Neuronal Excitability

Limited Efficacy of $\alpha$-Conopeptides, Vc1.1 and RglIA, To Inhibit Sensory Neuron CaV Current$^{1,2,3}$

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Abstract

Chronic pain is very difficult to treat. Thus, novel analgesics are a critical area of research. Strong preclinical evidence supports the analgesic effects of $\alpha$-conopeptides, Vc1.1 and RglIA, which block $\alpha_9\alpha_{10}$ nicotinic acetylcholine receptors (nAChRs). However, the analgesic mechanism is controversial. Some evidence supports the block of $\alpha_9\alpha_{10}$ nAChRs as an analgesic mechanism, while other evidence supports the inhibition of N-type CaV (CaV2.2) current via activation of GABA$_B$ receptors. Here, we reassess the effect of Vc1.1 and RglIA on CaV current in rat sensory neurons. Unlike the previous findings, we found highly variable effects among individual sensory neurons, but on average only minimal inhibition induced by Vc1.1, and no significant effect on the current by RglIA. We also investigated the potential involvement of GABA$_B$ receptors in the Vc1.1-induced inhibition, and found no correlation between the size of CaV current inhibition induced by baclofen (GABA$_B$ agonist) versus that induced by Vc1.1. Thus, GABA$_B$ receptors are unlikely to mediate the Vc1.1-induced CaV current inhibition. Based on the present findings, CaV current inhibition in dorsal root ganglia is unlikely to be the predominant mechanism by which either Vc1.1 or RglIA induce analgesia.

Key words: alpha9-alpha10 AChR current; analgesic mechanisms; baclofen; CaV2.2 current; rat sensory neurons

Significance Statement

Better analgesic drugs are desperately needed to help physicians to treat pain. While many preclinical studies support the analgesic effects of $\alpha$-conopeptides, Vc1.1 and RglIA, the mechanism is controversial. The development of improved $\alpha$-conopeptide analgesics would be greatly facilitated by a complete understanding of the analgesic mechanism. However, we show that we cannot reproduce one of the proposed analgesic mechanisms, which is an irreversible inhibition of CaV current in a majority of sensory neurons.
Introduction

Severe pain reduces the quality of life of millions of people each year (Cousins et al., 2004). Conventional treatment for chronic pain includes opiates and nonsteroidal anti-inflammatory agents. However, the therapeutic potential of these treatment options for chronic pain are often limited by the development of serious adverse effects and tolerance. Thus, the discovery of improved drug therapies is of great importance.

α-conopeptides are small, disulfide-rich peptides that are isolated from the venom of the Conus genus of carnivorous marine snails and that block nicotinic acetylcholine receptors (nAChRs) (McIntosh et al., 2009). Two α-conopeptides, Vc1.1 and RgIA, have been shown to display antinociceptive effects in animal models; however, the mechanism responsible for analgesia remains debated (Vincler et al., 2006; McIntosh et al., 2009; Napier et al., 2012). Early studies found these α-conopeptides to be potent antagonists of heterologously expressed and native α9α10 nAChRs (Ellison et al., 2006; Vincler et al., 2006). Other studies have found that Vc1.1, but not RgIA, also weakly antagonizes nAChRs subtypes expressed in the periphery containing the α3 subunit (Clark et al., 2006; Ellison et al., 2006). Analogs of Vc1.1 that retain their specificity for α9α10 nAChRs, but not nAChRs with the α3 subunit, are devoid of analgesic effects in animal pain models (Nevin et al., 2007). These findings indicate the possible involvement of off-target effects being responsible for analgesia. However, mice lacking α9 nAChRs have reduced mechanical hyperalgesia in both neuropathic and inflammatory pain models, supporting a role for α9α10 nAChRs as a target for treatment of chronic pain (Mohammadi and Christie, 2014).

One group has proposed that the antinociceptive effects of Vc1.1 and RgIA are elicited by inhibition of N-type CaV (CaV2.2) channels via activation of GABAB receptors (Callaghan et al., 2008; Callaghan and Adams, 2010; Klimis et al., 2011; Adams et al., 2012; Mohammadi and Christie, 2014). The analgesic effects of GABAB receptor activation by the specific GABAβ receptor agonist baclofen have been previously shown (Franek et al., 2004). Furthermore, GABAB receptor activation inhibits the activity of N-type CaV channels (CaV2.2) and inhibition of N-type channels expressed by nociceptors in the spinal cord dorsal horn is analgesic (Raingo et al., 2007). Pain relief comes from the reduction of excitatory neurotransmitter release (e.g., glutamate) from nociceptive nerve terminals when presynaptic N-channels are blocked (Elmslie, 2004; Miljanich, 2004; McIntosh et al., 2009). The inhibition of N-type CaV current by Vc1.1 and RgIA requires functional GABAB receptors since the effect can be blocked by either application of a GABAβ receptor antagonist (Callaghan et al., 2008) or the knockdown of GABAB receptors by siRNA (Cuny et al., 2012).

While inhibition of N-type CaV channel activity is a potential mechanism for Vc1.1- or RgIA-induced analgesia, this hypothesis is controversial (McIntosh et al., 2009). Whether Vc1.1 or RgIA were able to prevent the binding of a specific competitive antagonist to the human GABAβ receptor and both failed to activate GABAβ receptors expressed in Xenopus laevis oocytes (McIntosh et al., 2009). In addition, Vc1.1 failed to affect excitatory postsynaptic currents (eEPSCs) in the dorsal horn of rat spinal cord, which were almost completely blocked by baclofen (Napier et al., 2012). These findings are inconsistent with GABAβ receptor-induced inhibition of N-type CaV channels as the mechanism for analgesia produced by Vc1.1 and RgIA. Given these findings, there is a question of whether the CaV current inhibition in sensory neurons can be independently reproduced. The data presented here shows that the inhibition of CaV current in sensory neurons is on average either small (Vc1.1) or insignificant (RgIA), and that activation of GABAB receptors is not consistent with the small inhibition induced by Vc1.1.

Materials and Methods

Animals

All animal procedures were performed in accordance with the authors’ university animal care committee’s regulations and were consistent with the National Research Council Guide for the Care and Use of Laboratory Animals. Adult male Sprague Dawley rats (200 – 400 g; Hilltop Lab Animals) were used in these experiments. The rats were housed in a U.S Department of Agriculture-approved, Association for Assessment and Accreditation of Laboratory Animal Care-certified animal care facility at a constant temperature 24 ± 1°C, under controlled 12:12 h light-dark cycles, and fed a standard rat Chow diet and tap water ad libitum.

Isolation of DRG neurons

The rats were euthanized by CO2 inhalation followed by decapitation using a laboratory guillotine (Kent Scientific) (Ramachandra et al., 2012). The lumbar (L4) and L5 dorsal root ganglia (DRG) were isolated and dissociated in Earle’s balanced salt solution containing (in mg/ml): 0.7 collagenase, 1 trypsin, and 0.1 DNase at 37°C for 60 min (Ramachandra et al., 2012). The dissociated neurons were washed in minimum essential media (MEM) containing 10% fetal bovine serum (FBS) and plated onto polylysine-coated glass coverslips (Fisher Scientific). The isolated neurons were maintained overnight in a 5% CO2 incubation atmosphere.
tor at 37°C in MEM supplemented with 10% FBS and 1% penicillin-streptomycin and used within 12 – 24 h (Ramachandra et al., 2012).

**Electrophysiological recordings from sensory neurons**

The extracellular recording solution contained (in mM): 5 BaCl<sub>2</sub>, 145 NMG-Cl, 10 NMG-HEPES, and 15 glucose, with pH = 7.4 and osmolarity = 350 mOsm. The intracellular solution contained (mM): 104 NMG-Cl, 14 creatine·PO<sub>4</sub>, 6 MgCl<sub>2</sub>, 10 NMG-HEPES, 5 Tris-ATP, 10 NMG<sub>2</sub>·EGTA, and 0.3 Tris<sub>2</sub>·GTP with pH = 7.4 and osmolarity = 335 mOsm. In some experiments, 0.1 mg/ml bovine serum albumen (BSA) was added to the external solution along with Vc1.1, but no enhancement of the Ca<sub>v</sub> current inhibition was observed relative to Vc1.1 without BSA (same 5 neurons). Thus, the results combine conopeptide and baclofen data both with and without BSA.

Ionic currents were recorded using the whole-cell configuration of the patch-clamp technique with an Axopatch 200B amplifier (Molecular Devices) and digitized with an ITC-18 A/D converter (Instrutech Corp). Microelectrodes with a resistance of 2 – 5 MΩ were pulled from Schott 8250 glass (King Precision Glass) on a Sutter P-97 puller (Sutter Instruments). Series resistance was compensated by at least 80% using the electronic circuits of the Axopatch 200A amplifier. Neurons were voltage clamped at a holding potential of –80 mV and Ca<sub>v</sub> currents were assessed using a three-step voltage protocol that tests for voltage-dependence of Ca<sub>v</sub> channel inhibition (Elmslie et al., 1990; Ikeda, 1991; Ehrlich and Elmslie, 1995).

Experiments were controlled by a Power Macintosh computer (Apple Computer) running S5 data acquisition software written by Dr. Stephen Ikeda (NIH, NIAAA, Bethesda, MD). Leak current was subtracted from the step current using a −P/4 protocol. All experiments were conducted at room temperature (Ramachandra et al., 2012).

Data were analyzed with IgorPro (WaveMetrics) running on a Macintosh computer. Cell diameter was calculated from the cell capacitance as measured by the Axopatch circuitry, assuming a specific membrane capacitance of 1 μF/cm<sup>2</sup> and that the neuron was spherical (Ramachandra et al., 2012).

**Preparation and microinjection of oocytes**

Oocytes were prepared following a similar protocol as that described by Norimatsu et al. (2012). Female Xenopus laevis (Xenopus Express) were anesthetized by immersion in water containing tricaine (1.5 g/l) and sodium bicarbonate (0.2 g/l). The oocytes were removed through a small abdominal incision that was then closed by 4.0 nylon suture. Frogs were allowed to recover in their tanks.

Individual oocytes were placed in a 200 μL RC-1Z recording chamber (Warner Instruments) and gravity-perfused with Frog Ringer’s solution (98 mM NaCl, 2 mM KCl, 1 mM MgCl<sub>2</sub>, 1.8 mM CaCl<sub>2</sub>, and 5 mM HEPES hemisodium, pH 7.4) at ~1.5 ml/min. All solutions also contained 0.1 mg/ml BSA to reduce nonspecific adsorption of the peptide, as described by Vincler et al. (2006).

Membrane currents were recorded from oocytes with a two-electrode voltage-clamp amplifier (TEV-200; Dagan) at room temperature (~22 °C). Electrodes had resistances of 0.5 to 2 MΩ when filled with 3 M KCl. The membrane potential was clamped at ~70 mV. Data acquisition utilized an analog-to-digital converter (Digidata 1320A; Molecular Devices), and data acquisition as well as analysis was done on a Pentium-based microcomputer using pCLAMP software. Data were low-pass filtered (5 Hz cutoff) and digitized at a sampling frequency of 20 Hz.

To apply a pulse of ACh to the oocyte, the perfusion fluid was switched to one containing 10 μM ACh for 1 s. This was done at intervals of ~6 min and has previously been shown to allow reproducible control responses without substantial desensitization (Vincler et al., 2006). To measure block by α-conopeptides, the perfusion system was stopped, the solution from around the oocyte was removed via a mechanical pipetter, and the bath was filled with 200 μl of a solution containing one of the peptides (either 1 μM Vc1.1 or 100 nM RglA). The oocyte was incubated with the conopeptide for 5 min in the static bath. The perfusion system was then restarted with a 1 s pulse of ACh. The conopeptide dwell time was sufficiently long-lasting that the majority of α<sub>9α10</sub>nACHR were still blocked (~2 s of wash time) when the ACh pulse arrived at the oocyte (Vincler et al., 2006). Control ACh responses prior to peptide application were exposed to the same procedure except that control Frog Ringers was used instead of peptide-containing solution. Control ACh responses were measured from the average of two preceding responses and the first response following recovery from conopeptide block (~6 min of washout).

**Statistics**

All data are presented as mean ± SD. Two-tailed one-sample t tests (Excel) were used to determine significant differences (p < 0.05) versus zero of normally distributed data, while a Wilcoxon rank-sum analysis (IgorPro) was used to determine significant differences for data deviating from a normal distribution. The Pearson correlation test (Excel) was used to test for significant correlations between data sets.
MEM, FBS, and penicillin-streptomycin were purchased from Life Technologies. Liberase Blendzyme and collagenase were from Roche Molecular Biochemicals, and trypsin was from Worthington.

Drugs and chemicals
MEM, FBS, and penicillin-streptomycin were purchased from Life Technologies. Liberase Blendzyme and collagenase were from Roche Molecular Biochemicals, and trypsin was from Worthington. α-conopeptides Vc1.1 and RgIA were synthesized as reported previously (Cartier et al., 1996; Ellison et al., 2008). All other chemicals were obtained from Sigma-Aldrich.

Results
Inhibition of CaV current is one mechanism that has been proposed for analgesia induced by the α-conopeptides Vc1.1 and RgIA (Callaghan et al., 2008; Cuny et al., 2012). The effects of Vc1.1 and RgIA on CaV current were studied in sensory neurons dissociated from adult rats. Since the CaV current inhibition by these conopeptides has been reported to be mediated by GABAB receptors, the specific GABAB receptor agonist, baclofen, was used to test for functional presence of GABAB receptors by measuring CaV current inhibition (Tosetti et al., 1996). Thirty micromolar baclofen significantly inhibited prepulse currents by 19.2 ± 6.8% (mean ± SD, n = 21; Fig. 1). As expected, this inhibition was voltage-dependent since the postpulse current was inhibited by only 12.0 ± 5.5%, which was significantly smaller than the prepulse inhibition (p = 0.005). CaV current in 20/21 (95%) sensory neurons was inhibited by baclofen.

The effect of Vc1.1 (1 μM) and RgIA (1 μM) on CaV current differed from that of baclofen (Fig. 1). While inhibition was observed in some neurons by each conopeptide, the overall effect was a small but significant prepulse current inhibition by Vc1.1 (6.5 ± 12.7%, n = 21, p = 0.003; Fig. 1). This inhibition was not voltage-dependent since the postpulse current was inhibited by 6.2 ± 12.5%. There was no significant inhibition by RgIA of either the prepulse (5.5 ± 11.7%, n = 12, p = 0.077, n.s.) or postpulse current (5.3 ± 10.7%).

While the effect of Vc1.1 and RgIA was on average small or insignificant, there were a few neurons that responded with inhibitions >10%. This includes 4/21 (19%) neurons tested with Vc1.1 and 2/12 (17%) neurons tested with RgIA. This contrasts with the previous report showing...
that CaV current in ~75% of sensory neurons was inhibited by 100 nM Vc1.1 (Callaghan et al., 2008). We wanted to further investigate the peptide-induced inhibition to determine if the properties were similar to those reported previously (Callaghan et al., 2008). It was previously reported that CaV current block by Vc1.1 was irreversible, but we found that the block by Vc1.1 was readily reversible with an average recovery τ = 0.5 ± 0.2 min (n = 4; Fig. 2). Thus, this inhibition appears to be distinct from that previously reported (Callaghan et al., 2008).

Another question was if a particular group of neurons exhibited conopeptide sensitivity. One possibility is that the sensitive neurons were nociceptors, which would predict that the somal diameter of these neurons would be smaller (<35 μm) than the unresponsive, non-nociceptive neurons (Djouhri et al., 2003). This possibility was investigated by plotting the somal diameter versus percentage prepulse inhibition (Fig. 3). Against this prediction, the "responsive" neurons spanned the size range with large neurons (>35 μm) just as likely (n = 2) to respond to the conopeptides than small neurons (<30 μm, n = 2; Fig. 3A). Thus, the evidence suggests that the conopeptide-induced CaV current inhibition is not a marker for nociceptive sensory neurons.

Surprisingly, the baclofen-induced prepulse inhibition did not appear to correlate with that induced by Vc1.1 (Fig. 3A), as expected if GABA<sub>δ</sub> receptors mediate Vc1.1-induced inhibition. This relationship was more fully investigated by plotting the Vc1.1-induced CaV current inhibition versus that induced by baclofen (Fig 3B). Calculation of the Pearson correlation yielded R = 0.27 (n.s.). For completeness, the RgIA data are also plotted (Fig. 3B) and the Pearson correlation was R = 0.29 (n.s.). Thus, no correlation was found between the responses induced by either Vc1.1 or RgIA versus baclofen. Notably, the neuron with the largest baclofen response (34%) showed only a 3.5% CaV current inhibition by Vc1.1 (Fig. 3B). The neuron with the largest Vc1.1 (41%) and RgIA (35%) showed a 22% inhibition by baclofen. However, seven other neurons with baclofen responses ranging from 20 – 24% responded to Vc1.1 with an average 4.3 ± 6.6% inhibition of CaV current, while the four neurons also tested with RgIA responded with a 0.0 ± 7.6% effect. It appears that GABA<sub>δ</sub> receptors do not mediate the small CaV current inhibition induced by Vc1.1.

Interestingly, there was a significant correlation found between the CaV inhibitions induced by Vc1.1 and RgIA (R = 0.88, p < 0.05). This result suggests a common inhibitory mechanism for both Vc1.1 and RgIA, but as mentioned above, we could find no evidence that GABA<sub>δ</sub> receptors mediate this CaV current inhibition.

As a positive control, Xenopus oocytes expressing rat α9α10 nAChRs were exposed to Vc1.1 or RgIA to ensure the conopeptides were functional. ACh-induced currents were recorded using the two-electrode voltage-clamp method. Application of ACh (10 μM) was limited to 1 s in duration once every 5 – 6 min. This protocol yielded stable ACh-induced currents (Fig. 4) with average amplitude of 768 ± 275 nA. Consistent with other reports (Vincler et al., 2006), Vc1.1 (1 μM) and RgIA (100 nM) significantly

![Fig. 2](image_url) Rapid recovery from Vc1.1-induced inhibition. A, Example traces from a neuron with a 40% inhibition of CaV current induced by 1 μM Vc1.1 (blue trace). Note the almost full recovery from inhibition in the washout trace (Wash). B, The time course of inhibition by Vc1.1. The asterisks indicate the traces used in A. C, The average time constant (τ) for recovery from block by 1 μM Vc1.1 from the four neurons with inhibition >10%. Recovery τ was determined by fitting the Vc1.1 washout time course using a single exponential equation.


GABA<sub>B</sub> receptors was also assessed, but there was no correlation between the magnitudes of baclofen- and Vc1.1-induced inhibitions. These same α-conopeptides that minimally affected Ca<sub>V</sub> current strongly (>80%) inhibited α9α10 nAChRs expressed in Xenopus oocytes, which demonstrates the expected potency of these α9α10 nAChR antagonists. While our results fail to reproduce results reported in some previous publications (Callaghan et al., 2008; Callaghan and Adams, 2010), they do support other publications showing that RgIA and Vc1.1 do not activate GABA<sub>B</sub> receptors expressed in Xenopus oocytes (McIntosh et al., 2009) and showing that Vc1.1 does not affect excitatory neurotransmitter release from sensory nerve terminals that express GABA<sub>B</sub> receptors (Napier et al., 2012).

**Different effects**

While the overall inhibition was small, we found that these conopeptides could inhibit Ca<sub>V</sub> current (>10%) in a minority of sensory neurons (<20%). This fraction of sensitive neurons is much smaller than that previously reported (75%) (Callaghan et al., 2008). Other differences include a relative fast recovery from block versus irreversible block, and the apparent lack of GABA<sub>B</sub> receptor involvement (Callaghan et al., 2008). We were unable to identify a single neuronal group as conopeptide sensitive, since a few small (<30 μm), medium, and large (>40 μm) diameter neurons were found to be sensitive, while other neurons within the same size range were insensitive. As a result, it seems unlikely that nociceptors define the conopeptide-sensitive population.

We have no data to explain why we cannot reproduce the previously published Ca<sub>V</sub> current inhibitions (Callaghan et al., 2008; Callaghan and Adams, 2010). However, we can exclude some possibilities. First, the α-conopeptides used here were potent inhibitors of α9α10 nAChRs, which demonstrated that they were functional peptides. Second, the previous publications demonstrated that N-type Ca<sub>V</sub> channels were the Ca<sub>V</sub> channel type inhibited by Vc1.1 and RgIA (Callaghan et al., 2008), and we have demonstrated N-type channels comprise approximately half of the total Ca<sub>V</sub> current in rat sensory neurons (Ramachandra et al., 2012). Thus, the absence of the target channel cannot explain the differences. Finally, the GABA<sub>B</sub> receptors were functional in these neurons since the specific agonist, baclofen, inhibited Ca<sub>V</sub> current in 95% of neurons tested, which confirms the presence of the putative receptor that mediates the Ca<sub>V</sub> current inhibition induced by these α-conopeptides.

Differences among species have been proposed as a possible reason for differing results. McIntosh et al. (2009) demonstrated that both Vc1.1 and RgIA failed to block binding of [3H]CGP-54626, a specific competitive antagonist, to human GABA<sub>B</sub> receptors, and suggested that the human receptors were not a target for these conopeptides. However, Ca<sub>V</sub> current inhibition by Vc1.1 and RgIA has been shown in both rat and mouse sensory neurons (Callaghan et al., 2008; Callaghan and Adams, 2010), and our results from rat sensory neurons fit well with the human data. Thus, species differences are unlikely to explain these differences.
The sources of the conopeptide are different, but it is not clear how that would explain the different results. These peptides are synthesized by manual solid-phase synthesis. Disulfide bond formation is by directed synthesis and/or verified by NMR analysis. The peptides are purified in a similar manner between labs with reversed-phase high-performance liquid chromatography using trifluoracetic acid and acetonitrile buffer systems.

**Analgesic mechanisms**

Many experiments have demonstrated the analgesic properties of α-conopeptides that block α9α10 nAChR, including Vc1.1 and RgIA (Satkunanathan et al., 2005; Vincler et al., 2006; Napier et al., 2012; Di Cesare Mannelli et al., 2014). However, post-translational modifications of Vc1.1 that preserved α9α10 nAChR block eliminated the analgesic effects (Nevin et al., 2007). This suggested that the analgesic effect of Vc1.1 did not result from α9α10 nAChR block. Interestingly, the effect of Vc1.1 to inhibit Ca_{v} current was lost by these same post-translational modifications, which supported Ca_{v} current inhibition as an analgesic mechanism for Vc1.1 (Callaghan et al., 2008). However, the pharmacokinetic properties of this analog were not investigated, leaving open the possibility that RgIA and Vc1.1 are unlikely to cross the blood–brain barrier and reach spinal neuron synapses, which further suggests that RgIA and Vc1.1 induced analgesia may not involve Ca_{v} channels. In addition, the highly selective N-type Ca_{v} antagonist, ziconotide, did not decrease neuropathic pain when given peripherally by intravenous injection (Chaplan et al., 1994). This FDA-approved drug must be delivered by intrathecal administration for therapeutic effect (Sanford, 2013). Furthermore, N-type channel expression has been reported to be reduced in peripheral sensory neurons after nerve injury (McCallum et al., 2011). Together, these findings suggest non-Ca_{v} channel mechanisms are important for RgIA- and Vc1.1-induced analgesia.

Recent work has demonstrated a possible role of α9 nAChR in pain, since mechanical hyperalgesia was reduced in α9 nAChR knockout mice following chronic nerve constriction and in an inflammatory pain model (Mohammadi and Christie, 2014). In addition, a major effect of RgIA appears to be on the glial/immunological response to chronic nerve injury to prevent pathological changes within the nervous system that are thought to result in neuropathic pain (Di Cesare Mannelli et al., 2014). There are also small molecule antagonists of α9α10 nAChRs. These compounds have also been shown to be analgesic, lending further support for the importance of this mechanism.

**Fig. 4** Vc1.1 and RgIA potently block α9α10 nAChRs expressed in X. laevis oocytes. ACh-induced currents were measured from voltage-clamped oocytes as described in Materials and Methods. A, B, Representative traces of ACh-induced currents in the presence and absence of Vc1.1 or RgIA, respectively. C, The mean (± SD) inhibition of ACh-induced peak current amplitude by α-conopeptides Vc1.1 and RgIA. The numbers in bars reflects numbers of cells tested. * indicates significant inhibition (p < 0.05).
mechanism (Holtman et al., 2011; Zheng et al., 2011; Wala et al., 2012). While the present study does not allow us to identify the mechanism by which Vc1.1 or RgIA produce analgesia, our findings do not support a role for Ca2+ channel inhibition in sensory neurons as one of those mechanisms.

References

Adams DJ, Callaghan B, Berecki G (2012) Analgesic conotoxins: block and G protein-coupled receptor modulation of N-type (Ca,2.2) calcium channels. Br J Pharmacol 166:486–500. CrossRef Medline

Callaghan B, Adams DJ (2010) Analgesic α-conotoxins Vc1.1 and RgIA inhibit N-type calcium channels in sensory neurons of α9 nicotinic receptor knockout mice. Channels 4:31–54. Medline

Callaghan B, Haythornthwaite A, Berecki G, Clark RJ, Craik DJ, Adams DJ (2008) Analgesic alpha-conotoxins Vc1.1 and RgIA inhibit N-type calcium channels in rat sensory neurons via GABA<sub>A</sub> receptor activation. J Neurosci 28:10943–10951. CrossRef Medline

Cartier GE, Yoshikami D, Gray WR, Luo S, Olivera BM, McIntosh JM (1996) A new alpha-conotoxin which targets α3δ2 nicotinic acetylcholine receptors. J Biol Chem 271:7522–7528. Medline

Chaplan SR, Pogrel JW, Yaksh TL (1994) Role of voltage-dependent calcium channel subtypes in experimental tactile alldynia. J Pharmacol Exp Ther 269:1117–1123. Medline

Clark RJ, Fischer H, Nevin ST, Adams DJ, Craik DJ (2006) The synthesis, structural characterization, and receptor specificity of the α-conotoxin Vc1.1. J Biol Chem 281:23254–23263. CrossRef Medline

Cousins MJ, Brennan F, Carr DB (2004) Pain relief: a universal human right. Pain 112:1–4. CrossRef Medline

Cuny H, de Faoite A, Berta HE, Schilling R, Otsuka K, J passer P, Biondi G, Dhanabal M, Olivera BM, McIntosh JM (2006) The structural, functional and pharmacological characterization of the α9α10 nicotinic receptor in primary afferent myelinated fibers and dorsal root ganglion neurons. J Pharmacol Exp Ther 317:1197–1206. CrossRef Medline

Di Cesare Mannelli L, Cenci L, Micheli L, Zanardelli M, Pacini A, McIntosh JM, Ghelardini C (2014) α-conotoxin RgIA protects against the development of nerve injury-induced chronic pain and prevents both neuronal and glial derangement. Pain 155:1986–2002. CrossRef Medline

Djouhri L, Zhang X-P, Park AJ, Zhang X, Olivera BM, McIntosh JM (2011) Molecular mechanism for analgesia involving specific α9 α10 nicotinic acetylcholine receptors and the treatment of pain. Biochem Pharmacol 80:693–702. CrossRef Medline

Elmslie KS (2004) Calcium channel blockers in the treatment of peripheral neuropathy in the rat. Pharmacol Res 49:221–228. CrossRef Medline

Elmslie KS, Zhou W, Jones SW (1990) LHRH and GTP-gamma-S modify calcium current activation in bullfrog sympathetic neurons. Neuron 5:75–80. CrossRef Medline

Franke M, Vaculin S, Roktya R (2004) GABA<sub>B</sub> receptor agonist baclofen has non-specific antinociceptive effect in the model of peripheral neuropathy in the rat. Physiol Res 53:351–355. CrossRef Medline

Holtman JR, Dwoskin LP, Dowell C, Wala EP, Zhang Z, Crooks PA, McIntosh JM (2011) The novel small molecule α9α10 nicotinic acetylcholine receptor antagonist ZZ-204G is analgesic. Eur J Pharmacol 670:500–508. CrossRef Medline

Ikeda SR (1991) Double-pulse calcium channel current facilitation in adult rat sympathetic neurones. J Physiol 439:181–214. CrossRef Medline

Ikeda SR (1996) Voltage-dependent modulation of N-type calcium channels by G-protein beta gamma subunits. Nature 380:255–258. CrossRef Medline

Kim HS, Adams DJ, Callaghan B, Nevin S, Alewood PF, Vaughan CW, Mozar CA, Christie MJ (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. CrossRef Medline

McCallum JB, Wu HE, Tang Q, Kwok WM, Hogan QH (2011) Subtype-specific reduction of voltage-gated calcium current in medium-sized dorsal root ganglion neurons after painful peripheral nerve injury. Neuroscience 179:244–255. CrossRef Medline

McIntosh JM, Absalom N, Chebib M, Elgyohan AB, Vicinle M (2009) α9 nicotinic acetylcholine receptors and the treatment of pain. Biochem Pharmacol 78:693–702. CrossRef Medline

Miljanich GP (2004) Ziconotide: neuronal calcium channel blocker for treating severe chronic pain. Curr Med Chem 11:3029–3040. Medline

Mohammadi S, Christie MJ (2014) α9-nicotinic acetylcholine receptors contribute to the maintenance of chronic mechanical hyperalgesia, but not thermal or mechanical allodynia. Mol Pain 10:64.CrossRef Medline

Napier IA, Klimes H, Rycroft BK, Jin AH, Alewood PF, Motin L, Adams DJ, Christie MJ (2012) Intrathecal α-conotoxins Vc1.1, AuB and MII acting on distinct nicotinic receptor subtypes reverse signs of neuropathic pain. Neuropharmacology 62:2202–2207. CrossRef Medline

Nevin ST, Clark RJ, Klimis H, Christie MJ, Craik DJ, Adams DJ (2007) Are α9α10 nicotinic acetylcholine receptors a pain target for α-conotoxins? Mol Pharmacol 72:1406–1410. CrossRef Medline

Norimatsu Y, Morar AR, MacDonald KD (2012) Lubioprostone activates CFTR, but not CIC-2, via the prostaglandin receptor (EP4). Biochem Biophys Res Commun 426:374–379. CrossRef Medline

Raijo J, Castiglioni AJ, Lipscombe D (2007) Alternative splicing controls G protein-dependent inhibition of N-type calcium channels in nociceptors. Nat Neurosci 10:285–292. CrossRef Medline

Ramachandra R, McGrew SY, Baxter JC, Kieric E, Elmslie KS (2012) Tetrodotoxin-resistant voltage-dependent sodium channels in identified muscle afferent neurons. J Neurophysiol 108:2230–2241. CrossRef Medline

Sanford M (2013) Intrathecal ziconotide: a review of its use in patients with chronic pain refractory to other systemic or intrathecal analgesics. CNS Drugs 27:989–1002. CrossRef Medline

Sakunathanan N, Livett B, Gayler K, Sandall D, Down J, Khalil Z (2005) Alpha-conotoxin Vc1.1 alleviates neuropathic pain and accelerates functional recovery of injured neurons. Brain Res 1059:149–158. CrossRef Medline

Tosetti P, Turner T, Lu Q, Dunlap K (2002) Unique isoform of G<sub>alpha(i)2</g> interacting protein (RGS-GAIP) selectively discriminates between two Go-mediated pathways that inhibit Ca<sup>2+</sup> channels. J Biol Chem 277:46001–46009. CrossRef Medline

Vanegas H, Schaible HG (2000) Effects of antagonists to high-threshold calcium channels upon spinal mechanisms of pain, hyperalgesia and allodynia. Pain 85:9–18. Medline

Vicinle M, Wittenaer S, Parker R, Emslie KS, Olvera BM, McIntosh JM (2006) Molecular mechanism for analgesia involving specific antagonism of α9α10 nicotinic acetylcholine receptors. Proc Natl Acad Sci USA 103:17880–17884. CrossRef Medline

Wala EP, Elmslie KS, McIntosh JM, Holtman JR (2012) Novel small molecule α9α10 nicotinic receptor antagonist prevents and reverses chemotherapy-evoked neuropathic pain in rats. Anesth Analg 115:713–720. CrossRef Medline

Zheng G, Zhang Z, Dowell C, Wala E, Dwoskin LP, Holtman JR, McIntosh JM, Crooks PA (2011) Discovery of non-peptide, small molecule antagonists of α9α10 nicotinic acetylcholine receptors as novel analgesics for the treatment of neuropathic and tonic inflammatory pain. Bioorg Med Chem Lett 21:2476–2479. CrossRef Medline

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