken on cultured DRG, control slices and *in vivo* work suggest that gabapentin may also act postsynaptically to modulate either NMDA- or AMPA- mediated synaptic transmission variably [38,42,45]. In epilepsy, gabapentin exhibited similar actions to the L-type blocker, nimodipine, by preventing increased duration of seizure discharge in the rat hippocampus [46], yet did not alter Ca²⁺ channel currents recorded from hippocampal neurons taken from patients with temporal lobe epilepsy [47].

In addition to possible action at Ca²⁺ channel $\alpha_2\delta$ subunits, there is evidence for several alternative means by which gabapentin may exert its influence. Gabapentin can substitute as a substrate for the system L amino acid transporter [48] with its intracellular accumulation postulated to indirectly alter Ca²⁺ influx and/or other neuronal functions. Studies suggest that gabapentin can increase *in vitro* activity of the GABA synthesizing enzyme, glutamic acid decarboxylase [28] and enhance levels of GABA in the brains of epileptic patients [49]. Additional studies show an increase in the nonvesicular release of GABA [50]. Combined, the evidence provides support for a role for gabapentin in reducing overall neuronal excitability. While gabapentin exhibits clinical efficacy for a wide range of neuronal indications [29•], such diversity of action may well suggest that gabapentin acts via an assortment of different Ca²⁺ channel subunit combinations and/or additional mechanisms in distinct conditions.

Conclusions

Voltage-dependent Ca²⁺ channels are known to be critical in mediating both the development and maintenance of neuropathic pain and have provided attractive candidates for the development of analgesic drugs [7••]. Recent work on the α_{1E} Ca²⁺ channel [10•] and the search for specific low voltage-activated T-type channel ligands may also provide exciting new leads in the search for novel pain targets. Nevertheless, the ubiquitous distribution and multifaceted physiological roles played by Ca²⁺ channels also presents a number of challenges. Although narrow therapeutic windows and the relative safety of side effect

profiles need to be considered in the development of Ca²⁺ channel ligands, these limitations are not insurmountable and the continued development of specific Ca²⁺ channel ligands should provide a potentially rich source of novel therapies in the development of future analgesics.

Acknowledgements

TP Snutch is supported by a research grant from the Canadian Institutes for Health Research (CIHR) and is a CIHR Senior Scientist. GW Zamponi is supported by a research grant from the CIHR and Scholarship Awards from the EJLB Foundation, CIHR and the Alberta Heritage Foundation for Medical Research.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Miller RJ: **Multiple calcium channels and neuronal function.** *Science* 1987, **235**:46-52.
 2. Augustine GJ, Charlton MP, Smith SJ: **Calcium action in synaptic transmitter release.** *Annu Rev Neurosci* 1987, **10**:633-693.
 3. Janis RJ, Triggler DJ: *Calcium Channels: Their Properties, Functions, Regulation and Clinical Relevance.* London: CRC Press; 1991.
 4. Catterall WA: **Structure and regulation of voltage-gated Ca²⁺ channels.** *Annu Rev Cell Dev Biol* 2000, **16**:521-555.
A thorough review of the primary structure, nomenclature and molecular mechanisms of calcium channel modulation.
 5. Stea A, Soong TW, Snutch TP: **Voltage-gated calcium channels.** In *Handbook on Ion Channels.* London: CRC Press; 1994:113-151.
 6. Wilson SM, Toth PT, Oh SB, Gillard SE, Volsen S, Ren D, Philipson LH, Lee EC, Fletcher CF, Tessarollo L *et al.*: **The status of voltage-dependent calcium channels in α_{1E} knock-out mice.** *J Neurosci* 2000, **20**:8566-8571.
 7. Vanegas H, Schaible HG: **Effects of antagonists to high-threshold calcium channels upon spinal mechanisms of pain, hyperalgesia and allodynia.** *Pain* 2000, **85**:9-18.
An excellent review of the literature pertaining to the roles of calcium channels in central mechanisms of pain transmission.
 8. Malmberg AB, Yaksh TL: **Voltage-sensitive calcium channels in spinal nociceptive processing: blockade of N- and P-type channels inhibits formalin-induced nociception.** *J Neurosci* 1994, **14**:4882-4890.
 9. Sluka KA: **Blockade of N- and P/Q-type calcium channels reduces the secondary heat hyperalgesia induced by acute inflammation.** *J Pharmacol Exp Ther* 1998, **287**:232-237.
 10. Saegusa H, Kurihara T, Zong S, Minowa O, Kazuno A, Han W, Matsuda Y, Yamanaka H, Osanai M, Noda T, Tanabe T: **Altered pain responses in mice lacking α_{1E} subunit of the voltage-dependent Ca²⁺ channel.** *Proc Natl Acad Sci USA* 2000, **97**:6132-6137.
The paper shows that while α_{1E} mutant knockout mice are viable they also exhibit altered responses to somatic inflammatory pain responses.
 11. Zamponi GW: **Antagonist sites of voltage-dependent calcium channels.** *Drug Dev Res* 1997, **42**:131-143.
 12. Striessnig J, Hering S, Berger W, Catterall W, Glossmann H: **Ca antagonist binding domains of L-type Ca channels.** In *Ion Channels in the Cardiovascular System: Function and Dysfunction.* Edited by Spooner P. 1994:441-458.
 13. Heading C: **Ziconotide.** *Curr Opin CPNS Investigational Drugs* 1999, **1**:153-166.
A good summary of much of the preclinical and some of the clinical data concerning Ziconotide as a potential treatment for intractable pain and brain damage following head trauma.
 14. Wang YX, Pettus M, Gao M, Phillips C, Bowersox SS: **Effects of intrathecal administration of Ziconotide, a selective neuronal N-type calcium channel blocker, on mechanical allodynia and heat hyperalgesia in a rat model of postoperative pain.** *Pain* 2000, **84**:151-158.
A solid study demonstrating that intrathecal Ziconotide is antinociceptive in a model for postoperative pain and that the effects of Ziconotide are more potent and longer lasting than morphine.
 15. Ridgeway B, Wallace M, Gerayli A: **Ziconotide for the treatment of severe spasticity after spinal cord injury.** *Pain* 2000, **85**:287-289
 16. Penn RD, Paice JA: **Adverse effects associated with the intrathecal administration of Ziconotide.** *Pain* 2000, **85**:291-296.
Several case reports illustrating that some patients experience severe adverse reactions to intrathecal Ziconotide.
 17. Hu L, Ryder TR, Rafferty MF, Cody WL, Lotarski S, Mijanich GP, Millerman E, Rock DM, Song Y, Stoehr SJ *et al.*: **N,N-dialkyl-peptidylamines as novel N-type Ca channel blockers.** *Bioorg Med Chem Lett* 1999, **9**:907-912.
This paper identifies an entirely novel class of small organic molecule N-type calcium channel blockers.
 18. Ryder TR, Hu L, Rafferty MF, Millerman E, Szoke BG, Tarczy-Hornoch K: **Multiple parallel synthesis of N,N-dialkylaminepeptidylamines as N-type Ca channel blockers.** *Bioorg Med Chem Lett* 1999, **9**:1813-1818.
 19. Hu L, Ryder TR, Dooley DJ, Geer JJ, Lotarski SM, Milhanich GP, Millerman E, Rafferty MF, Rock DM, Stoehr SJ *et al.*: **Structure-activity relationship of N-methyl-N-alkyl-peptidylamines as novel N-type calcium channel blockers.** *Bioorg Med Chem Lett* 1999, **9**:1121.
 20. Hu L, Ryder TR, Rafferty MF, Feng MR, Lotarski SM, Rock DM, Sinz M, Stoehr SJ, Taylor CP, Weber ML: **Synthesis of a series of 4-benzoyloxyaniline analogs as neuronal N-type Ca channel blockers with improved anticonvulsant and analgesic properties.** *J Med Chem* 1999 **42**:4239-4249.
 21. Hu L, Ryder TR, Rafferty MF, Taylor CP, Feng MR, Kuo B, Lotarski S, Mijanich GP, Millerman E, Siebers KM, Szoke BG: **The discovery of 1-[4-Dimethylamino-benzyl]-piperidin-4-yl]-[4-(3,3-dimethyl-butyl)-phenyl]3-methyl-but-2-enyl-amine, an N-type Ca²⁺ channel blocker with oral activity for analgesia.** *Bioorg Med Chem Lett* 2000, **8**:1203-1212.
 22. Fraser DD, Hoehn K, Weiss S, MacVicar BA: **Aarachidonic acid inhibits sodium currents and synaptic transmission in cultures striatal neurons.** *Neuron* 1993, **11**:633-644.
 23. Xiao YF, Kang JX, Morgan JP, Leaf A: **Blocking effects of polyunsaturated fatty acids on Na⁺ channels of neonatal rat ventricular myocytes.** *Proc Natl Acad Sci USA* 1995, **92**:11000-11004.
 24. Leaf A: **Omega-3 fatty acids and prevention of ventricular fibrillation.** *Prostaglandins Leukot Essent Fatty Acids* 1995, **52**:197-198.
 25. Roulet JB, Luft UC, Xue H, Chapman J, Bychkov R, Roulet CM, Luft FC, Haller H, McCarron DA: **Farnesol inhibits L-type Ca²⁺ channels in vascular smooth muscle cells.** *J Biol Chem* 1997, **272**:32240-32246.
 26. Roulet J, Spaetgens RL, Burlingame T, Feng ZP, Zamponi GW: **Modulation of neuronal voltage-gated Ca channels by farnesol.** *J Biol Chem* 1999, **274**:25439-25446.
This paper describes a high affinity selective action of farnesol on N-type calcium channels and suggests the possibility that farnesol is an endogenous ligand for N-type channels.
 27. Beedle AM, Zamponi GW: **Block of voltage-dependent Ca channels by aliphatic monoamines.** *Biophys J* 2000, **79**:247-259.
 28. Taylor CP, Gee NS, Su TZ, Kocsis JD, Welty DF, Brown JP, Dooley DJ, Boden P, Singh L: **A summary of mechanistic hypotheses of gabapentin pharmacology.** *Epilepsy Res* 1998, **29**:233-249.
 29. Nicholson B: **Gabapentin use in neuropathic pain syndromes.** *Acta Neurol Scand* 2000, **101**:359-371.
Good overall review describing mechanisms involved in the development of neuropathic pain, use of Gabapentin in animal models of pain and summarizing recent clinical trials
 30. Di Trapani G, Mei D, Marra C, Mazza S, Capuano A: **Gabapentin in the prophylaxis of migraine: a double-blind randomized placebo-controlled study.** *Clin Ter* 2000, **151**:145-148.
 31. McClelland D, Herd MB, Martin DJ, Sutton KG, Lee, K, Scott, RH: **Comparisons between responses evoked by GABA receptor mechanisms and gabapentin in cultured neonatal rat dorsal root ganglion neurones.** *J Physiol* 2000, **528P**:C53.

32. Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN: **The novel anticonvulsant drug, gabapentin (neurontin), binds to the alpha2delta subunit of a calcium channel.** *J Biol Chem* 1996, **271**:768-776.
33. Philp L, Holloman E, Meecham K, Blyth K, Pinnock R, Hughes J, Williams R: **[³H]-Gabapentin binding and $\alpha_2\delta$ immunoreactivity in the spinal cord of the rat following chronic constriction injury of the sciatic nerve.** *Br Neurosci Assoc Abstr* 1999, **15**:46.08.
34. Field MJ, Hughes J, Singh L: **Further evidence for the role of the $\alpha_2\delta$ subunit of voltage dependent calcium channels in models of neuropathic pain.** *Br J Pharmacol* 2000, **131**:282-286.
- A structure-activity based paper in which the $\alpha_2\delta$ binding activity of stereospecific gabapentin analogues is shown to correlate with their analgesic action in animal models of neuropathic pain.
35. Walker D, De Waard M: **Subunit interaction sites in voltage-dependent Ca²⁺ channels: role in channel function.** *Trends Neurosci* 1998, **21**:148-154.
36. Klugbauer N, Lacinova L, Marais E, Hobom M, Hofmann F: **Molecular diversity of the calcium channel alpha2delta subunit.** *J Neurosci* 1999, **19**:684-691.
37. Stefani A, Spadoni F, Bernardi G: **Gabapentin inhibits calcium currents in isolated rat brain neurons.** *Neuropharmacology* 1998, **37**:83-91.
38. Rock DM, Kelly KM, Macdonald RL: **Gabapentin actions on ligand- and voltage-gated responses in cultured rodent neurons.** *Epilepsy Res* 1993, **16**:89-98.
39. Martin DJ, Ibbotson T, Scott RH: **The inhibitory effects of gabapentin on Ca²⁺ influx into cultured rat dorsal root ganglion neurones and F11 neuroblastoma cells.** *J Physiol* 2000, **528**:PC45.
40. Sutton KG, Scott RH, Lee K, Pinnock RD: **Gabapentin inhibits high threshold calcium channel currents in cultured rat dorsal root ganglia neurones.** *Soc Neurosci Abstr* 2000, **26**:234.4.
41. Patel MK, Gonzalez MI, Bramwell S, Pinnock RD, Lee K: **Gabapentin inhibits excitatory synaptic transmission in the hyperalgesic spinal cord.** *Br J Pharmacol* 2000, **130**:1731-1734.
- This paper presents evidence for a presynaptic site of action for gabapentin, which is dependent upon the hyperalgesic state of the animal.
42. Shimoyama M, Shimoyama N, Hori Y: **Gabapentin affects glutamatergic excitatory neurotransmission in the rat dorsal horn.** *Pain* 2000, **85**:405-414.
43. Meder WP, Dooley DJ: **Modulation of K⁺-induced synaptosomal calcium influx by gabapentin.** *Brain Res* 2000, **875**:157-159.
44. Fink K, Meder W, Dooley DJ, Gothert M: **Inhibition of neuronal Ca²⁺ influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices.** *Brit J Pharmacol* 2000, **130**:900-906.
- This paper provides evidence for gabapentin-dependent functional change in neuronal signal transmission processing. Data is presented showing gabapentin inhibition of presynaptic voltage-gated Ca influx and a reduction in neurotransmitter release.
45. Chizh BA, Scheede M, Schultz H: **Antinociception and (R,S)- α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid antagonism by gabapentin in the rat spinal cord in vivo.** *Naunyn Schmiedebergs Arch Pharmacol* 2000, **362**:197-200.
46. Stringer JL, Taylor CP: **The effects of gabapentin in the rat hippocampus are mimicked by two structural analogs, but not by nimodipine.** *Epilepsy Res* 2000, **41**:155-162.
47. Schumacher TB, Beck H, Steinhauser C, Schramm J, Elger CE: **Effects of phenytoin, carbamazepine and gabapentin on calcium currents in hippocampal granule cells from patients with temporal lobe epilepsy.** *Epilepsia* 1998, **39**:355-363.
48. Su T, Lunney E, Campbell G, Oxender DL: **Transport of gabapentin, a γ -amino acid drug, by system L α -amino acid transporters: a comparative study in astrocytes, synaptosomes and CHO cells.** *J Neurochem* 1995 **64**:2125-2131.
49. Petroff OA, Rothman, DL, Behar KL, Lamoureux D, Mattson RH: **The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy.** *Ann Neurol* 1996, **39**:95-99.
50. Honmou O, Oyelese AA, Kocsis D: **The anticonvulsant gabapentin enhances promoted release of GABA in hippocampus: a field potential analysis.** *Brain Res* 1995, **692**:273-277.