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Spinal 5-HT₃ receptors facilitate behavioral hypersensitivity induced by elevated calcium channel alpha-2-delta-1 protein

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Abstract

Background—Peripheral nerve injury induces upregulation of the calcium channel alpha-2-delta-1 proteins in the dorsal root ganglia and dorsal spinal cord that correlates with neuropathic pain development. Similar behavioral hypersensitivity was also observed in injury-free transgenic mice (TG) over-expressing the alpha-2-delta-1 proteins in neuronal tissues. To investigate pathways regulating alpha-2-delta-1 protein-mediated behavioral hypersensitivity, we examined whether spinal serotonergic 5-HT₃ receptors are involved similarly in the modulation of behavioral hypersensitivity induced by either peripheral nerve injury in a nerve injury model or neuronal alpha-2-delta-1 over-expression in the TG model.

Methods—The effects of blocking behavioral hypersensitivity in these two models by intrathecal or systemic injections of 5-HT₃ receptor antagonist, ondansetron, were compared.

Results—Our data indicated that the TG mice displayed similar behavioral hypersensitivities to non-painful mechanical stimulation (tactile allodynia) and painful thermal stimulation (thermal hyperalgesia) as that observed in the nerve injury model. Interestingly, tactile allodynia and thermal hyperalgesia in both models can be blocked similarly by intrathecal, but not systemic, injection of ondansetron.

Conclusions—Our data suggest that spinal 5-HT₃ receptors are likely play a role in alpha-2-delta-1-mediated behavioral hypersensitivities through a descending serotonergic facilitation.

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Introduction

The development of efficacious and safe medications for neuropathic pain management relies on our better understanding of neuropathic pain mechanisms. Data from previous studies have indicated that peripheral nerve injury causes upregulation of the calcium channel $\alpha_2\delta_1$ subunit proteins ($\text{Ca}_v\alpha_2\delta_1$) in dorsal root ganglia and dorsal spinal cord that plays a critical role in neuropathic pain development (Boroujerdi et al., 2008; Li et al., 2004; Li et al., 2006; Luo et al., 2002; Luo et al., 2001; Newton et al., 2001; Nguyen et al., 2009). It has been reported that a descending serotonergic modulatory pathway facilitates spinal nerve injury induced behavioral hypersensitivity through 5-HT₃ receptors in dorsal spinal cord (Bee and Dickenson, 2008; Suzuki et al., 2004; Zhuo and Gebhart, 1991). Interestingly, activation of descending 5-HT₃ facilitatory drive is required for the efficacy of pregabalin, an anti-neuropathic pain drug that binds to $\text{Ca}_v\alpha_2\delta_1$ (Gee et al., 1996), in a neuropathic pain model (Bee and Dickenson, 2008), suggesting that injury-induced dysregulation of $\text{Ca}_v\alpha_2\delta_1$ may mediate neuropathic pain states through a 5-HT₃ receptor dependent pathway. Since nerve injury also leads to dysregulation of a multitude of genes (Costigan et al., 2002; Kim et al., 2009; Valder et al., 2003; Wang et al., 2002), it is not clear if $\text{Ca}_v\alpha_2\delta_1$ upregulation is an essential and necessary factor in this neuropathic pain pathway. Therefore, we have generated a $\text{Ca}_v\alpha_2\delta_1$ overexpressing transgenic mouse line in which $\text{Ca}_v\alpha_2\delta_1$ gene overexpression in neuronal tissue alone is sufficient to drive dorsal horn neuron sensitization and behavioral hypersensitivities, mimicking that observed in spinal nerve injured neuropathic pain models (Li et al., 2006; Nguyen et al., 2009). To determine if $\text{Ca}_v\alpha_2\delta_1$ -mediated behavioral hypersensitivities in the transgenic model involve a 5-HT₃ receptor-dependent facilitatory pathway similar to that in the spinal nerve ligation neuropathic pain model (Bee and Dickenson, 2008; Suzuki et al., 2004), we compared the effects of a 5-HT₃ receptor antagonist, ondansetron, in blocking behavioral hypersensitivities observed in the $\text{Ca}_v\alpha_2\delta_1$ transgenic mouse model and unilateral spinal nerve ligation injury (SNL) mouse model.

Methods

Transgenic mice

$\text{Ca}_v\alpha_2\delta_1$ over-expressing transgenic mice (TG) were generated as described previously (Li et al., 2006). These mice were fertile, had normal growth rate, grooming, social interactions and showed no signs of 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.

Neuropathic lesions

The unilateral spinal nerve ligation (SNL) surgery was performed as described (Kim and Chung, 1992). Briefly, under isoflurane anesthesia, the mouse left L4 spinal nerve (Rigaud et al., 2008) were exposed and a tight ligation with a silk suture was made between the DRG and the conjunction where spinal nerves form the sciatic nerve. In sham operations, the same procedure was performed except that L4 spinal nerve was not ligated.

All animal care and experimental procedures were performed based on protocols approved by the Institutional Animal Care Committee of the University of California Irvine.

Drug injection

Ondansetron was dissolved in sterile saline, and injected either intraperitoneally (300 μL /mouse) or intrathecally (5 μL /mouse) between lumbar regions 4–5 (Nguyen et al., 2009). In the case of repetitive drug injections into the same group of animals, at least a drug-free

period of one day was introduced after the previous drug effect, if any, had completely dissipated (which occurred within a 3 hr post injection period).

Behavioral test

Hindpaw sensitivities to mechanical and thermal stimuli were tested blindly as described before and post drug treatments (Li et al., 2006).

Mechanical sensitivity—After acclimation for 1 hr in a clear plastic cage with a wire mesh bottom, the mice were tested for 50% paw withdrawal thresholds (PWT) to von Frey filament (Stoelting, Wood Dale, IL) stimulation using a modified up-down method (Dixon, 1980). Briefly, a set of filaments (starting with one that has a buckling weight of 0.41 g) was perpendicularly applied to the hindpaw plantar surface, in a consecutive order, with a slightly bending force. 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 paw lifting after 5 s was considered a negative response and led to the use of the next filament with increasing weight. The scores of six measurements, starting from the one prior to the first change in response, were used to calculate the 50% paw withdrawal thresholds as described (Li et al., 2004; Luo et al., 2002; Luo et al., 2001). In the case that four consecutive positive or three consecutive negative responses had occurred, a score of 0.01 g or 3 g, respectively, was assigned. Paw withdrawal thresholds from each hindpaw were recorded separately. Data from the injured and uninjured side in the sham or SNL groups or averaged from both hindpaws of the injury-free $Ca_v\alpha_2\delta_1$ TG and WT mice were used for comparing behavioral sensitivities between the injury and noninjury side in SNL or sham mice or between injury-free TG and WT mice, respectively, before or after systemic and intrathecal drug treatments.

Hot box test—Mouse hindpaw withdrawal latencies (PWL) to thermal stimuli were examined in a modified Hargreaves-type thermal testing device (Hargreaves et al., 1988). Briefly, after acclimation for at least 30 min in individual boxes on the glass surface of the hot box maintained at 30 °C, mouse planter surface of the hindpaw was aligned to a radiant light source underneath the glass surface. A timer was activated when the light source was turned on, and turned off when paw withdrawal from the light source occurred or at 20 s of light stimulation that turned off the light bulb. Paw withdrawal latencies from each hindpaw were recorded separately. Data from the injured and uninjured side in the sham or SNL groups or averaged from both hindpaws of the injury-free $Ca_v\alpha_2\delta_1$ TG and WT mice were used for comparing behavioral sensitivities between the injury and noninjury side in SNL or sham mice or between injury-free TG and WT mice, respectively, before or after systemic and intrathecal drug treatments.

Statistic analysis

Significant changes were determined by the two-way ANOVA followed by Bonferroni post-test analysis. A p value < 0.05 was considered statistically significant.

Results

Intrathecal, but not intraperitoneal, treatment with 5-HT₃ receptor antagonist reversed tactile allodynia in SNL and TG mice

As shown in Fig. 1, over-expression of $Ca_v\alpha_2\delta_1$ in neuronal cells of the TG mice induced a similar level of reduction in paw withdrawal thresholds to innocuous mechanical stimulation (tactile allodynia) as that in the injury side of the unilateral SNL injury model (before drug treatments), suggesting that elevated level of $Ca_v\alpha_2\delta_1$ that occurs in both models may play a similar role in mediating tactile allodynia. Since a spinal 5-HT₃ receptor mediated pathway is involved in tactile allodynia processing in the SNL model (Dogrul et al., 2009), we

studied if this pathway is modulated by increased spinal $\text{Ca}_v\alpha_2\delta_1$ in the SNL model by comparing whether intrathecal treatment with ondansetron can affect behavioral hypersensitivities similarly in both the SNL and $\text{Ca}_v\alpha_2\delta_1$ TG mouse models. As shown in Fig. 1A, intrathecal ondansetron could block $\text{Ca}_v\alpha_2\delta_1$ induced tactile allodynia in a dose-dependent and reversible manner in the TG mice compared with that in the age- and sex-matched WT littermates. A complete reversal of allodynia was achieved by 10 $\mu\text{g}/\text{mouse}$, which is consistent with the reported effective dose in blocking central sensitization (Rahman et al., 2004; Suzuki et al., 2004) and behavioral hypersensitivity (Green et al., 2000) in animal models. The anti-allodynic effects of 10 $\mu\text{g}/\text{mouse}$ intrathecal ondansetron were fast-in onset, peaked at 30 min post injection, and lasted for approximately two hrs. In contrast, a similar intrathecal injection with 0.1 $\mu\text{g}/\text{mouse}$ ondansetron or sterile saline was without effect, and the anti-allodynic effects of 1.0 $\mu\text{g}/\text{mouse}$ ondansetron was between that of 0.1 $\mu\text{g}/\text{mouse}$ and 10 $\mu\text{g}/\text{mouse}$. As indicated in Fig. 1B, similar dose- and time-dependent tactile allodynia reversals by intrathecal injection of ondansetron were observed in the SNL model. Baseline behavioral sensitivity in the WT mice and non-injury side of the SNL mice was not affected by the highest dose of ondansetron, nor saline. As shown in Fig. 2, intraperitoneal injections with the effective intrathecal dose, 10 $\mu\text{g}/\text{mouse}$, of ondansetron in the $\text{Ca}_v\alpha_2\delta_1$ TG (Fig. 2A) and SNL (Fig. 2B) mice failed to reverse tactile allodynia significantly in these models. Similar negative effects in allodynia reversal were observed in $\text{Ca}_v\alpha_2\delta_1$ TG mice injected with a three-times higher intraperitoneal ondansetron dose (30 $\mu\text{g}/\text{mouse}$, data not shown), suggesting that the negative effect was not likely due to insufficient systemic dosing.

Intrathecal, but not intraperitoneal, treatment with 5-HT₃ receptor antagonist reversed thermal hyperalgesia in SNL and TG mice

Over-expression of neuronal $\text{Ca}_v\alpha_2\delta_1$ in the TG mice also induced a reduced hindpaw withdrawal latency to thermal stimuli (thermal hyperalgesia) (Fig. 3A) similar to that in the injury side of the SNL, but not sham, injury model (Fig. 3B), suggesting that elevated level of $\text{Ca}_v\alpha_2\delta_1$ that occurs in both models may play a critical role in mediating thermal hyperalgesia. To determine whether a similar spinal 5-HT₃ receptor mediated pathway is also involved in thermal hyperalgesia processing in these models, we examined if treatment with ondansetron could reverse thermal hyperalgesia similarly in both models. As shown in Fig. 3A, intrathecal ondansetron at the dose (10 $\mu\text{g}/\text{mouse}$) that was effective in reversing tactile allodynia, could also reverse thermal hyperalgesia in the TG mice without affecting the baseline sensitivity in age- and sex-matched WT littermates. Similar intrathecal injection with sterile saline was without effects (data not shown). As indicated in Fig. 3B, a similar intrathecal ondansetron treatment in the SNL model led to a similar thermal hyperalgesia reversal without affecting the baseline behavioral sensitivity in the sham control or non-injury side of SNL mice. In contrast, intraperitoneal injections with the same dose of ondansetron in the $\text{Ca}_v\alpha_2\delta_1$ TG (Fig. 4A) or the SNL (Fig. 4B) mice failed to significantly reverse thermal hyperalgesia.

Discussion

While spinal cord 5-HT₃ receptors play a critical role in facilitating tactile allodynia in the SNL model (Bee and Dickenson, 2008; Dogrul et al., 2009; Leong et al., 2011; Suzuki et al., 2004), the detail mechanism underlying this pathway in neuropathic pain processing is not clear. It has been shown that pregabalin, a drug that binds to the $\text{Ca}_v\alpha_2\delta_1$ proteins, can block the facilitation mediated by spinal 5-HT₃ receptors (Bee and Dickenson, 2008), suggesting that $\text{Ca}_v\alpha_2\delta_1$ is involved in the regulation of this facilitation pathway. However, this has not been directly proven. Since nerve injury can also induce dysregulation of other genes (Kim et al., 2009; Valder et al., 2003; Wang et al., 2002), the involvement of $\text{Ca}_v\alpha_2\delta_1$ in this

facilitation pathway could be indirect. We tested this hypothesis by comparing data from the SNL neuropathic pain model with that from injury-free $\text{Ca}_v\alpha_2\delta_1$ overexpressing TG mice, which also display similar behavioral hypersensitivity as the SNL model but without any influence from other injury factors (Li et al., 2006). Our data have indicated that intrathecal, but not systemic, administration of the 5-HT₃ receptor antagonist - ondansetron results in a similar dose- and time-dependent reversal of behavioral hypersensitivities in both models, supporting that $\text{Ca}_v\alpha_2\delta_1$ protein, which is a common factor elevated in both models, plays a critical role in 5-HT₃ receptor mediated facilitation in neuropathic pain processing at the spinal cord level.

In contrast to other serotonin receptor subtypes that are G-protein-coupled, 5-HT₃ receptors are excitatory ionotropic receptors that enhance neurotransmitter release from spinal dorsal horn neurons due to activation of the descending facilitatory serotonergic pathway from the rostral ventromedial medulla (RVM) (Farber et al., 2004; Suzuki et al., 2002). 5-HT₃ receptors have been found on terminals of glutamate-releasing myelinated primary afferent fibers, excitatory interneurons, and NK1 receptor expressing projection neurons in lamina I/III (Conte et al., 2005; Kidd et al., 1993; Zeitz et al., 2002). It has been a controversial issue regarding whether serotonergic descending fibers from brain stem are inhibitory or facilitatory in modulating pain processing at the spinal cord level. Originally, it has been proposed that the serotonergic descending modulatory pathway is inhibitory (Basbaum and Fields, 1984) until the discovery of facilitation activity of the serotonergic descending pathway in pain processing (Suzuki et al., 2002; Zhuo and Gebhart, 1991). Findings from Leong et al (2011) recently shed some light on this issue by demonstrating that peripheral nerve injury (SNL) leads to loss of serotonergic inhibitory tone, most likely due to death of inhibitory serotonergic neurons in the RVM. However, the remaining serotonergic descending pathway becomes facilitatory. They concluded that injury-induced loss of brain stem inhibitory neurons shifts the balance of descending serotonergic modulation from inhibitory to facilitatory. Our data are consistent with their conclusions.

Since 5-HT₃ receptors are expressed in both central (Yakel and Jackson, 1988) and peripheral (Fozard, 1984) neurons, the lack of inhibitory effects after systemic ondansetron administration suggests that 5-HT₃ receptor-mediated facilitation is mainly at the spinal, but less likely at the supraspinal and peripheral levels. In addition, our data indicate that intrathecal ondansetron treatments in the WT and sham SNL mice do not affect significantly behavioral sensitivities to mechanical and thermal stimuli in these animals. This is consistent with findings from spinal cord recordings in normal animals (Green et al., 2000; Rahman et al., 2004), supporting a state-dependency of the drug action. Together, these data suggest that there is minimal 5-HT₃ receptor mediated facilitation at the spinal cord level in the absence of injury-induced plasticity changes as that seen in the SNL model, or of elevated $\text{Ca}_v\alpha_2\delta_1$ protein level as that seen in the TG mouse model, respectively. However, under certain pain-inducing conditions, such as peripheral nerve injury and/or elevated $\text{Ca}_v\alpha_2\delta_1$ levels in the sensory pathway, 5-HT₃ receptor-mediated facilitation at the spinal cord level is activated.

It is not clear why this 5-HT₃ receptor-mediated facilitatory pathway was not detectable in the rat SNL model treated with up to 100 $\mu\text{g}/\text{rat}$ of intrathecal ondansetron in a recent study (Peters et al., 2010). Overall species differences may not be the major factor since ondansetron sensitive, 5-HT₃ receptor mediated facilitation at the spinal cord level of the same rat model was detectable with a lower dose of intrathecal ondansetron in another study (Dogrul et al., 2009). In this study with mouse models, we observed complete reversal of tactile allodynia and thermal hyperalgesia with intrathecal ondansetron at a dose higher than that used in the latter study, but similar to the maximal dose used in the former study. The onset time of reversal in behavioral hypersensitivity in our study is faster than that in the rat

SNL model (Dogrul et al., 2009). It is possible that, in addition to other potential factors, such as sources and/or strains of experimental animals, local environment, variations in surgical procedures, and behavioral testing, that may contribute to the differences (Chesler et al., 2002), species differences in pharmacokinetics and pharmacodynamics may also contribute to this discrepancy.

Neuropathic pain models that involve altered influence of descending serotonergic activity on spinal 5-HT₃ receptors may mimic conditions in human neuropathic pain states that respond to 5-HT₃ receptor blockers. However, a possible anti-nociceptive role of 5-HT₃ receptor blockers has not been consistently supported by clinical data. Compared to placebo, a bolus intravenous injection of ondansetron has been shown to alleviate the overall pain experience by neuropathic pain patients (McCleane et al., 2003). Another study in postoperative pain after laminectomy did not show any difference in the amount of analgesic use for break-through pain control between patients that used intravenous ondansetron or saline control (Derbent et al., 2005). In a randomized, double-blind, placebo-controlled study of 15 patients with neuropathic pain associated with peripheral neuropathy, intravenous ondansetron had no influence on the intensity of brush-evoked or spontaneous ongoing pain in these patients when compared with saline control (Tuveson et al., 2011). The inconsistency in the effects of ondansetron in pain relief may result from differences in administration routes of ondansetron, as supported by our findings that 5-HT₃ receptor mediated facilitation at the spinal level can be reversed by intrathecal, but not systemic, administration of ondansetron.

How does injury-induced Ca_vα₂δ₁ proteins at the spinal cord level contribute to 5-HT₃ receptor-mediated facilitation? Previous findings indicate that peripheral nerve injury leads to an increased Ca_vα₂δ₁ expression in dorsal root ganglion (DRG) sensory neurons that results in elevated Ca_vα₂δ₁ proteins at the pre-synaptic sensory fiber terminals in dorsal spinal cord (Bauer et al., 2009; Li et al., 2004). This neuroplasticity can cause spinal neuron sensitization and behavioral hypersensitivity through enhanced pre-synaptic excitatory neurotransmitter release at the spinal level (Zhou et al., 2012), similar to that mediated by neuronal Ca_vα₂δ₁ over-expression (Nguyen et al., 2009; Zhou et al., 2012). Since 5-HT₃ receptor mediated facilitatory input into dorsal spinal cord is required for the anti-neuropathic pain effects of gabapentin to occur in the SNL model, it has been proposed that spinal interactions of 5-HT₃ and Ca_vα₂δ₁ are permissive for the anti-neuropathic pain actions of gabapentin (Rahman et al., 2009; Suzuki et al., 2005).

Even though pharmacology data may not allow the exclusion of possible involvement from other pathways since gabapentin may have effects independent from its binding to the Ca_vα₂δ₁ protein (Taylor, 2009), our data however have demonstrated that 5-HT₃ receptor-mediated spinal facilitation also mediates similar behavioral hypersensitivities in the Ca_vα₂δ₁ TG mice, which also display similar spinal pre-synaptic hyperexcitability as the SNL mice (Nguyen et al., 2009; Zhou et al., 2012). Therefore, our data confirm a critical involvement of spinal Ca_vα₂δ₁ in the 5-HT₃ receptor-mediated serotonergic circuits regulating central sensitization, behavioral hypersensitivities and gabapentin efficacy in neuropathic pain states. It seems that in the absence of injury/inflammatory insults that could trigger spinal 5-HT₃ receptor activation (as in the case of injury-free Ca_vα₂δ₁ TG mice), increased pre-synaptic excitatory input by elevated Ca_vα₂δ₁ levels is sufficient to activate this spinal-supraspinal descending serotonergic facilitatory pathway, presumably through activation of spinal post-synaptic NK1 receptor expressing neurons (Suzuki et al., 2005). This in turn activates spinal 5-HT₃ receptors that facilitates central sensitization and behavioral hypersensitivities. As summarized in Fig. 5, these spinal-supraspinal serotonergic circuits are likely turned on constantly in the SNL model after the onset of Ca_vα₂δ₁ upregulation that requires days to complete (Li et al., 2004; Luo et al., 2001). Even though

SNL also leads to substantial down-regulation of 5-HT₃ receptor in injured DRG (Kim et al., 2009; Valder et al., 2003; Wang et al., 2002) but no change in spinal cord (Wang et al., 2002), this neuroplasticity may result in reduced 5-HT₃ receptor levels in affected afferent central terminals in spinal dorsal horn. Thus, Ca_vα₂δ₁ upregulation post peripheral nerve injury may drive central sensitization and neuropathic pain states in neuropathy models mainly through activation of 5-HT₃ receptors on excitatory interneurons and projection neurons by this serotonergic descending facilitation pathway.

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Bulleted Statements**What's already known about this topic?**

- It is known that either peripheral nerve injury or elevated calcium channel alpha-2-delta-1 protein expression leads to behavioral hypersensitivity, and descending activation of 5-HT₃ receptors in spinal cord facilitates injury-induced pain states.

What does this study add?

- Data from this study added that elevated alpha-2-delta-1 protein is a critical contributor to spinal 5-HT₃ receptor-facilitated pain states post peripheral nerve injury.

Figure 1. Intrathecal administration of 5-HT₃ receptor antagonist ondansetron reversed tactile allodynia similarly in Ca_vα₂δ₁ TG and SNL mice in a dose-dependent manner

Hindpaw withdrawal thresholds to von Frey filament stimulation were examined before and after intrathecal ondansetron treatments in injury-free Ca_vα₂δ₁ TG, WT mice, and SNL or sham mice at least one-week post injury. **A.** intrathecal treatments with saline or ondansetron doses as indicated in WT and Ca_vα₂δ₁ TG mice. **B.** intrathecal treatments with saline or ondansetron doses as indicated in sham and SNL mice. Ip - ipsilateral to the injury; C – contralateral to the injury. Data presented are the Means ± SEM from at least eight mice in each group. ** P < 0.01, *** P < 0.001 compared with the pre-treatment level by two-way ANOVA with Bonferroni post-test.

Figure 2. Systemic administration of 5-HT₃ receptor antagonist ondansetron failed to reverse tactile allodynia in Ca_vα₂δ₁ TG and SNL mice

Hindpaw withdrawal thresholds to von Frey filament stimulation were examined before and one hour after intraperitoneal ondansetron treatments in injury-free Ca_vα₂δ₁ TG, WT mice, and SNL or sham mice at least one-week post injury. **A.** intraperitoneal treatments with ondansetron (10 μg/mouse) in WT and Ca_vα₂δ₁ TG mice. **B.** intraperitoneal treatments with ondansetron (10 μg/mouse) in sham and SNL mice. Ip - ipsilateral to the injury; C - contralateral to the injury. Data presented are the Means ± SEM from at least seven mice in each group. ** P < 0.01, *** P < 0.001 by two-way ANOVA with Bonferroni post-test.

Figure 3. Intrathecal administration of 5-HT₃ receptor antagonist ondansetron reversed thermal hyperalgesia similarly in Ca_vα₂δ₁ TG and SNL mice

Hindpaw withdrawal latency to thermal stimulation was examined before and 30 min after intrathecal ondansetron treatments in injury-free Ca_vα₂δ₁ TG, WT mice, and SNL or sham mice at least one-week post injury. **A.** intrathecal treatments with (10 μg/mouse) or without ondansetron in WT and Ca_vα₂δ₁ TG mice. **B.** intrathecal treatments with (10 μg/mouse) or without ondansetron in sham and SNL mice. Ip - ipsilateral to the injury; C – contralateral to the injury. Data presented are the Means ± SEM from at least eight mice in each group. *** P < 0.001 by two-way ANOVA with Bonferroni post-test.

Figure 4. Systemic administration of 5-HT₃ receptor antagonist ondansetron failed to reverse thermal hyperalgesia in Ca_vα₂δ₁ TG and SNL mice

Hindpaw withdrawal latency to thermal stimulation was examined before and one hour after intraperitoneal ondansetron treatments in injury-free Ca_vα₂δ₁ TG, WT mice, and SNL or sham mice at least one-week post injury. **A.** intraperitoneal treatments with (10 μg/mouse) or without ondansetron in WT and Ca_vα₂δ₁ TG mice. **B.** intraperitoneal treatments with (10 μg/mouse) or without ondansetron in sham and SNL mice. Ip - ipsilateral to the injury; C - contralateral to the injury. Data presented are the Means ± SEM from at least seven mice in each group. * P < 0.05, ** P < 0.01, *** P < 0.001 by two-way ANOVA with Bonferroni post-test.

Peripheral nerve injury induced $\text{Ca}_v\alpha_2\delta_1$ upregulation in DRG leads to increased $\text{Ca}_v\alpha_2\delta_1$ translocation to the dorsal spinal cord pre-synaptic terminals through the central axons of sensory neurons. This results in increased pre-synaptic excitatory input to activate spinal post-synaptic NK1 receptor expressing neurons (Suzuki et al., 2005), leading to activation of spinal-supraspinal descending serotonergic facilitatory pathway located in RVM. This in turn can activate spinal 5-HT₃ receptors on primary afferent fiber terminals, excitatory interneurons, and NK1 receptor expressing projection neurons (Conte et al., 2005; Kidd et al., 1993; Zeitz et al., 2002). Since spinal nerve injury also leads to substantial 5-HT₃ receptor down-regulation in injured DRG (Kim et al., 2009; Valder et al., 2003; Wang et al., 2002) that may result in reduced levels of 5-HT₃ receptors and facilitatory effects in affected afferent central terminals. Thus, this facilitatory pathway may lead to central sensitization and behavioral hypersensitivity mainly through activation of spinal 5-HT₃ receptors on excitatory interneurons, and projection neurons.