Title
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Journal
European Journal of Pain, 19(5)

ISSN
1090-3801

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Publication Date
2015-05-01

DOI
10.1002/ejp.585

Peer reviewed

HHS Public Access

Author manuscript

Eur J Pain. Author manuscript; available in PMC 2016 May 01.

Published in final edited form as:

Eur J Pain. 2015 May ; 19(5): 639–648. doi:10.1002/ejp.585.

Differential Effects of Voltage-Gated Calcium Channel Blockers on Calcium Channel Alpha-2-Delta-1 Subunit Protein Mediated Nociception

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Abstract

Background—Overexpression of the voltage gated calcium channel (VGCC) alpha-2-delta1 subunit protein ($Ca_v\alpha_2\delta_1$) has been shown to cause pain states. However, whether VGCC are involved in pain states driven by abnormal $Ca_v\alpha_2\delta_1$ expression is not known.

Methods—Intrathecal injection of N-, P/Q-, and L-type VGCC blockers were tested in two models: a transgenic neuronal $Ca_v\alpha_2\delta_1$ overexpression (TG) model with behavioral hypersensitivity and a spinal nerve ligation (SNL) model with $Ca_v\alpha_2\delta_1$ overexpression in sensory pathways and neuropathy pain states.

Results—The nociceptive response to mechanical stimuli was significantly attenuated in both models with ω-conotoxin GVIA (an N-type VGCC blocker) and nifedipine (a L-type VGCC blocker), in which ω-conotoxin GVIA appeared more potent than nifedipine. Treatments with ω-agatoxin IVA (P-VGCC blocker), but not ω-conotoxin MVIIC (Q-VGCC blocker) had similar potency in the TG model as the N-type VGCC blocker, while both ω-agatoxin IVA and ω-conotoxin MVIIC had minimal effects in the SNL model compared to controls.

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No conflict of interest to declare by any authors.

Author Contributions:

In addition to the following contributions from each author, all authors discussed the results, commented on the manuscript and approved for the submission.

E.C. contributed to conception, design of the study, data acquisition, analysis, and interpretation, drafting, editing the manuscript.

X.C., M.K, N. G. and S.B. contributed to the study design, data acquisition, analysis and interpretation.

Z.D.L. contributed to conception, design and overall supervision of the study. He also performed data analysis, interpretation, drafting and revising the manuscript.

Conclusion—These findings suggest that, at the spinal level, N- and L-type VGCC are likely involved in behavioral hypersensitivity states driven by $Ca_v\alpha_2\delta_1$ overexpression. Q-type VGCC have minimal effects in both models. The anti-nociceptive effects of P-type VGCC blocker in the $Ca_v\alpha_2\delta_1$ TG mice, but minimally at the SNL model with presynaptic $Ca_v\alpha_2\delta_1$ upregulation, suggest that its potential action site(s) is at the post-synaptic and/or supraspinal level. These findings support that N-, L- and P/Q-type VGCC have differential contributions to behavioral hypersensitivity modulated by $Ca_v\alpha_2\delta_1$ dysregulation at the spinal cord level.

Introduction

Chronic pain can adversely affect patients' quality of life, and also have psychosocial/economical consequences which, based on a recent survey, could cost up to $635 billion a year in medical expenses and lost of productivity (Gaskin and Richard 2012). Current pain medications, both opioid and non-opioid, provide only partial pain relief at best with intolerable side effects and adverse sequela. Therefore, there is a vital need to provide safer and more specific analgesic medications for chronic pain management.

Blocking high-threshold voltage-gated Ca^{2+} channels (VGCC) may hold part of the answer (Park and Luo 2010; Perret and Luo 2009). There are five distinct high-threshold VGCC subtypes identified as N-, P/Q-, L- and R- type based on their electrophysiological properties and sensitivity to specific antagonists (Park and Luo 2010). The N- and P/Q-types are the predominant VGCC found in the primary afferent fibers in the superficial dorsal horn with a greater number for the N-type VGCC (Westenbroek et al., 1998). N-type VGCC are mainly on the presynaptic terminals of primary sensory fibers and modulate peptidergic or non-peptidergic neurotransmitter release within the spinal cord (Gruner and Silva 1994; Kato et al., 2002; Westenbroek et al., 1998; Yu et al., 1992). P/Q-type VGCC are involved in glutamatergic, but not peptidergic, neurotransmission (Araque et al., 1994; Evans et al., 1996; Kato et al., 2002; Krieger et al., 1999; Matthews and Dickenson 2001; Takahashi and Momiyama 1993; Westenbroek et al., 1998). P/Q-type VGCC normally participate in inhibitory synaptic mechanisms under normal conditions, and participate in excitatory synaptic mechanisms under conditions leading to central sensitization (Vanegas and Schaible 2000). L-type VGCC can be found in postsynaptic membranes in cell bodies and dendrites throughout the dorsal horn (Murakami et al., 2004; Westenbroek et al., 1998). L-type VGCC blockers can modulate post-synaptic neurons in the dorsal horn and nociceptive responses induced by pain-inducing peptide Substance P (Kato et al., 2002).

Data from previous studies in an unilateral spinal nerve ligation (SNL) model have indicated a critical role of upregulated dorsal root ganglion and spinal cord VGCC alpha-2-delta-1 subunit ($Ca_v\alpha_2\delta_1$) in neuropathic pain development (Bauer et al., 2009; Boroujerdi et al., 2008; Li et al., 2004; Luo et al., 2002; Luo et al., 2001; Newton et al., 2001). It is not clear, however, if elevated spinal $Ca_v\alpha_2\delta_1$ mediates neuropathic pain states through differential modulation of VGCC or a mechanism independent of VGCC functions (Eroglu et al., 2009; Zhou and Luo 2013). Since SNL leads to an upregulation of a large number of genes (Costigan et al., 2002; Kim et al., 2009; Valder et al., 2003; Wang et al., 2002), which could contribute to nociception through different pathways, the SNL model alone is not suitable to address this question. In this study, we compared the effects of N-, L- and P/Q-type VGCC

blockers in $Ca_v\alpha_2\delta_1$-mediated behavioral hypersensitivities between the SNL model and a $Ca_v\alpha_2\delta_1$ overexpressing transgenic (TG) mouse line with dorsal horn neuron sensitization and similar behavioral hypersensitivities as the SNL model (Chang et al., 2013; Li et al., 2006; Nguyen et al., 2009; Zhou and Luo 2014).

Methods

Animals

Adult male mice with 129SV background were purchased from Charles River Laboratories International, Inc. (Hollister, CA). The $Ca_v\alpha_2\delta_1$ TG mice were bred in house and characterized and reported previously (Li et al., 2006). These mice developed normally, groomed appropriately, and were fertile. They showed no signs of distress, ataxia, motor function defects, tremor, seizure, or other abnormalities (Li et al., 2006). Only adult male TG mice and their wild type (WT) littermates were used for the experiments. Food and water were provided to the animals *ad libitum*. All the animal care and experimental procedures were performed based on protocols approved by the Institutional Animal Care Committee of the University of California, Irvine.

Neuropathic lesions

The SNL surgery was performed as described (Kim and Chung 1992). Briefly, the mouse left L4 spinal nerve (Rigaud et al., 2008) was exposed, and tightly ligated under isoflurane anesthesia with a 7.0 silk suture between the DRG and the junction where spinal nerves form the sciatic nerve. In sham operations, the same procedure as described above was performed except that the L4 spinal nerve was left intact.

Drug injection

ω-conotoxin GVIA (Sigma-Aldrich U.S.A.), ω-conotoxin MVIIC (Alomone Labs, Jerusalem, Israel), and ω-agatoxin IVA (Sigma-Aldrich U.S.A.) were dissolved in sterile saline. Nifedipine (Sigma-Aldrich U.S.A.) was first dissolved in a stock solution of 10 mM with 50% DMSO in saline. This was further diluted to the appropriate testing dilutions with saline to a final DMSO concentration <0.0001%, which did not affect the behavioral sensitivity of animals (data not shown). These drug solutions were injected intrathecally (5 μL/mouse) between lumbar regions L4–L5 via a 30-gauge, 1/2-inch needle attached to a microinjector (Tritech Research Inc, Los Angeles, CA) (Inoue et al., 2004). In the case that mice were used for repetitive injections, a drug-free period of at least 48 hours after the last drug injection was introduced. Molarity of injected drugs was calculated based on estimated 40 μL mouse cerebrospinal fluid (Johanson et al., 2008).

Behavioral Test

Animals were tested between the hours of 9am to 5pm. Briefly, the hindpaw withdrawal sensitivities to von Frey filament (mechanical) stimuli were tested blindly before and after drug treatments as described previously (Li et al., 2006). After acclimatization for 1 hour in a plastic box with a wire mesh bottom, the mice were tested for the 50% paw withdrawal thresholds (PWT) to von Frey filament (Stoelting, Wood Dale, IL) stimulation using a modified up-down method (Dixon 1980). In a consecutive order, a set of 6 von Frey

filaments, starting with the one with 0.40 grams, was applied perpendicularly to the hindpaw plantar surface of each mouse until the filament was slightly bent. Lifting of the hindpaw within 5 s was considered a positive response and led to the application of the next weaker filament. Absence of a response within 5 seconds led to the use of the next stiffer filament. The mice were tested until six scores, starting from the one prior to the first change in response, were obtained that were used to calculate the 50% paw withdrawal thresholds as described previously (Li et al., 2004; Luo et al., 2002; Luo et al., 2001). In the case of four consecutive positive or three consecutive negative responses, a score of 0.01 g or 3.0 g, respectively, was assigned. Paw withdrawal thresholds from injured and uninjured hindpaws of the SNL model were recorded individually, and data from the sham or SNL groups were used for comparisons between injury and noninjury side before or after intrathecal drug treatments. Data from each hindpaw of the injury-free $Ca_v\alpha_2\delta_1$ TG and wild-type (WT) mice were collected, and averaged for comparisons between the TG and WT mice before or after intrathecal drug treatments.

Statistic analysis

Significant changes from baseline for the time-course compared to pre-treatment and among different dosages of toxin were determined by using Kruskal-Wallis test followed by Dunn's post-test analysis. Wilcoxon signed rank test was used for pair-wise comparison with the pretreatment level. A p value < 0.05 was considered statistically significant.

Results

Previous data have shown that $Ca_v\alpha_2\delta_1$ over-expression in neuronal cells of the transgenic mice and the injury side of the SNL model induces similar dorsal horn neuron sensitization (Zhou and Luo 2014), that leads to reduced paw withdrawal thresholds to innocuous mechanical stimuli (tactile allodynia) to a similar degree in both models (Chang et al., 2013; Li et al., 2006), which is confirmed in this study (Fig. 1). Intrathecal treatments with ω-conotoxin GVIA in TG mice, ranging from 1.25 fM – 1.25 pM, induced a statistically significant, dose-dependent, reversal of the allodynia state compared with that at the pre-treatment level and in saline treated TG mice. The window between no statistical significance and potentially a full reversal was narrow, between 1.25 fM and 12.5 fM (Fig. 1A). At the highest dose tested (1.25 pM), the anti-allodynic effects of ω-conotoxin GVIA in TG mice were fast in onset and short in duration, with the peak effects occurring 30 min post injection, and lasted for less than 2 hours (Fig. 1B). These findings are consistent with the reported anti-nociceptive effects of ω-conotoxin GVIA in SNL and inflammatory pain models with no motor function side effects (Bowersox et al., 1996; Chaplan et al., 1994; Fukuizumi et al., 2003a; Murakami et al., 2004; Sluka 1997; 1998; Yamamoto and Sakashita 1998).

Similar efficacy of intrathecal ω-conotoxin in allodynia reversal was observed in the SNL model (Fig. 1C). At the highest dose tested (1.25 pM), the anti-allodynic effect of ω-conotoxin GVIA peaked 30–60 minutes post injection, and lasted about 2 hrs (Fig. 1D). For both models, the baseline behavioral sensitivity in age- and sex-matched WT littermates, sham, and non-injury side of the SNL mice was not affected by the highest dose of ω-

conotoxin GVIA. Similar saline injection did not cause allodynia reversal in the TG mice, nor in the injury side of the SNL mice (Fig 1B and 1D).

Next, we examined the role of L-type VGCC on $Ca_V\alpha_2\delta_1$ protein mediated behavioral hypersensitivity states using nifedipine, an L-type VGCC blocker. Data from i.t. nifedipine treatments on TG mice showed that nifedipine appeared less potent than ω-conotoxin GVIA in reversing allodynia states in the TG mice. A statistically significant reversal of the allodynia state was observed at the dose of 1.25 nM compared to saline treatment, and at a lower dose, 312.5 pM, when compared with the pre-treatment level within the same group of animals (Fig. 2A). At the highest dose tested, 1.25 nM, the anti-allodynic effects of nifedipine were fast in onset and short in duration, with the peak effects occurring 30 minutes post-injection, and lasting less than 1 hour (Fig. 2B). Similar intrathecal nifedipine treatments in the SNL model caused a statistically significant reversal of allodynia at the dose of 1.25 nM compared with the saline treated SNL mice (Fig. 2C). At this dose (1.25 nM), a complete allodynia reversal occurred at 30 min post-injection, and the anti-allodynic effects lasted about 2 hours (Fig. 2D). However, nifedipine appeared less potent than ω-conotoxin in reversing allodynia states. For both models, the baseline behavioral sensitivity was not affected by the highest dose of nifedipine in age- and sex-matched WT littermates, sham mice, and non-injury side of the SNL mice. Similar intrathecal saline injections with diluted DMSO (<0.0001%) did not cause allodynia nor reverse pain states in TG, WT, sham, or injury side of the SNL mice (data not shown).

Finally, we investigated the role of P/Q-type VGCC in $Ca_V\alpha_2\delta_1$ protein mediated behavioral hypersensitivity states in the TG and SNL models, respectively. Similar to i.t. ω-conotoxin GVIA and nifedipine treatments (Fig 3A), intrathecal ω-agatoxin, a P-type VGCC blocker at the dosages used (Nimmrich and Gross 2012), caused a statistically significant, dose-dependent reversal of allodynia states, starting at the dose of 0.125 pM, compared to the saline treated TG mice. The window between no statistical significance and potentially full reversal was narrow when compared to saline, between 12.5 fM– 0.125 pM (Fig. 3A). The anti-allodynic effects of ω-agatoxin started at a lower dose (12.5 fM) when compared with the pre-treatment level within the same group of animals. At the highest dose tested (1.25 pM), the anti-allodynia effects of ω-agatoxin were fast in onset and short in duration, with the peak effect occurring 30 min post injection, and lasting for less than 2 hours (Fig 3B). On the contrary, intrathecal injection of various doses (12.5 fM – 1.25 pM) of ω-agatoxin into the SNL model did not produce a significant allodynia reversal compared to saline treated SNL mice (Fig. 3C), though at the maximum dose (1.25 pM) tested, there was a paw withdrawal threshold reversal to <21% of the maximum possible effect compared to the pre-treatment level in the same group of mice (Figure 3C and Figure 3D). Baseline behavioral sensitivity in age- and sex-matched WT, sham, and non-injury side of the SNL littermates were not affected by the highest dose of ω-agatoxin nor saline (Fig. 3B, 3D).

To investigate Q-type VGCC function, we utilized ω-conotoxin MVIIC intrathecally to block Q-type VGCC (Hillyard et al., 1992; McDonough et al., 1996; Nimmrich and Gross 2012). We repeated the experiments with the highest dose of ω-conotoxin MVIIC (6 μM) reported without producing side effects (Chaplan et al., 1994; Dalmolin et al., 2011; Malmberg and Yaksh 1994) and found no significant anti-allodynic effect in both the TG

(Figure 4A) and SNL (Figure 4B) models. This supports similar findings from other groups that Q-type VGCC play a limited role in peripheral nerve injury-induced pain states (Chaplan et al., 1994; Malmberg and Yaksh 1994; Nimmrich and Gross 2012). Again, we found that baseline behavioral sensitivity in age- and sex-matched WT, sham, and non-injury side of the SNL littermates were not affected by ω-conotoxin MVIIC nor saline (Fig. 4).

Administrations of VGCC antagonists in high doses can affect normal spinal processing of somatosensory, including nociceptive, information, and elicit motor function deficit and other side effects. However, the dosages used for the N-, L-, and P/Q- type VGCC blockers in this study are at least three magnitudes smaller than other studies (Bowersox et al., 1996; Chaplan et al., 1994; Fukuizumi et al., 2003b; Jayamanne et al., 2013; Kato et al., 2002; Malmberg and Yaksh 1994; Murakami et al., 2004; Sluka 1997; 1998; Yamamoto and Sakashita 1998), so no motor function deficits were observed in treated mice at any dose.

Discussion

The detailed mechanism underlying neuropathic pain processing in the SNL model has not been fully elucidated. $Ca_v\alpha_2\delta_1$ upregulation in this model may contribute to neuropathic pain processing (Bauer et al., 2009; Li et al., 2004; Luo et al., 2001; Newton et al., 2001), but it is not clear if this neuroplasticity mediates abnormal sensations through a VGCC-dependent or VGCC-independent pathway. Since SNL injury leads to dysregulation of other genes in addition to the $Ca_v\alpha_2\delta_1$ gene (Kim et al., 2009; Valder et al., 2003; Wang et al., 2002), using this model alone is not sufficient to address this question. By comparing VGCC blocker effects in allodynia reversal between the SNL model and the injury-free $Ca_v\alpha_2\delta_1$ TG model, which has similar behavioral hypersensitivity states as the SNL model but lack other injury-induced factors, we have assessed the influence of different VGCC in $Ca_v\alpha_2\delta_1$-mediated behavioral hypersensitivity.

One of the differences between these two models is that the $Ca_v\alpha_2\delta_1$ TG model has increased $Ca_v\alpha_2\delta_1$ proteins in the central and peripheral nervous systems (Li et al., 2006), while the SNL model has increased $Ca_v\alpha_2\delta_1$ expression in dorsal root ganglion sensory neurons that results in elevated $Ca_v\alpha_2\delta_1$ transportation to their pre-synaptic terminals in dorsal spinal cord (Bauer et al., 2009; Li et al., 2004). In both models, increased $Ca_v\alpha_2\delta_1$ expression has been shown to increase behavioral hypersensitivity and the frequency, but not amplitude, of dorsal horn neuron miniature excitatory post-synaptic currents, a reflection of increased pre-synaptic excitatory input. This suggests that enhanced pre-synaptic excitatory neurotransmitter release is likely contributing to $Ca_v\alpha_2\delta_1$ mediated behavioral hypersensitivity in both models (Nguyen et al., 2009; Zhou and Luo 2014).

Our data indicate that ω-conotoxin GVIA has the highest efficacy and potency in allodynia reversal among VGCC blockers tested, supporting that N-type VGCC play a critical role in pain processing in these models. This is supported by findings that null N-type VGCC expression in mice results in diminished pain states (Saegusa et al., 2001). Administration of N-type VGCC antagonists leads to mechanical allodynia relief in rat models of painful peripheral neuropathy (Xiao and Bennett 1995) and SNL (Bowersox et al., 1996; Chaplan et

al., 1994). Since N-type VGCC are mainly located pre-synaptically in spinal cord and involved in regulating excitatory neurotransmitter release (Evans et al., 1996; Maggi et al., 1990; McGivern and McDonough 2004; Santicioli et al., 1992), blocking N-type VGCC may lead to a reversal of behavioral hypersensitivity by normalizing abnormal pre-synaptic neurotransmitter release in the spinal cord (Jayamanne et al., 2013; Matthews and Dickenson 2001; Motin and Adams 2008). However, our data would not allow us to determine if increased $Ca_V\alpha_2\delta_1$ mediates abnormal pre-synaptic neurotransmitter release and behavioral hypersensitivity through modulating pre-synaptic N-type VGCC expression/function or an N-type VGCC independent pathway such as increasing excitatory synapse formation (Eroglu et al., 2009).

Compared with ω-conotoxin GVIA, intrathecal L-type VGCC antagonist nifedipine has a similar efficacy, but lower potency, in allodynia reversal in both models. Since L-type VGCC are primarily on the soma and indirectly involved in neurotransmitter release, the inhibitory effects of L-type VGCC antagonists on nociceptive responses are theorized to act through modulation of post-synaptic dorsal horn neurons, and secondarily through projection neurons and interneurons (Kato et al., 2002).

L-type VGCC are composed of three subtypes (Cav1.1, Cav1.2 and Cav1.3), which could be dysregulated in DRG and spinal cord of neuropathic pain models. Cav1.2 is upregulated in the spinal cord and correlates with neuropathic pain states in the SNL model (Fossat et al., 2010). However, Cav1.2 and Cav1.3 are down regulated in rat DRG neurons following chronic constriction injury of the sciatic nerve (Kim et al., 2001). It has been reported that knocking down Cav1.2 with intrathecal anti-sense or small interfering RNA can lead to a reversal of dorsal horn hyperexcitability and pain states in the SNL model, suggesting that injury-induced Cav1.2 upregulation may contribute to the maintenance of chronic pain states (Fossat et al., 2010). These findings are consistent with our findings, but contradict with findings showing that intrathecal L-type VGCC blockers (verapamil, diltiazem, and nimodipine) are not effective in blocking neuropathic pain states in models of diabetic, and vincristine-induced neuropathies (Calcutt and Chaplan 1997; Fukuizumi et al., 2003a) or SNL (Chaplan et al., 1994). These discrepancies may be due to differences in Cav1 subtype specific regulation among rat models and in species sensitivity of L-type VGCC blockers between rat and mouse models. In addition, various other inflammatory mediators at peripheral sites post-injury may elicit different patterns of excitation of phenotypically heterogeneous sensory neurons (Shibata et al., 1989) thus contributing to these discrepancies (Kato et al., 2002).

Our data indicates that P/Q-type VGCC appear to play a less critical role in modulating neuropathic pain states in the SNL model. Highest dose treatments of ω-agatoxin or ω-conotoxin MVIIC, when compared with the pre-treatment level, did not inhibit pain states in the SNL model significantly. For the TG model, ω-agatoxin produced dose-dependent pain state reversal, but ω-conotoxin MVIIC failed to do so even at a much higher dose. These suggest that P-type VGCC may contribute to sensory processing in the TG, but not SNL model, while the contribution from Q-type VGCC is minimum in both models.

This is consistent with the current literature showing that blocking spinal P/Q-type VGCC has no effect on mechanical allodynia and thermal hyperalgesia in chronic pain models of peripheral neuropathies (Chaplan et al., 1994; Yamamoto and Sakashita 1998). P/Q-type VGCC are expressed at the pre-synaptic terminals of afferents in laminae II–VI of spinal dorsal horn (Westenbroek et al., 1998). They are less likely involved in release of pain-inducing peptides, such as substance P and CGRP, from primary afferents since they show little colocalization with pain-inducing peptides, and ω-agatoxin treatment does not affect the release of these peptides from dorsal horn neurons (Evans et al., 1996; Westenbroek et al., 1998). However, P/Q-type VGCC are likely participate in release of both excitatory and inhibitory neurotransmitters (Araque et al., 1994; Takahashi and Momiyama 1993) from dorsal horn interneurons. It has been reported that ω-agatoxin has a strong effect on polysynaptic nociceptive transmission, but a minimal effect on monosynaptic inputs from nociceptive C- and Aδ-fibers (Heinke et al., 2004), suggesting that P/Q-type VGCC are likely involved in the modulation of synaptic transmission among spinal cord interneurons (Araque et al., 1994; Heinke et al., 2004; McCallum et al., 2011; Park and Luo 2010; Takahashi and Momiyama 1993). In addition, P/Q-type VGCC in the rostral ventromedial medulla have been shown to contribute to tactile allodynia via modulation of descending facilitatory systems (Porreca et al., 2002; Urban et al., 2005). Our data indicate that intrathecal ω-agatoxin at a dose blocking P-type VGCC can reverse behavioral hypersensitivity in the $Ca_v\alpha_2\delta_1$ TG mice. Thus, $Ca_v\alpha_2\delta_1$ overexpression may induce P-type VGCC function changes in this model, likely in spinal interneurons and/or descending facilitatory pathways, that may contribute to behavioral hypersensitivity. However, the role of P/Q VGCC in sensory transmission is not changed post SNL as demonstrated by findings from in vivo spinal neuron recording (Matthews and Dickenson 2001), thus ω-agatoxin is no effective in the SNL model.

The antinociceptive effects of VGCC blockers from our study support a state-dependent function of VGCC at the spinal cord level since the drugs are effective only after SNL or $Ca_v\alpha_2\delta_1$ overexpression. This is consistent with a similar role of N-type VGCC in long-term potentiation of rat spinal cord neurons (Ohnami et al., 2011), and changes in functional roles of N-type VGCC in spinal sensory transmission after nerve injury (Matthews and Dickenson 2001) that may contribute to behavioral hypersensitivity in animal models (Bowersox et al., 1996; Chaplan et al., 1994; Jayamanne et al., 2013; Vanegas and Schaible 2000). Our findings support that different VGCC may play distinct roles in behavioral hypersensitivity mediated by $Ca_v\alpha_2\delta_1$ dysregulation. Targeting selective VGCC for pain management, even at the spinal level, is often associated with severe side effects due to widely distribution and various functional roles of VGCC (Penn and Paice 2000). However, SNL-induced $Ca_v\alpha_2\delta_1$ upregulation at the pre-synaptic terminals of sensory fibers could be one form of plasticity that potentiates the inhibitory effects of low dose of N-type VGCC blockers on central sensitization (Matthews and Dickenson 2001), which could contribute to the anti-hyperalgesic effects of low doses of N-type VGCC toxin demonstrated in our study. Interestingly, $Ca_v\alpha_2\delta_1$ has been shown to modulate the affinity and reversibility of selective ω-conotoxin binding to recombinant N-type VGCC in vitro (Brust et al., 1993; Mould et al., 2004). It seems that further investigations are necessary to confirm the effects of $Ca_v\alpha_2\delta_1$ upregulation on inhibitory effects of VGCC toxins in vivo. While the transient nature of the

anti-hyperalgesic effects of low dose toxin seems not suitable for therapeutic application, it maybe possible to improve the efficacy and/or duration of actions of selective VGCC toxins by blocking $Ca_v\alpha_2\delta_1$ with gabapentinoids in combination treatment of pain states. Further investigation regarding the beneficial effects of such combination therapy will help to explore the potential of this alternative approach in pain management.

Acknowledgments

Funding Source: Supported in part by NIH grants: NS064341 and DE021847 (Z.D. Luo). E.Y. Chang is the recipient of Rehabilitation Medicine Scientist Training Program fellowship (K12 HD001097) and Institute for Clinical and Translational Science (UL1 TR000153) faculty career development fellowship (KL-2).

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What's already known about this topic?

- It is known that increased voltage gated calcium channel (VGCC) alpha-2-delta-1 subunit protein in either a transgenic neuronal overexpression model or a spinal nerve ligation model leads to behavioral hypersensitivities. It is not known whether spinal N-, L-, and P/Q-type VGCC contribute to the behavioral hypersensitivity modulated specifically by this plasticity.

What does this study add?

- Findings from this study support that different-type of VGCC have differential contributions to the behavioral hypersensitivity modulated by alpha-2-delta-1 dysregulation at the spinal cord level.

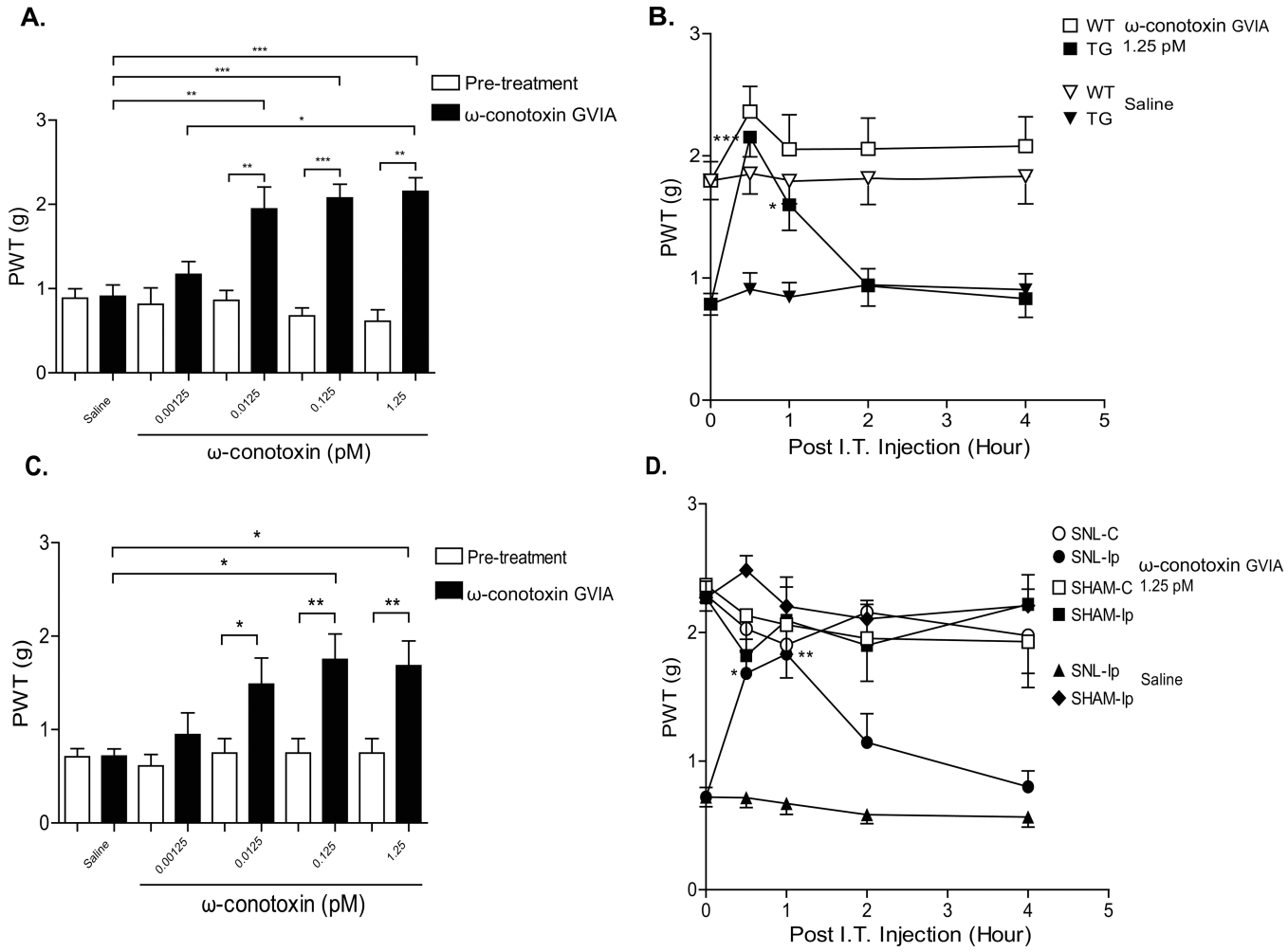

Figure 1.

Intrathecal administration of ω-conotoxin reversed tactile allodynia similarly in $Ca_V\alpha_2\delta_1$ transgenic (TG) and unilateral spinal nerve ligation (SNL) mice. Hindpaw withdrawal thresholds (PWT) to mechanical von Frey filament stimulation were tested prior to and after i.t. ω-conotoxin treatments in injury-free $Ca_V\alpha_2\delta_1$ TG or WT mice, and SNL or sham mice at least 1 week post-injury. (A) PWT in TG mice after dose-dependent treatments with ω-conotoxin or saline for 30 min. (B) Time-dependent effects of 1.25 pM ω-conotoxin GVIA or saline on PWT in TG and WT mice. (C) PWT in SNL mice after dose-dependent treatments with ω-conotoxin GVIA or saline for 30 min. (D) Time-dependent effects of 1.25 pM ω-conotoxin GVIA or saline in SNL or sham mice. Ip, ipsilateral to the injury; C, contralateral to the injury. Data presented are the means ± SEM from 7–11 mice for the ω-conotoxin treated groups, 17–19 mice for the saline treated groups. *p<0.05, **p<0.01, ***p<0.001 by Wilcoxon signed rank test for pair-wise comparisons or by Kruskal-Wallis Test with Dunn's post-test compared with the pre-treatment level.

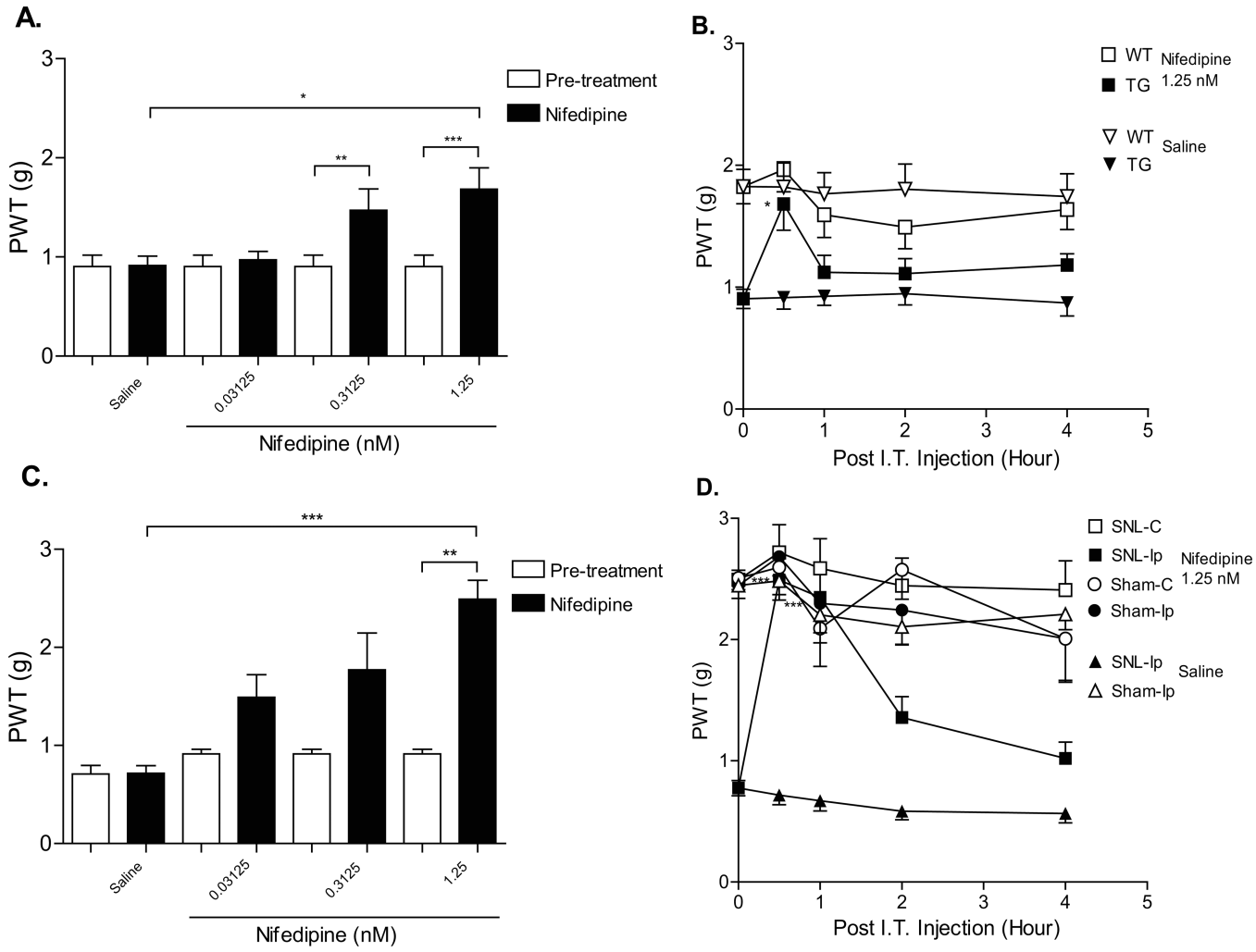

Figure 2.

Intrathecal administration of nifedipine reversed tactile allodynia similarly in $Ca_v\alpha_2\delta_1$ transgenic (TG) and unilateral spinal nerve ligation (SNL) mice. Hindpaw withdrawal thresholds (PWT) to mechanical von Frey filament stimulation were tested prior to and after i.t. nifedipine treatments in injury-free $Ca_v\alpha_2\delta_1$ TG or WT mice, and unilateral SNL or sham mice at least 1 week post-injury. (A) PWT in TG mice after dose-dependent treatments with nifedipine or saline for 30 min. (B) Time-dependent effects of 1.25 nM nifedipine or saline on PWT in TG and WT mice. (C) PWT in SNL mice after dose-dependent treatments of nifedipine or saline for 30 min. (D) Time-dependent effects of 1.25 nM nifedipine or saline in SNL or sham mice. Ip, ipsilateral to the injury; C, contralateral to the injury. Data presented are the means ± SEM from 9–12 mice for the nifedipine treated groups, 19 mice for the saline treated groups. *p<0.05, **p<0.01, ***p<0.001 by Wilcoxon signed rank test for pair-wise comparisons or by Kruskal-Wallis Test with Dunn's post-test compared with the pre-treatment level.

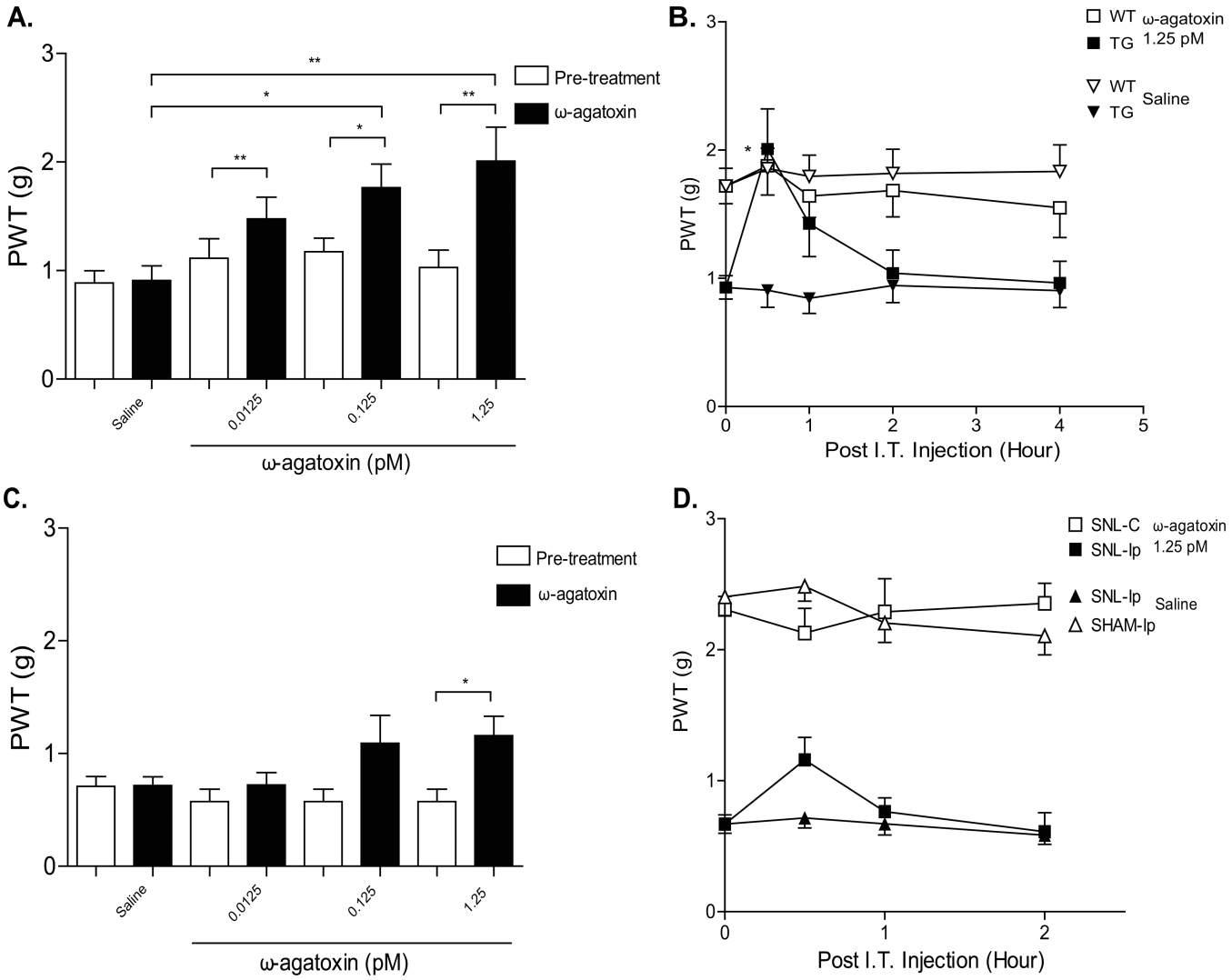

Figure 3.

Intrathecal administration of ω-agatoxin IVA reversed tactile allodynia in $Ca_v\alpha_2\delta_1$ transgenic (TG), but not in the unilateral spinal nerve ligation (SNL) mice. Hindpaw withdrawal thresholds (PWT) to von Frey filament stimulation were tested prior to and after i.t. ω-agatoxin IVA treatments in injury-free $Ca_v\alpha_2\delta_1$ TG mice or WT mice, and SNL or sham mice at least 1 week post-injury. (A) PWT in TG mice after dose-dependent treatments with ω-agatoxin IVA or saline for 30 min. (B) Time-dependent effects of 1.25 pM ω-agatoxin IVA or saline on PWT in TG and WT mice. (C) PWT in SNL mice after dose-dependent treatments with ω-agatoxin IVA or saline for 30 min. (D) Time-dependent effects of 1.25 pM ω-agatoxin IVA or saline in SNL or sham mice. Ip, ipsilateral to the injury; C, contralateral to the injury. Data presented are the means ± SEM from 8 mice for the ω-agatoxin treated groups, 17–19 mice for the saline treated groups. *p<0.05, **p<0.01, ***p<0.001 by Wilcoxon signed rank test for pair-wise comparisons or by Kruskal-Wallis Test with Dunn's post-test compared with the pre-treatment level.

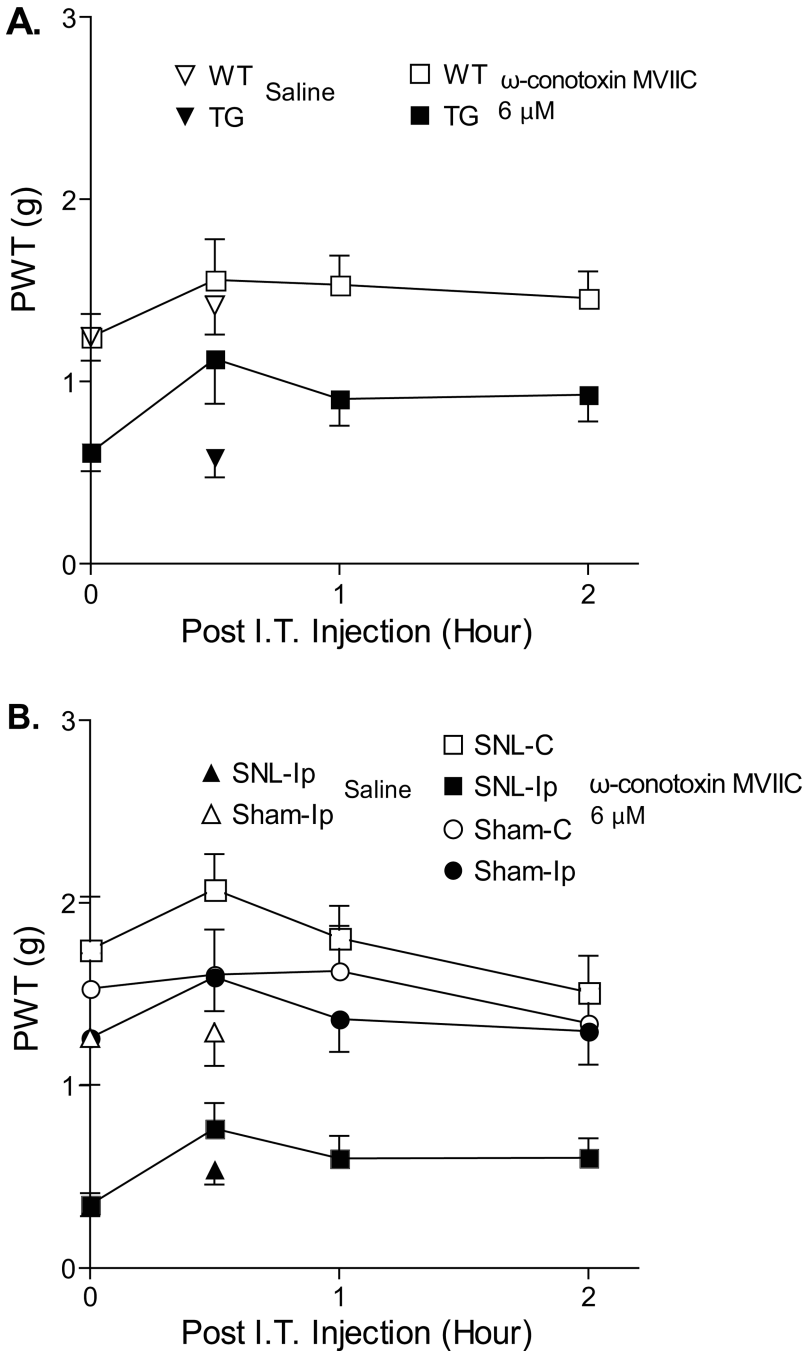

Figure 4.

Intrathecal administration of ω-conotoxin MVIIC did not reverse tactile allodynia in $Ca_v\alpha_2\delta_1$ transgenic (TG) nor in unilateral spinal nerve ligation (SNL) mice. Hindpaw withdrawal thresholds (PWT) to von Frey filament stimulation were tested prior to and after i.t. ω-conotoxin MVIIC treatments in injury-free $Ca_v\alpha_2\delta_1$ TG mice or WT mice, and SNL or sham mice at least 1 week post-injury. (A) Time-dependent effects of 6 μM ω-conotoxin MVIIC or saline on PWT in TG and WT mice. (B) Time-dependent effects of 6 μM ω-conotoxin MVIIC or saline in SNL or sham mice. Ip, ipsilateral to the injury; C,

contralateral to the injury. Data presented are the means ± SEM from 8–10 mice for the ω-conotoxin MVIIC treated groups, 8–12 mice for the saline treated groups. *p<0.05, **p<0.01, ***p<0.001 by Kruskal-Wallis Test with Dunn's post-test compared with the pre-treatment level.