Novel coupling is painless

Ann R. Rittenhouse

Department of Microbiology and Physiological Systems, Program in Neuroscience, University of Massachusetts Medical School, Worcester, MA 01605

The first report that several neurotransmitters, including γ-aminobutyric acid (GABA), decreased neuronal action potential duration in dorsal root ganglia (DRG) neurons appeared more than 35 years ago (Dunlap and Fischbach, 1978). Dunlap and Fischbach (1978) realized that the effects of GABA could not occur via the ionotropic GABA receptor—at that time the only known GABA receptor—and that the target ion channel was most likely a voltage-gated calcium (Ca_V) channel, rather than a Na_V or K_V channel. They correctly concluded that another type of GABA receptor must exist, which we now know is the G protein–coupled GABA type B ($GABA_B$) receptor. The modulated channel was later identified as the chick homologue of the N-type Ca^{2+} channel $Ca_V2.2$ (α_1B) (Cox and Dunlap, 1992), one of three members of the Ca_V2 family. $GABA_B$ receptors in human and rodent sensory neurons and in various expression systems were shown subsequently to inhibit native N-current and recombinant $Ca_V2.2$ current, respectively (Raingo et al., 2007; Callaghan et al., 2008; Adams and Berecki, 2013). Inhibition primarily occurs by a voltage-dependent mechanism common to various neurotransmitters whereby Gβγ binds to $Ca_V2.2$ slowing channel opening, whereas positive voltage steps relieve this inhibition (Marchetti et al., 1986). The closely related P/Q-type (α_1A) channel, $Ca_V2.1$, exhibits similar modulation by GABA (Mintz and Bean, 1993). The third member of the Ca_V2 family, $Ca_V2.3$ (α_1E), is less susceptible to direct Gβγ modulation than the other two family members (Shekter et al., 1997). The revelation that mice with a deletion in either $Ca_V2.2$ or in $Ca_V2.3$ exhibited reduced neuropathic pain–like behavior, indicating that these channels participate in pain sensation signaling (Saegusa et al., 2000, 2001), sparked great interest in the regulation of Ca_V2 inhibition by $GABA_B$ receptors in DRG neurons. Astonishingly, however, the precise mechanism of $GABA_B$ receptor modulation of $Ca_V2.3$ channels has remained ill defined.

Given $Ca_V2.3$'s functional importance in pain pathways, the uncertainty surrounding whether $GABA_B$ receptors modulate $Ca_V2.3$ seems remarkable. Several observations may provide insights as to why this question still awaits an answer. In neurons, native $Ca_V2.3$ is referred to as R-type current, which is poorly defined as the current remaining after blocking the activity of T channels (Ca_V3) with nickel, L channels (Ca_V1) with dihydropyridines, and N, P, and Q channels with ω-conotoxins. Because selective and complete pharmacological blockage of any target rarely occurs, doubt has surrounded the notion that native neuronal "R-type" current arises from a pure population of $Ca_V2.3$ channels (Wilson et al., 2000; Yang and Stephens, 2009); $Ca_V2.3$ activation occurs over a range of relatively negative voltages (approximately −40 to −50 mV), which has been used as an additional defining characteristic; however, this activation profile overlaps with that of other Ca_V currents (Williams et al., 1994). A further confounding issue is that R current often contributes a small percentage of the total whole cell Ca_V current, making it difficult to measure its modulation accurately. In any event, the $GABA_B$ receptor's ability to modulate R-type current has not been adequately examined in neurons, nor has its ability to modulate $Ca_V2.3$ been tested in a recombinant system.

In searching for novel treatments of neuropathic pain, in this issue Berecki et al. have now answered the long-languishing question of whether $GABA_B$ receptors modulate $Ca_V2.3$ activity. In so doing, they have advanced three distinct research fields: (1) development of synthetic forms of naturally occurring toxins for treatment of neuropathic pain, (2) Ca_V current modulation, and (3) $GABA_B$ receptor function. They demonstrate that cyclized Vc1.1 (cVc1.1), an orally active compound, derived by the cyclization of the synthetic α-conotoxin peptide Vc1.1 (Clark et al., 2010), selectively activates $GABA_B$ receptors to inhibit recombinant $Ca_V2.3$ activity. $Ca_V2.3$ inhibition occurs by a voltage-independent, pertussis toxin (PTX)-sensitive mechanism that requires c-src kinase, a nonreceptor tyrosine kinase (see Okada, 2012), and channel phosphorylation in its proximal C terminus. The signaling pathway is similar to one that mediates voltage-independent inhibition of the $Ca_V2.2e$[37a] splice variant observed in a subpopulation of nociceptive DRG neurons after $GABA_B$ receptor stimulation (Bell et al., 2004; Raingo et al., 2007). In characterizing

Correspondence to Ann R. Rittenhouse: Ann.Rittenhouse@umassmed.edu

The Rockefeller University Press
J. Gen. Physiol. Vol. 143 No. 4 443–447
www.jgp.org/cgi/doi/10.1085/jgp.201411190

this mechanism, the authors identify cVc1.1's inhibition of $Ca_V2.3$ activity via $GABA_B$ receptor activation as a potential therapeutic strategy for treating certain forms of neuropathic pain.

Pain sensation is complicated. Initially, pain is perceived by nociceptive sensory neurons whose cell bodies reside in DRG (see Costigan et al., 2009; Woolf, 2010). These neurons project to lamina I and II in the dorsal horn to trans-synaptically stimulate ascending spinal neurons. The signal then travels to the thalamus where sensory information is distributed to higher cortical areas. Nociceptive pain is characterized as a high threshold pain activated by immediate, intense stimuli such as noxious heat or a sharp prick. If an injury is sustained, inflammatory pain will occur, which is characterized by hypersensitivity or tenderness from an immune response in the area of an injury that may last for days but usually is reversible. Inflammatory pain, like nociceptive pain, is protective because it serves to discourage use and promote healing. In contrast, neuropathic pain, a disease state of the nervous system (Woolf, 2010), is maladaptive in that pain sensation remains despite the disappearance of the original insult. Often neurons sustain an injury such that the pain threshold decreases so that innocuous stimuli are perceived as painful and are characterized by increased intensity and duration. These changes may persist or become irreversible (Costigan et al., 2009).

Neuropathic pain is remarkably difficult to treat perhaps because of its varied quality, intensity, and source (Costigan et al., 2009). Not surprisingly, several ion channels have been implicated in mediating pain including Na_V, Ca_V, HCN, TRPV1, and various K^+ channels, NMDA receptors, and nicotinic receptors (Raouf et al., 2010). Therapeutically targeting any one of these channels to produce analgesia has undesirable side effects because of their widespread distribution both peripherally and centrally. Moreover, developing highly selective channel ligands is difficult, and their off-target binding contributes to further unwanted actions. Lastly, once a promising drug is identified, delivering it to its site of action in patients is problematic because of high biodegradability within and low cellular uptake through the gut, difficulty crossing the blood–brain barrier, and short half-life (Adams and Berecki, 2013). An example of the promises and difficulties of developing effective treatment for pain is the Conus snail peptide toxin MVIIA, a highly selective antagonist of $Ca_V2.2$ activity with FDA approval for use as a non-opiate analgesic and marketed under several names (SNX-111, Prialt, Ziconotide). By blocking $Ca_V2.2$ activity, MVIIA reduces transmitter release from nociceptive nerve endings (McGivern, 2006). However MVIIA must be injected intrathecally for treating neuropathic pain and has a narrow therapeutic range.

Despite limitations in using toxins to treat neuropathic pain, David Adams' laboratory in collaboration with many colleagues has synthesized various native and modified Conus snail toxins and tested them for analgesic actions (Clark et al., 2010; Adams and Berecki, 2013). Vc1.1 is a 16–amino acid peptide, originally isolated and purified from *Conus victoriae* venom, but with the native posttranslational modifications of the toxin removed. It is classified as a cholinergic α-conotoxin based on sequence homology and its pattern of disulfide bridges (Sandall et al., 2003). Vc1.1 selectively antagonizes neuronal α9α10 nicotinic ACh receptor functioning. In vivo, Vc1.1 acts as an effective and long-lasting analgesic in the chronic constriction injury and partial nerve ligation rat models of human neuropathic pain (Sandall et al., 2003; Satkunanathan et al., 2005). It also accelerates recovery from nerve damage in these two models. When Adams and colleagues tested Vc1.1, they found what seemed to be a second, more potent action of Vc1.1. Vc1.1 appeared to selectively inhibit high threshold (HVA) but not low threshold Ca_V currents in DRG neurons. At that time, only one other α-conotoxin, Rg1A, was known to inhibit HVA Ca_V current in DRG neurons; native, reduced, or alkylated forms of Vc1.1 as well as five other α-conotoxins exhibited no inhibitory action on Ca_V currents. When $Ca_V2.2$ activity was blocked with the selective ω-conotoxin CVID, Vc1.1 lost its inhibitory action on DRG neuron HVA Ca_V currents, identifying this modulated HVA Ca_V current as N current (Callaghan et al., 2008).

Further characterization of Vc1.1's actions on DRG neurons revealed that N-current inhibition by Vc1.1 occurred independently of rises in intracellular Ca^{2+}, but instead depended on a G protein–dependent signaling cascade because current inhibition was lost in the presence of GDP-β-S, PTX, or pp60c-src tyrosine kinase inhibitory peptide (Callaghan et al., 2008). From this unusual profile, the group screened a variety of receptors expressed in DRG neurons that might mediate Vc1.1's inhibitory actions on $Ca_V2.2$ activity. Surprisingly, only $GABA_B$ receptor antagonists occluded N-current inhibition by Vc1.1 and Rg1A (Callaghan et al., 2008; Callaghan and Adams, 2010). Concomitantly, the $GABA_B$ receptor agonist baclofen and Vc1.1 were nonadditive in their inhibitory effects. Vc1.1 and Rg1A inhibited N current in mouse DRG neurons from α9 nicotinic receptor subunit knockout mice, confirming that Vc1.1's actions occurred independently of nicotinic receptors because α10 is unable to form functional channels without the α9 subunit. A $GABA_B$ receptor antagonist blocked toxin-mediated relief from allodynic pain in animal studies (Klimis et al., 2011), consistent with Vc1.1 somehow acting via $GABA_B$ receptors and independently of nicotinic receptor subunits.

In most DRG neurons, $GABA_B$ receptors inhibit N current by a Gβγ-mediated, voltage-dependent mechanism as noted above. However, in a subpopulation of DRG neurons, baclofen modulates N current by an

additional PTX-sensitive, Gβγ-independent and voltage-independent mechanism involving c-src kinase (Diversé-Pierluissi et al., 1997; Raingo et al., 2007). The Lipscombe laboratory and their colleagues previously identified a $Ca_V2.2$ splice variant selectively enriched in capsaicin-responsive DRG nociceptive neurons that exhibits both voltage-dependent and independent inhibition of N current by baclofen (Bell et al., 2004; Raingo et al., 2007). In exon [37a] of $Ca_V2.2$, a 14–amino acid insert in the proximal end of the carboxy-terminal tail contains two tyrosine residues (Y1743 and Y1747), whereas a phenylalanine has replaced the second tyrosine in the mutually exclusive exon [37b]. Voltage-independent inhibition of $Ca_V2.2e[37a]$ requires phosphorylation of the second but not the first tyrosine by c-src kinase. If mutated to phenylalanine (Y1747F) as is found in $Ca_V2.2e[37b]$, voltage-independent inhibition is lost and only voltage-dependent inhibition remains (Raingo et al., 2007). In contrast, complete relief of $Ca_V2.2e[37b]$ current inhibition by baclofen occurs with positive voltage, indicating that the 37b splice variant is insensitive to voltage-independent inhibition by c-src kinase. Collectively, the findings suggest that as with baclofen, Vc1.1 binds to $GABA_B$ receptors activating the same voltage-independent signaling pathway in DRG neurons to selectively inhibit $Ca_V2.2e[37a]$. Whether other Ca_V2 family members could be modulated by this pathway remained an open question.

Here, Berecki et al. (2014) tested human recombinant $Ca_V2.1$ and $Ca_V2.3$ channels expressed in HEK 293T cells along with the $GABA_B$ receptor to answer the question of whether other Ca_V2 family members are sensitive to baclofen and/or Vc1.1 modulation by $GABA_B$ receptors (Fig. 1 A). Although the traditional $GABA_B$ receptor agonist baclofen inhibited currents from both channels, Vc1.1 and its cyclized form, cVc1.1, inhibited $Ca_V2.3$ but not $Ca_V2.1$ currents. The $GABA_B$ receptor antagonist CGP55845 had no effect on currents itself but antagonized the actions of cVc1.1, indicating that it too must somehow activate the $GABA_B$ receptor. The characteristics of $Ca_V2.1$ modulation by baclofen fit classical Gβγ-mediated, voltage-dependent inhibition (Fig. 1 B), as prepulses relieved all the inhibition (Diversé-Pierluissi et al., 1997). In contrast, $Ca_V2.3$ current inhibition by either baclofen or cVc1.1 shows no voltage-dependent modulation; prepulses were unable to relieve any of the inhibition. As with $Ca_V2.2$, inhibition requires c-src kinase because coexpression with a double mutant, inactive src kinase or synthetic pp60c-src kinase inhibitory peptide blocked $Ca_V2.3$ current inhibition, whereas the opposite treatment, overexpression of wild-type c-src kinase, enhanced inhibition (Fig. 1 C).

Sequence analysis revealed that $Ca_V2.3$ has two tyrosines (Y1761 and Y1765) in its C-terminal tail homologous to the two tyrosines in $Ca_V2.2$ e[37a]'s proximal C terminus (Raingo et al., 2007). $Ca_V2.3$ mutagenesis revealed that the first (Y1761) but not the second (Y1765) tyrosine is required for voltage-independent current inhibition, presumably because its phosphorylation is required. In contrast, the critical tyrosine-containing amino acid motif found in the C termini of both $Ca_V2.2e[37a]$ and Cav2.3 is absent in $Ca_V2.1$, which may explain this channel's insensitivity to voltage-independent modulation. To test that possibility, a tyrosine residue was swapped in to either of the two homologous sites in the C terminus of $Ca_V2.1$, and current was retested for sensitivity to cVc1.1. A small amount of current inhibition was now

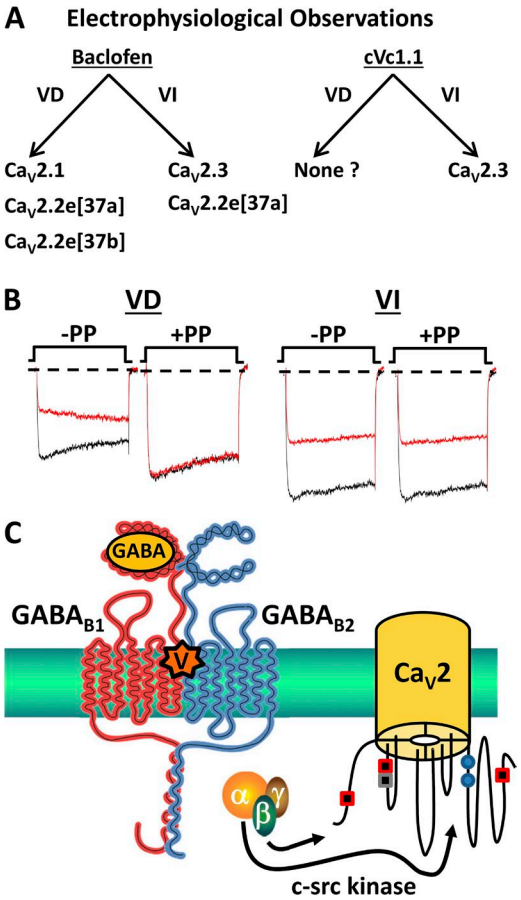

Figure 1. $GABA_B$ receptor activation elicits both voltage-dependent and voltage-independent inhibition of Ca_V2 current. (A) After $GABA_B$ receptor activation, Ca_V2 family members exhibit distinct modulation by voltage-dependent (VD) and/or voltage-independent (VI) current inhibition. (B) Example current traces illustrate the two forms of modulation. (Left traces) VD inhibition exhibits slowed kinetics (red traces) compared with control (black) currents that is relieved with positive prepulses (PP). (Right traces) VI inhibition (VI) remains following a PP. (C) Schematic of the proposed mechanism for Ca_V2 inhibition by the two pathways. $GABA_{B1}$ and $GABA_{B2}$ subunits form the $GABA_B$ receptor. GABA or baclofen binds to the B1 subunit, activating the receptor. cVc1.1 (V) binds at an undefined site within the interface of the two subunits. Activated Gβγ binds to multiple sites (red squares), whereas Gα stimulates c-src kinase to phosphorylate tyrosine residues (blue circles) in the C-terminal tail of certain Ca_V2 family members. Black squares, $Ca_V\beta$ high affinity–binding sites.

observed with each point mutation, indicating that tyrosine phosphorylation in the proximal C-terminal tail is critical for c-src kinase modulation of channels.

These and previous findings from Adams and colleagues (Callaghan et al., 2008; Callaghan and Adams, 2010; Clark et al., 2010; Cuny et al., 2012) associate three toxins, Rg1A, Vc1.1, and cVc1.1, with $GABA_B$ receptor activation; however, whether they bind directly to the receptor or to another target protein remains controversial (McIntosh et al., 2009). One B1 and one B2 subunit heterodimerize to form a functional $GABA_B$ receptor (White and Cousins, 1998). B1 binds GABA in its large extracellular N-terminal domain. B2 is required for high affinity agonist binding to B1, contains a binding site for allosteric modulators, and recruits the G protein (Benke, 2013). Studies using siRNA to lower either B1 or B2 levels found that baclofen, Vc1.1, and Rg1A require both B1 and B2 subunits for N-current inhibition in DRG neurons, consistent with binding to $GABA_B$ heterodimers (Cuny et al., 2012). When Berecki et al. (2014) transfected HEK 293T cells with only the B1 subunit of the $GABA_B$ receptor, $Ca_V2.3$ inhibition by both baclofen and cVc1.1 was lost, supporting—but not proving—the notion that the toxin is somehow interacting with the receptor to elicit its activation. Potentially confounding this notion are findings that very high concentrations of Vc1.1 cannot not displace ^{3}H-CGP-5462 binding from human recombinant B1b/B2 $GABA_B$ receptors (McIntosh et al., 2009) or from binding in DRG neurons (Adams and Berecki, 2013). These findings indicate that Vc1.1 does not compete with baclofen for a binding site but raise several questions surrounding Vc1.1's site of action. In particular, if Vc1.1 does bind to the $GABA_B$ receptor, where is the binding site located, and how does toxin binding activate the receptor? Recently, molecular dynamic simulation studies of toxin binding to $GABA_B$ receptors identified a putative toxin-binding site at the internal B1/B2 heterodimerization interface (Adams and Berecki, 2013). Whether toxins do indeed bind an ectodomain to activate $GABA_B$ receptors awaits further binding, structure–function, and crystal structure studies. Once the toxin-binding site is confirmed, functional questions can be addressed, including whether other splice variants of $Ca_V2.3$, which contain the critical tyrosine residue in the C terminus tail, are similarly modulated, and where in the pain pathway are $Ca_V2.3$ and $GABA_B$ receptors found within the same macromolecular signaling complex.

This insightful study by Berecki et al. (2014) demonstrates how the elaborate details of signaling by individual G protein–coupled receptors (GPCRs), in this case $GABA_B$ receptors, can give rise to a remarkable level of control over specific members of the Ca_V2 family. By characterizing the details of each branch of $GABA_B$ receptor signaling, potential therapeutic targets can be identified to strategically treat specific types of pain. Indeed, behavioral studies using siRNAs for $Ca_V2.2$e[37a] indicate that this channel selectively participates in mediating normal thermal nociception as well as thermal hyperalgesia that accompanies nerve injury (Altier et al., 2007; Andrade et al., 2010). Splice variants of Ca_V2 channels exhibit different voltage and gating properties that will affect transmitter release and membrane excitability (Bell et al., 2004). Furthermore, whether a channel can be modulated will depend on its splicing and on the colocalization of critical signaling molecules such as c-src kinase within a sensory neuron. Lastly, expression of a particular GPCR within any given sensory neuron will determine whether modulation could be initiated by a transmitter such as GABA or a ligand such as cVc1.1. In this study, rather than using a toxin to directly inhibit ion channel activity, a toxin was used to activate a GPCR to inhibit the activity of a subset of Ca_V2 channels. Moreover, cVc1.1 is ∼1,000 times more potent than baclofen in inhibiting $Ca_V2.3$ currents. Most exciting is previous findings that oral delivery of cVc1.1 produces significant pain relief in the chronic constriction injury rat model of neuropathic pain (Clark et al., 2010). Whether cVc1.1 will find its way into the pharmacological toolbox of treatments for clinical neuropathic pain awaits future studies. Who knew that such a simple question of whether GABA modulates $Ca_V2.3$ currents would yield a new target, ligand, and potential mechanism for treating neuropathic pain?

I thank Diane Lipscombe and Sonia Ortiz-Miranda for helpful discussion and comments on the manuscript. I also thank Dietmar Benke for providing a schematic of the $GABA_B$ receptor.

The author declares no competing financial interests.

Sharona E. Gordon served as editor.

REFERENCES

Adams, D.J., and G. Berecki. 2013. Mechanisms of conotoxin inhibition of N-type ($Ca_v2.2$) calcium channels. *Biochim. Biophys. Acta.* 1828:1619–1628. http://dx.doi.org/10.1016/j.bbamem.2013.01.019

Altier, C., C.S. Dale, A.E. Kisilevsky, K. Chapman, A.J. Castiglioni, E.A. Matthews, R.M. Evans, A.H. Dickenson, D. Lipscombe, N. Vergnolle, and G.W. Zamponi. 2007. Differential role of N-type calcium channel splice isoforms in pain. *J. Neurosci.* 27:6363–6373. http://dx.doi.org/10.1523/JNEUROSCI.0307-07.2007

Andrade, A., S. Denome, Y.Q. Jiang, S. Marangoudakis, and D. Lipscombe. 2010. Opioid inhibition of N-type Ca^{2+} channels and spinal analgesia couple to alternative splicing. *Nat. Neurosci.* 13:1249–1256. http://dx.doi.org/10.1038/nn.2643

Bell, T.J., C. Thaler, A.J. Castiglioni, T.D. Helton, and D. Lipscombe. 2004. Cell-specific alternative splicing increases calcium channel current density in the pain pathway. *Neuron.* 41:127–138. http://dx.doi.org/10.1016/S0896-6273(03)00801-8

Benke, D. 2013. $GABA_B$ receptor trafficking and interacting proteins: Targets for the development of highly specific therapeutic strategies to treat neurological disorders? *Biochem. Pharmacol.* 86:1525–1530. http://dx.doi.org/10.1016/j.bcp.2013.09.016

Berecki, G., J.R. McArthur, H. Cuny, R.J. Clark, and D.J. Adams. 2014. Differential $Ca_v2.1$ and $Ca_v2.3$ channel inhibition by

baclofen and α-conotoxin Vc1.1 via $GABA_B$ receptor activation. *J. Gen. Physiol.* 143:465–479.

Callaghan, B., and D.J. Adams. 2010. Analgesic α-conotoxins Vc1.1 and RgIA inhibit N-type calcium channels in sensory neurons of α9 nicotinic receptor knockout mice. *Channels (Austin).* 4:51–54. http://dx.doi.org/10.4161/chan.4.1.10281

Callaghan, B., A. Haythornthwaite, G. Berecki, R.J. Clark, D.J. Craik, and D.J. Adams. 2008. Analgesic α-conotoxins Vc1.1 and Rg1A inhibit N-type calcium channels in rat sensory neurons via $GABA_B$ receptor activation. *J. Neurosci.* 28:10943–10951. http://dx.doi.org/10.1523/JNEUROSCI.3594-08.2008

Clark, R.J., J. Jensen, S.T. Nevin, B.P. Callaghan, D.J. Adams, and D.J. Craik. 2010. The engineering of an orally active conotoxin for the treatment of neuropathic pain. *Angew. Chem. Int. Ed. Engl.* 49:6545–6548. http://dx.doi.org/10.1002/anie.201000620

Costigan, M., J. Scholz, and C.J. Woolf. 2009. Neuropathic pain: A maladaptive response of the nervous system to damage. *Annu. Rev. Neurosci.* 32:1–32. http://dx.doi.org/10.1146/annurev.neuro.051508.135531

Cox, D.H., and K. Dunlap. 1992. Pharmacological discrimination of N-type from L-type calcium current and its selective modulation by transmitters. *J. Neurosci.* 12:906–914.

Cuny, H., A. de Faoite, T.G. Huynh, T. Yasuda, G. Berecki, and D.J. Adams. 2012. γ-Aminobutyric acid type B ($GABA_B$) receptor expression is needed for inhibition of N-type ($Ca_v2.2$) calcium channels by analgesic α-conotoxins. *J. Biol. Chem.* 287:23948–23957. http://dx.doi.org/10.1074/jbc.M112.342998

Diversé-Pierluissi, M., A.E. Remmers, R.R. Neubig, and K. Dunlap. 1997. Novel form of crosstalk between G protein and tyrosine kinase pathways. *Proc. Natl. Acad. Sci. USA.* 94:5417–5421. http://dx.doi.org/10.1073/pnas.94.10.5417

Dunlap, K., and G.D. Fischbach. 1978. Neurotransmitters decrease the calcium component of sensory neurone action potentials. *Nature.* 276:837–839. http://dx.doi.org/10.1038/276837a0

Klimis, H., D.J. Adams, B. Callaghan, S. Nevin, P.F. Alewood, C.W. Vaughan, C.A. Mozar, and M.J. Christie. 2011. A novel mechanism of inhibition of high-voltage activated calcium channels by α-conotoxins contributes to relief of nerve injury-induced neuropathic pain. *Pain.* 152:259–266. http://dx.doi.org/10.1016/j.pain.2010.09.007

Marchetti, C., E. Carbone, and H.D. Lux. 1986. Effects of dopamine and noradrenaline on Ca channels of cultured sensory and sympathetic neurons of chick. *Pflugers Arch.* 406:104–111. http://dx.doi.org/10.1007/BF00586670

McGivern, J.G. 2006. Targeting N-type and T-type calcium channels for the treatment of pain. *Drug Discov. Today.* 11:245–253. http://dx.doi.org/10.1016/S1359-6446(05)03662-7

McIntosh, J.M., N. Absalom, M. Chebib, A.B. Elgoyhen, and M. Vincler. 2009. α9 nicotinic acetylcholine receptors and the treatment of pain. *Biochem. Pharmacol.* 78:693–702. http://dx.doi.org/10.1016/j.bcp.2009.05.020

Mintz, I.M., and B.P. Bean. 1993. $GABA_B$ receptor inhibition of P-type Ca^{2+} channels in central neurons. *Neuron.* 10:889–898. http://dx.doi.org/10.1016/0896-6273(93)90204-5

Okada, M. 2012. Regulation of the SRC family kinases by Csk. *Int. J. Biol. Sci.* 8:1385–1397. http://dx.doi.org/10.7150/ijbs.5141

Raingo, J., A.J. Castiglioni, and D. Lipscombe. 2007. Alternative splicing controls G protein-dependent inhibition of N-type calcium channels in nociceptors. *Nat. Neurosci.* 10:285–292. http://dx.doi.org/10.1038/nn1848

Raouf, R., K. Quick, and J.N. Wood. 2010. Pain as a channelopathy. *J. Clin. Invest.* 120:3745–3752. http://dx.doi.org/10.1172/JCI43158

Saegusa, H., T. Kurihara, S. Zong, O. Minowa, A. Kazuno, W. Han, Y. Matsuda, H. Yamanaka, M. Osanai, T. Noda, and T. Tanabe. 2000. Altered pain responses in mice lacking α_{1E} subunit of the voltage-dependent Ca^{2+} channel. *Proc. Natl. Acad. Sci. USA.* 97:6132–6137. http://dx.doi.org/10.1073/pnas.100124197

Saegusa, H., T. Kurihara, S. Zong, A. Kazuno, Y. Matsuda, T. Nonaka, W. Han, H. Toriyama, and T. Tanabe. 2001. Suppression of inflammatory and neuropathic pain symptoms in mice lacking the N-type Ca^{2+} channel. *EMBO J.* 20:2349–2356. http://dx.doi.org/10.1093/emboj/20.10.2349

Sandall, D.W., N. Satkunanathan, D.A. Keays, M.A. Polidano, X. Liping, V. Pham, J.G. Down, Z. Khalil, B.G. Livett, and K.R. Gayler. 2003. A novel α-conotoxin identified by gene sequencing is active in suppressing the vascular response to selective stimulation of sensory nerves in vivo. *Biochemistry.* 42:6904–6911. http://dx.doi.org/10.1021/bi034043e

Satkunanathan, N., B. Livett, K. Gayler, D. Sandall, J. Down, and Z. Khalil. 2005. Alpha-conotoxin Vc1.1 alleviates neuropathic pain and accelerates functional recovery of injured neurones. *Brain Res.* 1059:149–158. http://dx.doi.org/10.1016/j.brainres.2005.08.009

Shekter, L.R., R. Taussig, S.E. Gillard, and R.J. Miller. 1997. Regulation of human neuronal calcium channels by G protein βγ subunits expressed in human embryonic kidney 293 cells. *Mol. Pharmacol.* 52:282–291.

White, D.M., and M.J. Cousins. 1998. Effect of subcutaneous administration of calcium channel blockers on nerve injury-induced hyperalgesia. *Brain Res.* 801:50–58. http://dx.doi.org/10.1016/S0006-8993(98)00539-3

Williams, M.E., L.M. Marubio, C.R. Deal, M. Hans, P.F. Brust, L.H. Philipson, R.J. Miller, E.C. Johnson, M.M. Harpold, and S.B. Ellis. 1994. Structure and functional characterization of neuronal α_{1E} calcium channel subtypes. *J. Biol. Chem.* 269:22347–22357.

Wilson, S.M., P.T. Toth, S.B. Oh, S.E. Gillard, S. Volsen, D. Ren, L.H. Philipson, E.C. Lee, C.F. Fletcher, L. Tessarollo, et al. 2000. The status of voltage-dependent calcium channels in α_{1E} knock-out mice. *J. Neurosci.* 20:8566–8571.

Woolf, C.J. 2010. What is this thing called pain? *J. Clin. Invest.* 120:3742–3744. http://dx.doi.org/10.1172/JCI45178

Yang, L., and G.J. Stephens. 2009. Effects of neuropathy on high-voltage-activated Ca^{2+} current in sensory neurones. *Cell Calcium.* 46:248–256. http://dx.doi.org/10.1016/j.ceca.2009.08.001