

Sigma-1 receptor regulates p-PKC α and P2X₃ expression in dorsal root ganglia to attenuate neuropathic pain in rats

Shuang-shuang Liu^{1,2,3}, Xuan Yu⁴, Xue Li⁵, Qing-mei Li⁶, Qing-xiang Mao³, Jie Yuan^{1,2*}, Bang-yong Qin^{1*}

¹ Department of Pain Medicine, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China

² Guizhou Key Laboratory of Basic Research on Anesthesia and Organ Protection, Zunyi, Guizhou, China

³ Department of Anesthesiology, Daping Hospital, Army Medical University, Chongqing, China

⁴ Department of Anesthesiology, The Second Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China

⁵ Department of Anesthesiology, Hospital of Stomatology, Zunyi Medical University, Zunyi, Guizhou, China

⁶ Department of Anesthesiology, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, China

*Email: yuanjie@zmu.edu.cn; qbyzmczy@163.com

The present study aims to elucidate the role of the Sigma-1 receptor in the pathogenesis of neuropathic pain and evaluate its potential therapeutic implications. To systematically assess the effects of the Sigma-1 receptor, neuropathic pain was induced in rats using the chronic constriction injury (CCI) model. Subjects were subsequently divided into three groups: Sham, CCI, and CCI+BD1047 (where BD1047 is a Sigma-1 receptor antagonist). Following intrathecal administration of the respective agents, thermal withdrawal latency (TWL) and mechanical withdrawal threshold (MWT) were measured. Additionally, Western blotting was utilized to examine Sigma-1 receptor, phosphorylated protein kinase C α (p-PKC α), and P2X₃ receptor expression in the dorsal root ganglia (DRG). Immunofluorescence techniques were employed to examine p-PKC α and P2X₃ receptor expression. The results indicate a direct correlation between Sigma-1 receptor activity and pain perception, evidenced by changes in TWL and MWT. In the CCI group, both TWL and MWT were significantly reduced compared to the Sham group. Furthermore, protein levels of the Sigma-1 receptor, p-PKC α , and P2X₃ receptor in the DRG were elevated, and immunofluorescence expression of p-PKC α and the P2X₃ receptor also increased. Conversely, in the CCI+BD1047 group, TWL and MWT were significantly enhanced. Additionally, protein levels of the Sigma-1 receptor, p-PKC α , and P2X₃ receptor in the DRG decreased, along with reduced immunofluorescence expression of p-PKC α and P2X₃ receptor. The findings indicate that neuropathic pain is intricately associated with the Sigma-1 receptor, p-PKC α , and P2X₃ receptor in the dorsal root ganglia. Notably, the Sigma-1 receptor regulates the expression of p-PKC α and P2X₃ receptor, presenting a novel therapeutic target for neuropathic pain management.

Key words: neuropathic pain, dorsal root ganglia, Sigma-1 receptor, p-PKC α , P2X₃ receptor

INTRODUCTION

Neuropathic pain (NP) is a chronic pain condition resulting from damage or disease affecting the somato-sensory nervous system. It is characterized by an unpleasant sensory and emotional experience associated with actual or potential tissue injury (Raja et al., 2020). NP is highly prevalent, with an estimated incidence of

7% to 10% in the general population (Smith et al., 2020). The dorsal root ganglia (DRG), located along the spinal cord, contain the cell bodies of sensory neurons and other neuron types, playing a crucial role in transmitting neuropathic pain signals. The occurrence and progression of neuropathic pain are closely linked to nerve injury, inflammation, and altered molecular expression within the DRG (Esposito et al., 2019).

Specifically, abnormal expression of the Sigma-1 receptor, phosphorylated protein kinase α (p-PKC α), and P2X₃ receptors enhances pain signaling. Understanding the molecular mechanisms underlying neuropathic pain, particularly the role of specific receptors, is essential for developing effective treatments.

The Sigma-1 receptor is an endoplasmic reticulum membrane protein that functions as a molecular chaperone and is widely distributed throughout the central and peripheral nervous systems. It has a molecular mass ranging from 25 to 30 kDa and comprises 223 amino acids. In recent years, the Sigma-1 receptor has garnered attention for its potential role in modulating pain across various models, including neuropathic pain. Under conditions of cellular stress, characterized by elevated intracellular calcium ions, the Sigma-1 receptor becomes activated and translocates to the plasma membrane, where it physically interacts with various membrane proteins. Furthermore, Sigma-1 receptor activation is associated with changes in ion channel activity, monoamine neurotransmitter levels, and the functioning of opioid receptor G proteins. This includes modulation of transient receptor potential vanilloid 1 (TRPV1), N-methyl-D-aspartate (NMDA) receptors, and adrenergic receptors, which may contribute to increased pain perception (Entrena et al., 2009; Alvarez-Perez et al., 2022).

The P2X₃ receptor is the most significant contributor to chronic constriction injury (CCI)-induced neuropathic pain among all ion channels (Reinhold et al., 2015). Tissue injury results in the release of ATP from various cells, which activates P2X₃ receptors at the damaged ends of DRG neurons (Paukert et al., 2001). Studies indicate that P2X₃ receptors enhance pain signaling through ATP activation, promoting central sensitization and interacting with signaling pathways such as protein kinase C (PKC) (Gu et al., 2016). Various PKC isoforms, including PKC γ , PKC ϵ , PKC α , and PKC δ , play crucial roles in the regulation of neuropathic pain (Hirai & Chida, 2003; Gu et al., 2016; Wang et al., 2018; Sun et al., 2019). In unstimulated cells, PKC is primarily localized in an inactive form within the cytoplasm. Upon stimulation by antigens, inflammatory mediators, or phorbol esters, phospholipids in the cell membrane are hydrolyzed, producing diacylglycerol (DAG). The accumulation of DAG facilitates the translocation of PKC from the cytoplasm to the membrane, a key indicator of PKC activation. Once activated, PKC phosphorylates various substrate proteins, thereby regulating receptor desensitization, membrane events, gene transcription, immune responses, and cell growth (Wu et al., 2018).

Given the established role of the Sigma-1 receptor in pain modulation and the significance of p-PKC α and P2X₃ receptors in the DRG (Guzman-Lenis et al., 2009;

Gonzalez-Cano et al., 2020), this study hypothesizes that the Sigma-1 receptor alleviates neuropathic pain by modulating the expression of these targets. Furthermore, the study aims to advance understanding of the interplay between the Sigma-1 receptor and critical neuropathic pain signaling molecules, specifically p-PKC α and P2X₃ receptor.

To investigate this hypothesis, we employed a rat model of CCI of the sciatic nerve to induce neuropathic pain. Pain behavior was assessed by measuring thermal withdrawal latency (TWL) and mechanical withdrawal threshold (MWT) in the ipsilateral hind paw at various time points before and after surgery. Western blotting and immunofluorescence were utilized to evaluate Sigma-1 receptor, p-PKC α , and P2X₃ receptor expression in the DRG. The selective Sigma-1 receptor antagonist BD1047 was administered to examine its regulatory effects on p-PKC α and P2X₃ receptor expression, as this compound exhibits established anti-nociceptive effects across pain models. Behavioral experiments demonstrated that Sigma-1 receptor inhibition significantly reduced mechanical allodynia and thermal hyperalgesia in CCI rats. These findings indicate that the Sigma-1 receptor not only modulates pain but represents a therapeutic target for neuropathic pain through its regulatory effects on p-PKC α and P2X₃ receptors in the DRG.

METHODS

Ethical approval and animal preparation

All animals procedures were approved by the Experimental Animal Care and Use Committee of Zunyi Medical University (Approval No. 2020-032).

Forty healthy male Sprague-Dawley rats (7 weeks old, 180-200 g) were housed under controlled conditions (22-26°C, 12-h light/dark cycle) with *ad libitum* access to food and water. Experiments commenced following a 1-week acclimatization period.

Intrathecal catheterization and drug administration

Rats were anesthetized via intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg) according to established methods. A 2-cm longitudinal skin incision was made at the L5-L6 level, followed by tissue dissection. A PE-10 catheter was inserted rostrally for 2 cm. Upon confirmation of cerebrospinal fluid outflow, the catheter was secured (Milligan et al., 1999). Twenty-four hours post-catheterization, motor function

was assessed; rats exhibiting motor deficits were excluded. Remaining rats received intrathecal lidocaine (2%, 10 μ l), which induced bilateral hindlimb paralysis within 30 s that resolved within 30 min, confirming catheter functionality. Successfully catheterized rats received daily intraperitoneal penicillin (200,000 U) for 3 days to prevent infection. The Sigma-1 receptor antagonist BD1047 (MedChemExpress, batch HY-16996A) was administered intrathecally using a 50- μ l Hamilton syringe connected to the catheter.

Experimental groups and treatments

Rats were stratified into five groups ($n=8$ /group) based on surgical intervention, treatment. Sham: Sham surgery + intrathecal saline (20 μ l, twice daily, postoperative days 1-14 (POD 1-14)); CCI7d: CCI + intrathecal saline (20 μ l, twice daily, POD 1-7); CCI14d: CCI + intrathecal saline (20 μ l, twice daily, POD 1-14); CCI+BD1047 7d: CCI + intrathecal BD1047 (100 nmol in 20 μ l saline, twice daily, POD 1-7); CCI+BD1047 14d: CCI + intrathecal BD1047 (100 nmol in 20 μ l saline, twice daily, POD 1-14). BD1047 (MedChemExpress, batch HY-16996A; 100 nmol dose) was selected based on established efficacy in neuropathic pain models (Choi et al., 2013), Sham and CCI control groups received volume-matched saline vehicle.

CCI model establishment

Rat CCI models were established 24 hours post-intrathecal catheterization using the Bennett and Xie (1988) method. Anesthesia was induced via intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg). The surgical site was shaved and disinfected. After incision, the right sciatic nerve was exposed and ligated at four points (1-mm intervals) with 4-0 chromic catgut. Ligation tightness was adjusted to elicit mild calf muscle twitching. Postoperatively, all ligated rats developed neuropathic pain indicators (ipsilateral lameness, lower limb flexion), confirming successful model establishment. Sham group rats underwent blunt dissection of the sciatic nerve through the biceps femoris muscle without ligation prior to wound closure.

Pain threshold assessment

TWL and MWT was assessed 30 min postintrathecal injection at baseline (presurgery) and on POD 1, 3, 7, 10, and 14. For TWL, rats were individually placed in transparent glass chambers. A radiant heat source was

directed at the mid-plantar surface of the ipsilateral hind paw. TWL was recorded over five trials at 5-min intervals, with the mean value calculated. For MWT, rats were similarly placed in chambers with wire-mesh floors. Calibrated von Frey filaments were applied perpendicularly to the ipsilateral hind paw until withdrawal occurred. Five measurements at 5-min intervals were averaged to determine MWT.

Western blotting

Ipsilateral L4-L6 DRG were harvested on postoperative days 7 and 14. Tissues were homogenized in RIPA buffer, vortexed, and centrifuged at 12,000 $\times$ rpm for 15 min. Protein concentrations were determined by BCA assay. Equal amounts of protein (30 μ g/lane) were separated by 10% SDS-PAGE and transferred to PVDF membranes. After blocking with 5% non-fat milk for 2 h at 25°C, membranes were incubated overnight at 4°C with primary antibodies: rabbit anti-Sigma-1 (1:500, Affinity, DF7363), rabbit anti-p-PKC α (1:1000, Aifang, AF301054), and rabbit anti-P2X₃ (1:1000, Aifang, AF06653). Membranes were washed with TBST and incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:5000, Proteintech) for 1 h at 25°C. Protein bands were detected using ECL substrate and quantified with ImageJ (v1.53).

Immunofluorescence staining

Ipsilateral L4-L6 DRGs were harvested on postoperative days 7 and 14. Paraffin-embedded sections underwent antigen retrieval. After three 3-min PBST washes, slides were encircled with a hydrophobic barrier. Following three additional 5-min PBST washes, nonspecific sites were blocked with 3% bovine serum albumin (BSA) for 30 min. Sections were incubated overnight at 4°C with primary antibodies: rabbit anti-P2X₃ (1:1000, AiFang, AF06653) and rabbit anti-p-PKC α (1:100, AiFang, AF301054). After PBST washes, CY3 goat anti-rabbit secondary antibody (1:300, Servicebio, GB21303) was applied for 1 h in darkness. Slides were imaged using a Nikon upright fluorescence microscope (Nikon Eclipse C1).

Statistical analysis

Data were analyzed using GraphPad Prism (v8.0; GraphPad Software, USA). Results are expressed as mean $\pm$ standard deviation (SD). Intergroup differences were assessed by one-way or two-way ANOVA. Statistical significance was defined as $p<0.05$.

RESULTS

Behavioral Attenuation of Neuropathic Pain *via* Sigma-1 Receptor Antagonism in a CCI Rat Model

BD1047, a Sigma-1 receptor antagonist, significantly attenuated thermal and mechanical hypersensitivity in CCI rats, as demonstrated by increased TWL and MWT following intrathecal administration (Fig. 1A, B). Sham-operated rats exhibited no neuropathic pain behaviors after intrathecal injection, confirming that surgical procedures alone did not induce hypersensitivity. In contrast, CCI rats developed characteristic neuropathic pain behaviors (toe curling, licking, and back flexion) within 24 h post-surgery, without motor dysfunction or autonomic abnormalities. Compared to Sham controls, CCI rats showed significantly reduced TWL and MWT on postoperative days 1, 3, 7, 10, and 14 (Fig. 1A, B). BD1047 treatment significantly elevated TWL relative to CCI controls at all measured time points (Fig. 1A). BD1047 administration significantly increased MWT on days 3, 7, 10, and 14 (Fig. 1B).

Sigma-1 Receptor Antagonism Regulates Molecular Pathways in CCI-Induced Neuropathic Pain

We examined the molecular underpinnings of the observed behavioral changes. At the molecular level, CCI surgery induced robust upregulation of Sigma-1, p-PKC α , and P2X $_3$ proteins in the DRG of CCI rats (Fig. 2A). Specifically, on postoperative days 7 and 14, expression levels of these proteins in the L4-L6 DRGs were significantly elevated in CCI rats compared to the Sham group. Notably, intrathecal injection of BD1047 significantly attenuated this upregulation. In the CCI+BD1047 group, marked reductions in Sigma-1, p-PKC α , and P2X $_3$ expression were observed on postoperative days 7 and 14 relative to the CCI group (Fig. 2B, C, D).

To further validate our findings, immunofluorescence analysis was performed. This revealed that CCI surgery increased p-PKC α /P2X $_3$ co-localization in DRGs (Fig. 3). In the CCI group, p-PKC α (red) and P2X $_3$ (green) expression showed significant upregulation in L4-L6 DRGs on postoperative days 7 and 14 (Fig. 3A), with pronounced co-localization (Fig. 3B, C, D). Conversely, the CCI+BD1047 group exhibited marked reductions in

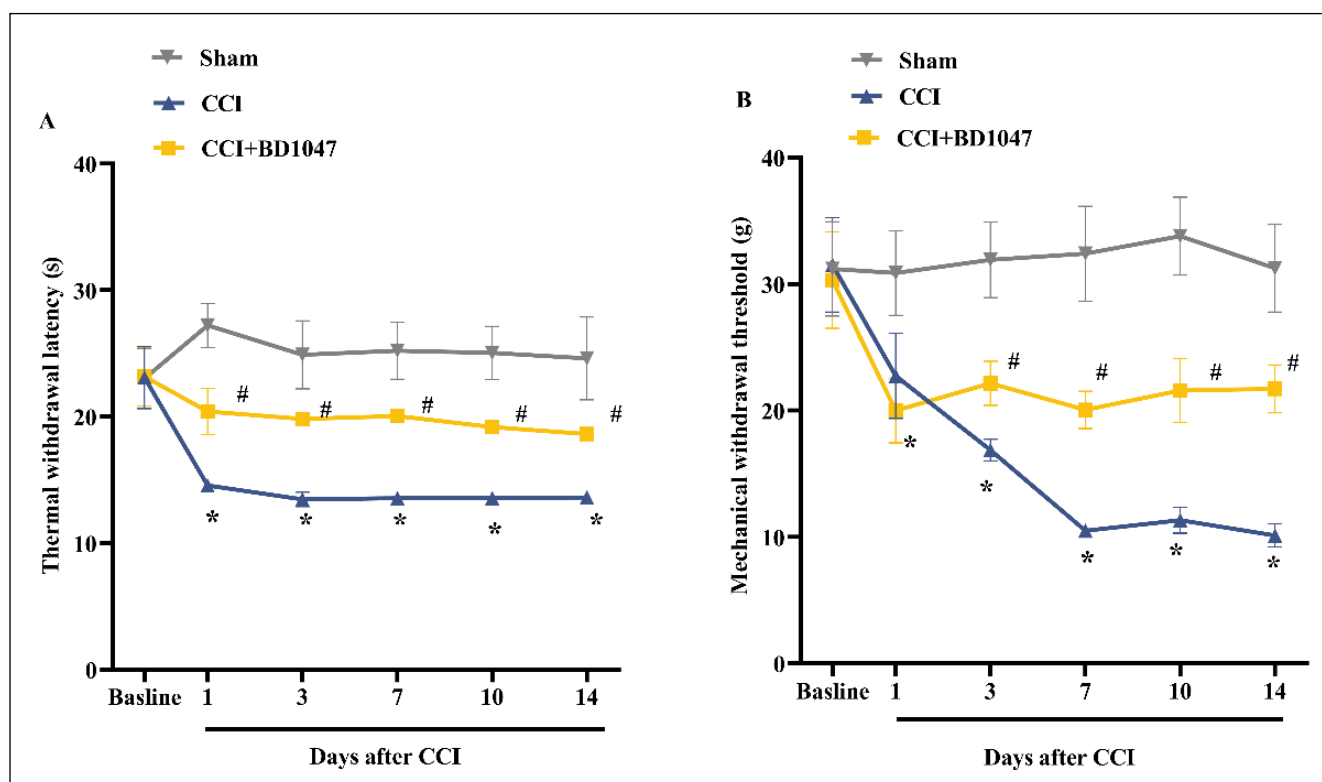


Fig. 1. Sigma-1 receptor antagonism attenuates CCI-induced hypersensitivity. Intrathecal BD1047 (100 nmol) significantly increased (A) thermal withdrawal latency (TWL) and (B) mechanical withdrawal threshold (MWT) in chronic constriction injury (CCI) rats *versus* saline-treated CCI controls. TWL effects observed at postoperative days 1, 3, 7, 10, and 14; MWT effects at days 3, 7, 10, and 14 (Day 1 excluded). Data = mean $\pm$ SD; *p < 0.05 vs. Sham group; #p < 0.05 vs. CCI group (two-way ANOVA, Tukey's test).

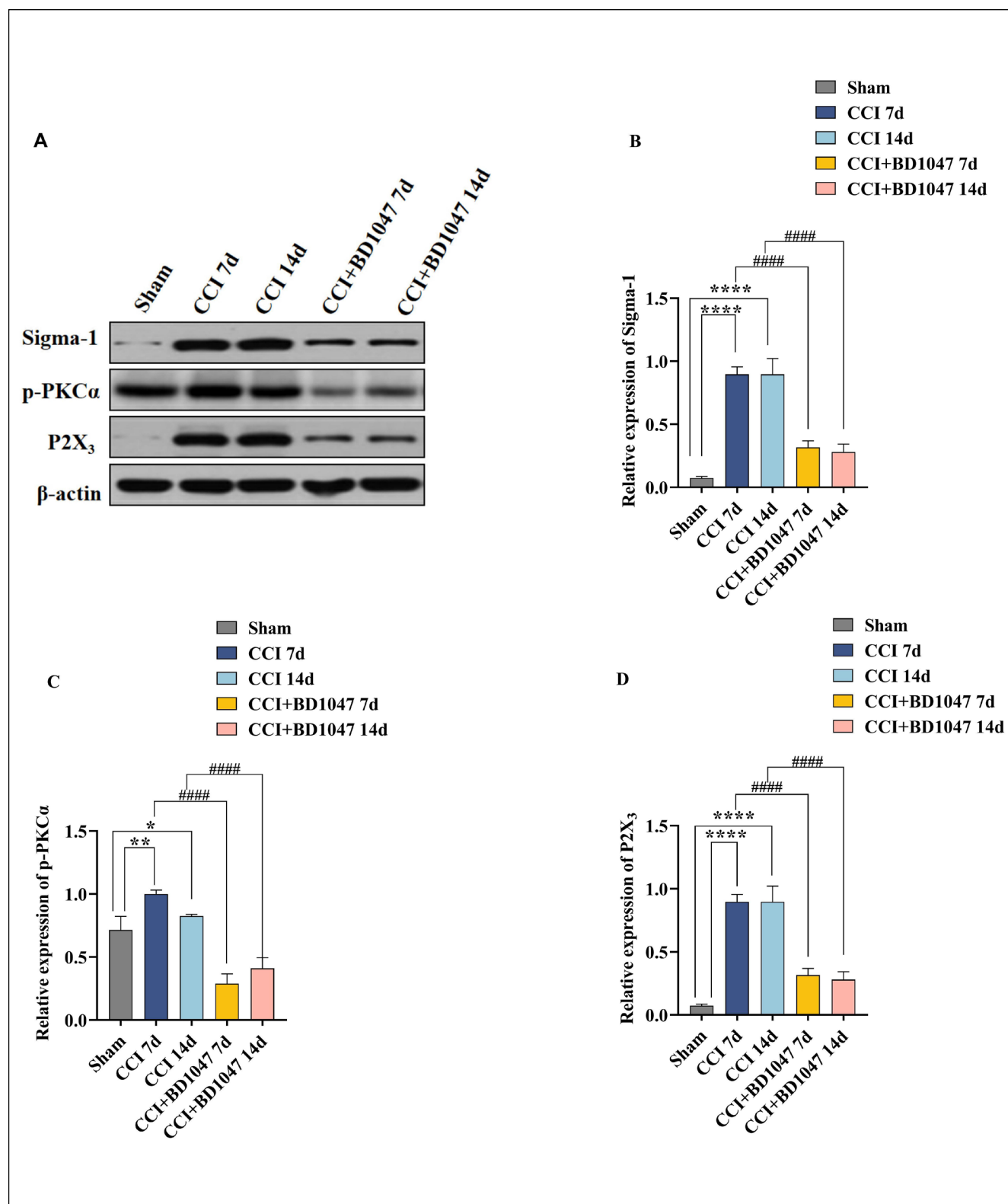


Fig. 2. Sigma-1 receptor antagonism suppresses CCI-induced overexpression of pain mediators in DRG. (A) Representative Western blots of Sigma-1 receptor, p-PKC α , P2X₃, and β -actin loading control in L4-L6 DRG. (B-D) Quantitative analysis of (B) Sigma-1, (C) p-PKC α , and (D) P2X₃ expression normalized to β -actin. Chronic constriction injury (CCI) significantly upregulated all targets *versus* Sham at postoperative days 7 and 14 (* p <0.05 vs. Sham group). BD1047 treatment reversed these elevations at both time points (* p <0.05 vs. CCI). Data = mean $\pm$ SD (n =8/group; one-way ANOVA, Tukey's test).

both expression and co-localization of these proteins at the same time points, indicating BD1047 effectively disrupted their interaction (Fig. 3B, C, D).

In summary, these results demonstrate that the Sigma-1 receptor antagonist BD1047 effectively alleviates thermal and mechanical hypersensitivity in a chronic

constriction injury rat model. This effect is likely mediated by downregulating Sigma-1, p-PKC α , and P2X₃ protein expression in dorsal root ganglia. These findings provide mechanistic insights into the therapeutic potential of Sigma-1 receptor antagonists for neuropathic pain management.

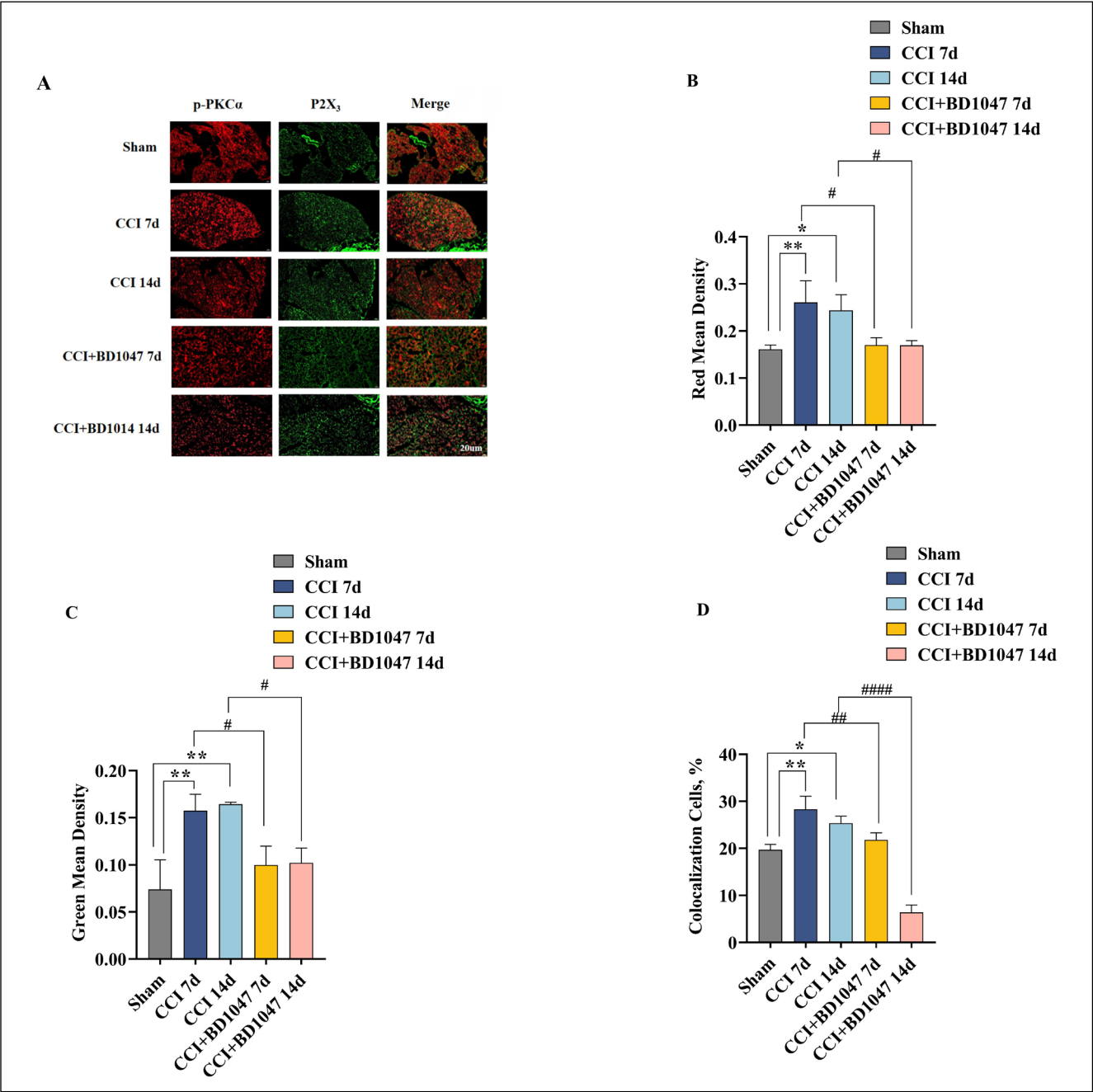


Fig. 3. BD1047 reduces CCI-induced p-PKC α /P2X₃ co-localization in DRG neurons. (A) Representative immunofluorescence images of L4-L6 DRG sections showing p-PKC α (red), P2X₃ (green) in Sham, CCI (7d/14d), and CCI+BD1047 (7d/14d) groups. (B) Quantitative analysis of p-PKC α fluorescence intensity. (C) P2X₃ fluorescence intensity. (D) Pearson's co-localization coefficient. CCI significantly increased p-PKC α /P2X₃ expression and co-localization *versus* Sham at postoperative days 7 and 14 (* p <0.05 vs. Sham group). BD1047 treatment reversed these increases at both time points (# p <0.05 vs. CCI). Data = mean $\pm$ SD (n=8 rats/group). Scale bar: 20 μ m.

DISCUSSION

Neuropathic pain, characterized by chronic pain resulting from nerve injury, significantly impairs quality of life. Understanding its molecular mechanisms is essential for developing effective therapies. Research indicates that under neuropathic pain conditions, certain pain- and inflammation-associated pathways are regulated, while others present under normal physiology are downregulated. This suggests altered communication patterns between DRG and spinal cord dorsal horn. Furthermore, intercellular communication shows plasticity in neuropathic pain conditions (Dong et al., 2025). The DRG emerges as a pivotal site where peripheral nerve injury plays critical roles in both initiating and maintaining neuropathic pain.

The Sigma-1 receptor, an endoplasmic reticulum membrane protein, modulates intracellular signaling pathways and is implicated in neuroinflammation and neuropathic pain. In DRG neurons, Sigma-1 receptor activation influences macrophage infiltration and neuroinflammatory responses following nerve injury (Bravo-Caparrós et al., 2020). Moreover, receptors such as p-PKC α and P2X₃ expressed in DRG neurons serve as key mediators in pain transmission. Upregulation of p-PKC α and P2X₃ in dorsal root ganglia of CCI rats contributes to neuropathic pain development, with phosphorylated PKC α modulating P2X₃ activity (Gu et al., 2016; Li et al., 2020).

In this study, intrathecal administration of the Sigma-1 antagonist BD1047 significantly increased response thresholds to thermal and mechanical stimuli in CCI rats. This treatment reduced Sigma-1, p-PKC α , and P2X₃ expression in L4-L6 dorsal root ganglia and decreased p-PKC α /P2X₃ co-localization. These findings suggest that PKC and P2X₃ pathways contribute to Sigma-1-mediated neuropathic pain pathogenesis following nerve injury.

The CCI model is a well established approach for studying neuropathic pain. Our results showed significantly reduced TWL and MWT at all postoperative time points, confirming successful model establishment. BD1047, a selective Sigma-1 receptor antagonist, effectively attenuated mechanical hypersensitivity in this model (Almansa & Vela, 2014). CCI rats exhibited progressive pain sensitization, evidenced by decreased TWL and MWT on postoperative days 1, 3, 7, 10, and 14, concurrent with increased Sigma-1 receptor expression in DRG tissue on days 7 and 14. In contrast, intrathecal administration of BD1047 reversed neuropathic pain behaviors – evidenced by increased TWL on postoperative days 1, 3, 7, 10, and 14, and elevated MWT on days 3, 7, 10, and 14 – and reduced Sigma-1 receptor expression on days 7 and 14.

These findings underscore the critical role of Sigma-1 receptors in neuropathic pain development and maintenance.

Crosstalk between neuronal and non-neuronal cells is mediated by neuronal ATP synthesis and subsequent activation of ATP receptors such as P2X₃ (Ren & Dubner, 2010). ATP functions as a neurotransmitter transmitting peripheral information to the spinal cord (Ballini et al., 2011). Our group previously established a CCI model and demonstrated through immunofluorescence and co-immunoprecipitation that Sigma-1 receptors regulate P2X₃ in dorsal root ganglia. This study reveals increased P2X₃ receptor expression in DRG tissues on postoperative days 7 and 14 correlating with heightened pain sensitivity in CCI rats. Intrathecal BD1047 administration attenuated neuropathic pain and reduced both Sigma-1 and P2X₃ receptor expression at these timepoints. These findings indicate that P2X₃ receptors significantly contribute to neuropathic pain regulation, with Sigma-1 receptors modulating P2X₃ expression in the CCI model.

We additionally investigated p-PKC α 's role. Previous studies demonstrate Sigma-1 receptor activation stimulates phospholipase C (PLC), producing DAG and inositol 1,4,5-trisphosphate (IP₃) (Morin-Surun et al., 1999). DAG critically activates PKC, which regulates neuropathic pain. Our data show increased p-PKC α levels in DRGs on postoperative days 7 and 14 following CCI. Conversely, intrathecal BD1047 decreased p-PKC α levels, indicating Sigma-1 receptor modulation of p-PKC α activity. Roh et al. (2008) further reported that Sigma-1 receptor agonists promote PKC translocation from cytoplasm to membrane, enhancing PKC activity. PKC α , a serine/threonine kinase regulating ion channels and nociceptors, participates in diverse pain states including neuropathic pain (Chang et al., 2021).

On postoperative days 7 and 14 after CCI surgery, p-PKC α and P2X₃ receptor expression in DRG was elevated. Immunofluorescence analysis revealed increased p-PKC α /P2X₃ co-localization at these timepoints, suggesting pathway interaction. Conversely, BD1047-administered rats showed reduced immunofluorescence intensity for both proteins on days 7 and 14 post-surgery, with decreased co-localization indicating disrupted interaction.

Given the multifunctional nature of the Sigma-1 receptor, we propose that it modulates P2X₃ receptor activity through multiple mechanisms. First, through its association with endoplasmic reticulum (ER) calcium signaling, the Sigma-1 receptor may indirectly regulate PKC and P2X₃ receptor function by controlling ER calcium storage and release. Second, Sigma-1 receptor activation alters intracellular signaling molecules, in-

cluding PKC and PLC. Activated PKC phosphorylates various substrates, such as plasma membrane calcium channels, thereby enhancing calcium influx and subsequently modulating P2X₃ activity.

CONCLUSION

Our findings establish that neuropathic pain in CCI rats involves Sigma-1 receptor-mediated regulation of p-PKC α and P2X₃ receptor in dorsal root ganglia. The Sigma-1 receptor appears to critically modulate the expression of both p-PKC α and P2X₃. Future studies should elucidate the precise mechanisms underlying Sigma-1 receptor-mediated regulation of these targets. Furthermore, therapeutic strategies targeting Sigma-1 receptors warrant investigation for neuropathic pain management.

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REFERENCES

- Almansa, C., & Vela, J. M. (2014). Selective sigma-1 receptor antagonists for the treatment of pain. *Future Med Chem*, 6(10), 1179–1199. <https://doi.org/10.4155/fmc.14.54>
- Alvarez-Perez, B., Bago-Mas, A., Deulofeu, M., Vela, J. M., Merlos, M., Verdu, E., & Boadas-Vaello, P. (2022). Long-Lasting Nociceptive Pain Modulation by Repeated Administration of Sigma-1 Receptor Antagonist BD1063 in Fibromyalgia-like Mouse Models. *Int J Mol Sci*, 23(19). <https://doi.org/10.3390/ijms23191933>
- Bai, P., Gomm, A., Yoo, C. H., Mondal, P., Lobo, F. M., Meng, H., Lan, Y. (2025). Development of Carbon-11 Labeled Pyrimidine Derivatives as Novel Positron Emission Tomography (PET) Agents Enabling Brain Sigma-1 Receptor Imaging. *Adv Sci (Weinh)*, 12(21), e2414827. <https://doi.org/10.1002/adv.202414827>
- Ballini, E., Virginio, C., Medhurst, S. J., Summerfield, S. G., Aldegheri, L., Buson, A., Jarolimek, W. (2011). Characterization of three diaminopyrimidines as potent and selective antagonists of P2X₃ and P2X_{2/3} receptors with in vivo efficacy in a pain model. *Br J Pharmacol*, 163(6), 1315–1325. <https://doi.org/10.1111/j.1476-5381.2011.01322.x>
- Bennett, G. J., & Xie, Y. K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain*, 33(1), 87–107. [https://doi.org/10.1016/0304-3959\(88\)90209-6](https://doi.org/10.1016/0304-3959(88)90209-6)
- Bravo-Caparrós, I., Ruiz-Cantero, M. C., Perazzoli, G., Cronin, S. J. F., Vela, J. M., Hamed, M. F., Nieto, F. R. (2020). Sigma-1 receptors control neuropathic pain and macrophage infiltration into the dorsal root ganglion after peripheral nerve injury. *FASEB J*, 34(4), 5951–5966. <https://doi.org/10.1096/fj.201901921R>
- Chang, C., Liu, H. K., Yeh, C. B., Yang, M. L., Liao, W. C., Liu, C. H., & Tseng, T. J. (2021). Cross-Talk of Toll-Like Receptor 5 and Mu-Opioid Receptor Attenuates Chronic Constriction Injury-Induced Mechanical Hyperalgesia through a Protein Kinase C Alpha-Dependent Signaling. *Int J Mol Sci*, 22(4). <https://doi.org/10.3390/ijms22041891>
- Choi, S. R., Roh, D. H., Yoon, S. Y., Kang, S. Y., Moon, J. Y., Kwon, S. G., Lee, J. H. (2013). Spinal sigma-1 receptors activate NADPH oxidase 2 leading to the induction of pain hypersensitivity in mice and mechanical allodynia in neuropathic rats. *Pharmacol Res*, 74, 56–67. <https://doi.org/10.1016/j.phrs.2013.05.004>
- Dong, F. L., Yu, L., Feng, P. D., Ren, J. X., Bai, X. H., Lin, J. Q., Jiang, B. C. (2025). An atlas of neuropathic pain-associated molecular pathological characteristics in the mouse spinal cord. *Commun Biol*, 8(1), 70. <https://doi.org/10.1038/s42003-025-07506-0>
- Entrena, J. M., Cobos, E. J., Nieto, F. R., Cendan, C. M., Gris, G., Del Pozo, E., Baeyens, J. M. (2009). Sigma-1 receptors are essential for capsaicin-induced mechanical hypersensitivity: studies with selective sigma-1 ligands and sigma-1 knockout mice. *Pain*, 143(3), 252–261. <https://doi.org/10.1016/j.pain.2009.03.011>
- Esposito, M. F., Malayil, R., Hanes, M., & Deer, T. (2019). Unique Characteristics of the Dorsal Root Ganglion as a Target for Neuromodulation. *Pain Med*, 20(Suppl 1), S23–S30. <https://doi.org/10.1093/pm/pnz012>
- Gonzalez-Cano, R., Artacho-Cordon, A., Romero, L., Tejada, M. A., Nieto, F. R., Merlos, M., Baeyens, J. M. (2020). Urinary bladder sigma-1 receptors: A new target for cystitis treatment. *Pharmacol Res*, 155, 104724. <https://doi.org/10.1016/j.phrs.2020.104724>
- Gu, Y., Li, G., Chen, Y., & Huang, L. M. (2016). Epac-protein kinase C alpha signaling in purinergic P2X₃R-mediated hyperalgesia after inflammation. *Pain*, 157(7), 1541–1550. <https://doi.org/10.1097/j.pain.0000000000000547>
- Guzman-Lenis, M. S., Navarro, X., & Casas, C. (2009). Selective sigma receptor agonist 2-(4-morpholinethyl)-1-phenylcyclohexanecarboxylate (PRE084) promotes neuroprotection and neurite elongation through protein kinase C (PKC) signaling on motoneurons. *Neuroscience*, 162(1), 31–38. <https://doi.org/10.1016/j.neuroscience.2009.03.067>
- Hirai, T., & Chida, K. (2003). Protein kinase C ζ (PKC ζ): activation mechanisms and cellular functions. *J Biochem*, 133(1), 1–7. <https://doi.org/10.1093/jb/mvg017>
- Li, X., Yuan, J., Yu, X., Zhang, Q., & Qin, B. (2020). Effect of PKC/NF-kappaB on the Regulation of P2X₃ Receptor in Dorsal Root Ganglion in Rats with Sciatic Nerve Injury. *Pain Res Manag*, 2020, 7104392. <https://doi.org/10.1155/2020/7104392>
- Milligan, E. D., Hinde, J. L., Mehmert, K. K., Maier, S. F., & Watkins, L. R. (1999). A method for increasing the viability of the external portion of lumbar catheters placed in the spinal subarachnoid space of rats. *J Neurosci Methods*, 90(1), 81–86. [https://doi.org/10.1016/S0165-0270\(99\)00075-8](https://doi.org/10.1016/S0165-0270(99)00075-8)
- Morin-Surun, M. P., Collin, T., Denavit-Saubie, M., Baulieu, E. E., & Monnet, F. P. (1999). Intracellular sigma1 receptor modulates phospholipase C and protein kinase C activities in the brainstem. *Proc Natl Acad Sci U S A*, 96(14), 8196–8199. <https://doi.org/10.1073/pnas.96.14.8196>
- Paukert, M., Osteroth, R., Geisler, H. S., Brandle, U., Glowatzki, E., Ruppersberg, J. P., & Grunder, S. (2001). Inflammatory mediators potentiate ATP-gated channels through the P2X₃ subunit. *J Biol Chem*, 276(24), 21077–21082. <https://doi.org/10.1074/jbc.M101465200>
- Qin, L., Zhang, X., & Li, J. (2025). Mechanism of heat treatment on exercise pressor reflex in hindlimb ischemia-reperfusion: Does the temperature

- gradient matter? *Auton Neurosci*, 259, 103290. <https://doi.org/10.1016/j.autneu.2025.103290>
- Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain*, 161(9), 1976–1982. <https://doi.org/10.1097/j.pain.0000000000001939>
- Reinhold, A. K., Batti, L., Bilbao, D., Buness, A., Rittner, H. L., & Heppenstall, P. A. (2015). Differential transcriptional profiling of damaged and intact adjacent dorsal root ganglia neurons in neuropathic pain. *PLoS One*, 10(4), e0123342. <https://doi.org/10.1371/journal.pone.0123342>
- Ren, K., & Dubner, R. (2010). Interactions between the immune and nervous systems in pain. *Nat Med*, 16(11), 1267–1276. <https://doi.org/10.1038/nm.2234>
- Roh, D. H., Kim, H. W., Yoon, S. Y., Seo, H. S., Kwon, Y. B., Kim, K. W., Lee, J. H. (2008). Intrathecal administration of sigma-1 receptor agonists facilitates nociception: involvement of a protein kinase C-dependent pathway. *J Neurosci Res*, 86(16), 3644–3654. <https://doi.org/10.1002/jnr.21802>
- Smith, B. H., Hebert, H. L., & Veluchamy, A. (2020). Neuropathic pain in the community: prevalence, impact, and risk factors. *Pain*, 161 Suppl 1, S127–S137. <https://doi.org/10.1097/j.pain.0000000000001824>
- Sun, H. J., Wang, S. S., Li, X. Y., Du, J. Y., Fang, J. Q., & Fang, J. F. (2019). [Advances of researches on peripheral PKCepsilon pathway during transformation from acute to chronic pain and possibility of application of electroacupuncture intervention]. *Zhen Ci Yan Jiu*, 44(7), 543–547. <https://doi.org/10.13702/j.1000-0607.180542>
- Wang, W., Ma, X., Luo, L., Huang, M., Dong, J., Zhang, X., Xu, T. (2018). Exchange factor directly activated by cAMP-PKCepsilon signalling mediates chronic morphine-induced expression of purine P2X3 receptor in rat dorsal root ganglia. *Br J Pharmacol*, 175(10), 1760–1769. <https://doi.org/10.1111/bph.14191>
- Wu, Y., Bai, X., Li, X., Zhu, C., & Wu, Z. P. (2018). Overexpression of sigma-1 receptor in MCF-7 cells enhances proliferation via the classic protein kinase C subtype signaling pathway. *Oncol Lett*, 16(5), 6763–6769. <https://doi.org/10.3892/ol.2018.9448>