RESEARCH PAPER

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Sigma-1 receptor regulates p-PKCα and $P2X_3$ expression in dorsal root ganglia to attenuate neuropathic pain in rats

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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 Cα (p-PKCα), and $P2X_3$ receptor expression in the dorsal root ganglia (DRG). Immunofluorescence techniques were employed to examine p-PKCα and $P2X_3$ 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-PKCα, and $P2X_3$ receptor in the DRG were elevated, and immunofluorescence expression of p-PKCα and the $P2X_3$ receptor also increased. Conversely, in the CCI+BD1047 group, TWL and MWT were significantly enhanced. Additionally, protein levels of the Sigma-1 receptor, p-PKCα, and $P2X_3$ receptor in the DRG decreased, along with reduced immunofluorescence expression of p-PKCα and $P2X_3$ receptor. The findings indicate that neuropathic pain is intricately associated with the Sigma-1 receptor, p-PKCα, and $P2X_3$ receptor in the dorsal root ganglia. Notably, the Sigma-1 receptor regulates the expression of p-PKCα and $P2X_3$ receptor, presenting a novel therapeutic target for neuropathic pain management.

Key words: neuropathic pain, dorsal root ganglia, Sigma-1 receptor, p-PKCα, $P2X_3$ receptor

INTRODUCTION

Neuropathic pain (NP) is a chronic pain condition resulting from damage or disease affecting the somatosensory 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).

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Specifically, abnormal expression of the Sigma-1 receptor, phosphorylated protein kinase Cα (p-PKCα), and $P2X_3$ 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 $P2X_3$ 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 $P2X_3$ receptors at the damaged ends of DRG neurons (Paukert et al., 2001). Studies indicate that $P2X_3$ 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 PKCγ, PKCε, PKCα, and PKCδ, 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-PKCα and $P2X_3$ 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-PKCα and $P2X_3$ 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-PKCα, and $P2X_3$ receptor expression in the DRG. The selective Sigma-1 receptor antagonist BD1047 was administered to examine its regulatory effects on p-PKCα and $P2X_3$ 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-PKCα and $P2X_3$ 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 µl), 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-µl 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 µl, twice daily, postoperative days 1-14 (POD 1-14)); CCI7d: CCI + intrathecal saline (20 µl, twice daily, POD 1-7); CCI14d: CCI + intrathecal saline (20 µl, twice daily, POD 1-14); CCI+BD1047 7d: CCI + intrathecal BD1047 (100 nmol in 20 µl saline, twice daily, POD 1-7); CCI+BD1047 14d: CCI + intrathecal BD1047 (100 nmol in 20 µl 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×rpm for 15 min. Protein concentrations were determined by BCA assay. Equal amounts of protein (30 µg/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-PKCα (1:1000, Aifang, AF301054), and rabbit anti-$P2X_3$ (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-$P2X_3$ (1:1000, AiFang, AF06653) and rabbit anti-p-PKCα (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 Prisms (v8.0; GraphPad Software, USA). Results are expressed as mean ± 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-PKCα, 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-PKCα, 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-PKCα/$P2X_3$ co-localization in DRGs (Fig. 3). In the CCI group, p-PKCα (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

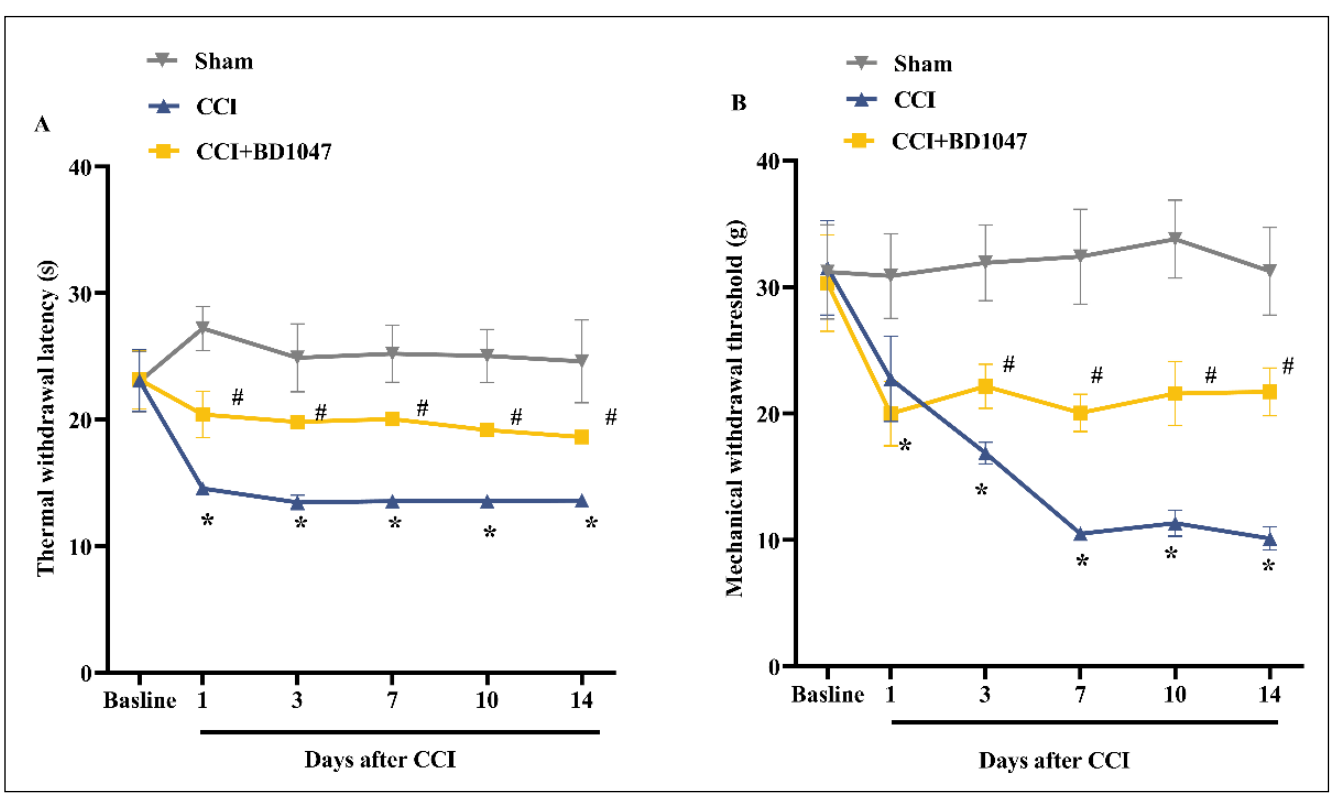

Fig. 1. Sigma-1 receptor antagonism attenuates CCI-induced hypersensitivity. Intrathecal BD1047 (100 nmol) significantly increased (A) thermal withdrawal latency (TWL) and (B) mechanical withdrawal threshold (MWT) in chronic constriction injury (CCI) rats *versus* saline-treated CCI controls. TWL effects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

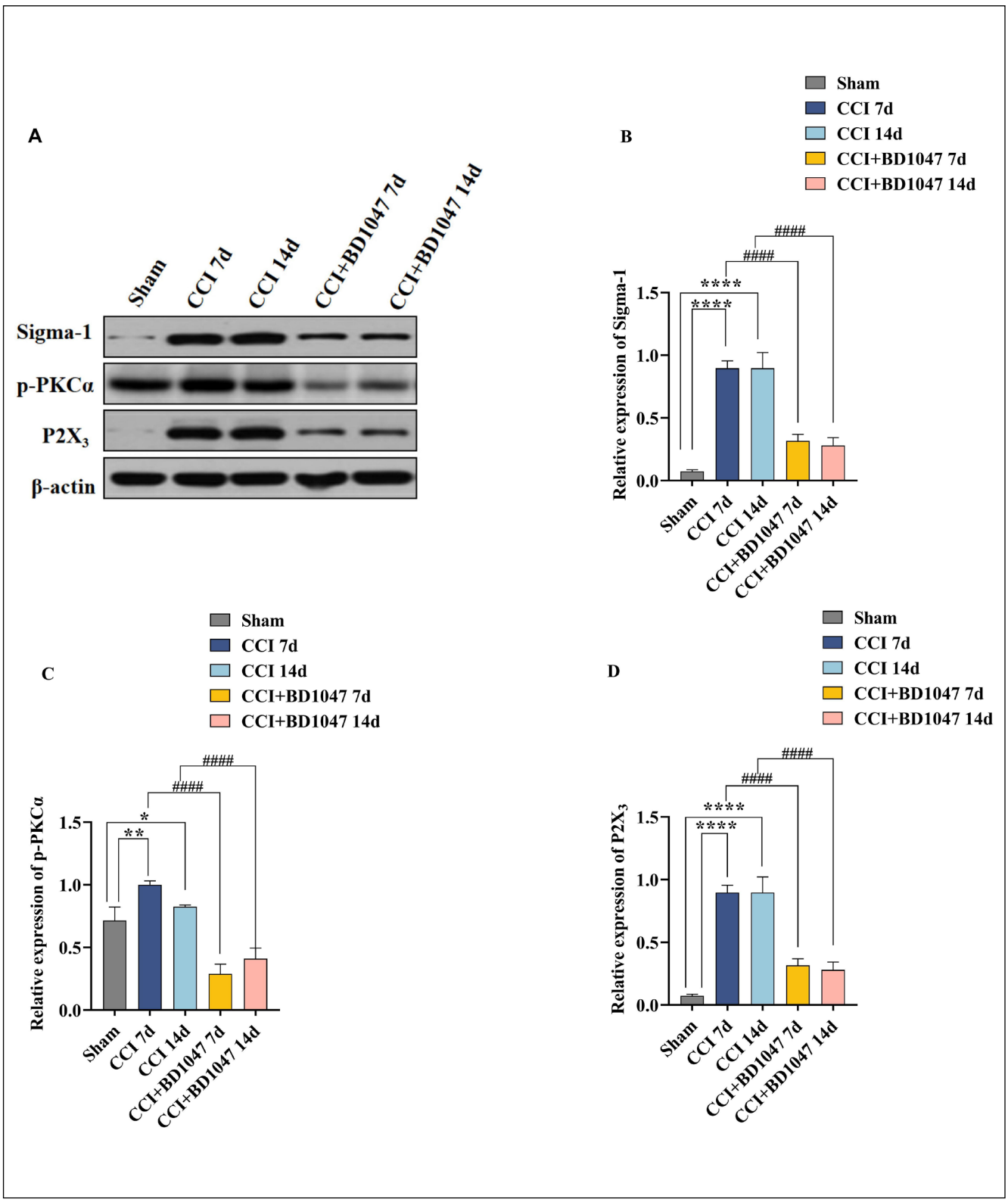

Fig. 2. Sigma-1 receptor antagonism suppresses CCI-induced overexpression of pain mediators in DRG. (A) Representative Western blots of Sigma-1 receptor, p-PKCα, $P2X_3$, and β-actin loading control in L4-L6 DRG. (B-D) Quantitative analysis of (B) Sigma-1, (C) p-PKCα, and (D) $P2X_3$ expression normalized 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 ± 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-PKCα, and $P2X_3$ protein expression in dorsal root ganglia. These findings provide mechanistic insights into the therapeutic potential of Sigma-1 receptor antagonists for neuropathic pain management.

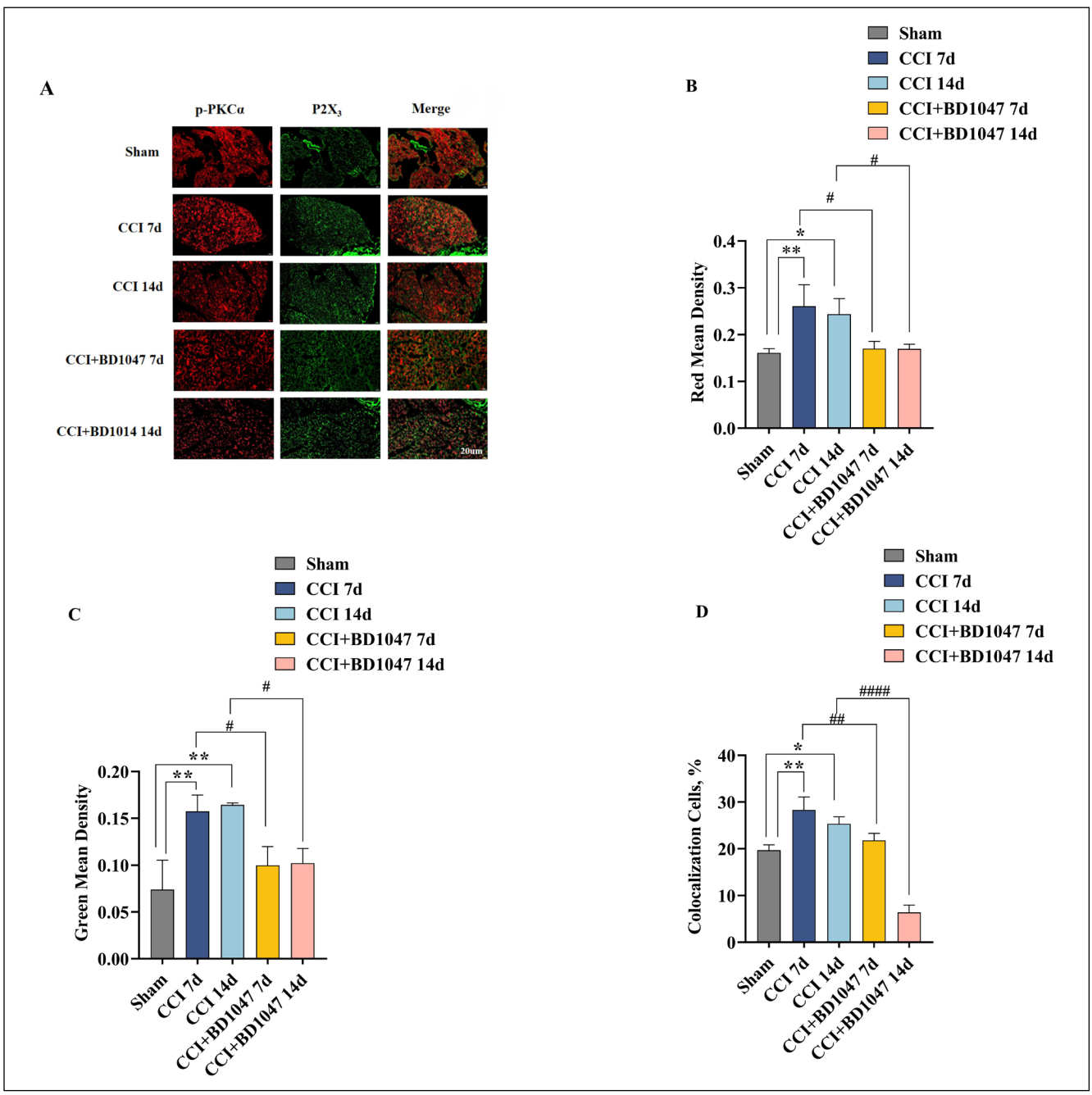

Fig. 3. BD1047 reduces CCI-induced p-PKCα/$P2X_3$ co-localization in DRG neurons. (A) Representative immunofluorescence images of L4-L6 DRG sections showing p-PKCα (red), $P2X_3$ (green) in Sham, CCI (7d/14d), and CCI+BD1047 (7d/14d) groups. (B) Quantitative analysis of p-PKCα fluorescence intensity. (C) $P2X_3$ fluorescence intensity. (D) Pearson's co-localization coefficient. CCI significantly increased p-PKCα/$P2X_3$ 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 ± SD (n=8 rats/group). Scale bar: 20 μm.

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-Caparros et al., 2020). Moreover, receptors such as p-PKCα and $P2X_3$ expressed in DRG neurons-serve as key mediators in pain transmission. Upregulation of p-PKCα and $P2X_3$ in dorsal root ganglia of CCI rats contributes to neuropathic pain development, with phosphorylated PKCα modulating $P2X_3$ 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-PKCα, and $P2X_3$ expression in L4-L6 dorsal root ganglia and decreased p-PKCα/$P2X_3$ co-localization. These findings suggest that PKC and $P2X_3$ 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 $P2X_3$ (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 $P2X_3$ in dorsal root ganglia. This study reveals increased $P2X_3$ 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 $P2X_3$ receptor expression at these timepoints. These findings indicate that $P2X_3$ receptors significantly contribute to neuropathic pain regulation, with Sigma-1 receptors modulating $P2X_3$ expression in the CCI model.

We additionally investigated p-PKCα's role. Previous studies demonstrate Sigma-1 receptor activation stimulates phospholipase C (PLC), producing DAG and inositol 1,4,5-trisphosphate (IP_3) (Morin-Surun et al., 1999). DAG critically activates PKC, which regulates neuropathic pain. Our data show increased p-PKCα levels in DRGs on postoperative days 7 and 14 following CCI. Conversely, intrathecal BD1047 decreased p-PKCα levels, indicating Sigma-1 receptor modulation of p-PKCα activity. Roh et al. (2008) further reported that Sigma-1 receptor agonists promote PKC translocation from cytoplasm to membrane, enhancing PKC activity. PKCα, 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-PKCα and $P2X_3$ receptor expression in DRG was elevated. Immunofluorescence analysis revealed increased p-PKCα/$P2X_3$ 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 $P2X_3$ receptor activity through multiple mechanisms. First, through its association with endoplasmic reticulum (ER) calcium signaling, the Sigma-1 receptor may indirectly regulate PKC and $P2X_3$ 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 $P2X_3$ activity.

CONCLUSION

Our findings establish that neuropathic pain in CCI rats involves Sigma-1 receptor-mediated regulation of p-PKCα and $P2X_3$ receptor in dorsal root ganglia. The Sigma-1 receptor appears to critically modulate the expression of both p-PKCα and $P2X_3$. 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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