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ORIGINAL RESEARCH

Dexmedetomidine attenuates persistent postsurgical pain by upregulating K^+–Cl^- cotransporter-2 in the spinal dorsal horn in rats

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Background: Dexmedetomidine (DEX) could have an analgesic effect on pain transmission through the modulation of brain-derived neurotrophic factor (BDNF). In addition, KCC2-induced shift in neuronal Cl- homeostasis is crucial for postsynaptic inhibition mediated by GABAA receptors. Accumulating evidence shows that nerve injury, peripheral inflammation and stress activate the spinal BDNF/TrkB signal, which results in the downregulation of KCC2 transport and expression, eventually leads to GAGAergic disinhibition and hyperalgesia. The aim of this experiment was to explore the interaction between DEX and KCC2 at a molecular level in rats in the persistent postsurgical pain (PPSP).

Methods: PPSP in rats was evoked by the skin/muscle incision and retraction (SMIR). Mechanical hypersensitivity was assessed with the Dynamic Plantar Aesthesiometer. Western blot and immunofluorescence assay were used to assess the expressions of related proteins.

Results: In the first part of our experiment, the results revealed that the BDNF/TrkB-KCC2 signal plays a critical role in the development of SMIR-evoked PPSP; the second part showed that intraperitoneal administrations of 40 μg/kg DEX at 15 min presurgery and 1 to 3 days post-surgery significantly attenuated SMIR-evoked PPSP. Simultaneously, SMIR-induced KCC2 downregulation was partly reversed, which coincided with the inhibition of the BDNF/TrkB signal in the spinal dorsal horn. Moreover, intrathecal administrations of KCC2 inhibitor VU0240551 significantly reduced the analgesic effect of DEX on SMIR-evoked PPSP.

Conclusion: The results of our study indicated that DEX attenuated PPSP by restoring KCC2 function through reducing BDNF/TrkB signal in the spinal dorsal horn in rats, which provides a new insight into the treatment of chronic pain in clinical postsurgical pain management.

Keywords: skin/muscle incision and retraction, persistent postsurgical pain, dexmedetomidine, KCC2, BDNF/TrkB signal

Introduction

Persistent postsurgical pain (PPSP) is a serious issue in clinical pain management because it slows surgical recovery, prolongs hospitalization, lowers the patient's quality of life, and increases economic consequences.[1] Moreover, PPSP has a high prevalence,[2] particularly in certain surgeries that require essential prolonged tissue retraction, such as thoracotomy and inguinal hernia repair;[3,4] However, the process of PPSP is still poorly understood.

In recent years, there has been sustained and enhanced interest among researchers and clinicians in exploring the role of GABAergic disinhibition in a series of pathological states, such as hyperalgesia.[5–10] One of the most important areas in this

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study is GABAergic disinhibition caused by the activation of the spinal or supraspinal brain-derived neurotrophic factor (BDNF)/TrkB-KCC2 signal.[7,10–12]

The pain modulatory actions of α2-adrenoceptors at both the spinal[13–15] and supraspinal[16] levels have been extensively explored. The activation of α2-adrenoceptors by agonists was effective in alleviating the neuropathic pain state in both animals[14,17] and humans.[18] Dexmedetomidine (DEX), a highly selective agonist of α2-adrenergic receptors with sedative properties, has been promoted for its palpable analgesic effect through intrathecal or systemic administration.[16,19–24] A recent study demonstrated that intraperitoneal (IP) injections of 40 μg/kg DEX for 14 consecutive days clearly reversed sciatic nerve spared nerve injury (SNI)-induced neuropathic pain through the downregulation of P2×4Rs, p-p38 and BDNF in the microglia of the spinal dorsal horn.[22] Even though the proposed mechanisms concerning DEX-induced analgesia in other pain models have been studied, some possible mechanisms between DEX and KCC2 in the skin/muscle incision and retraction (SMIR)-evoked PPSP have not been elucidated. To address this question, we first investigated the role of the BDNF/TrkB-KCC2 signal in SMIR-evoked PPSP in the spinal dorsal horn in rats. We then explored the effects of DEX on the pain behaviors of SMIR-evoked PPSP and the underlying relations with KCC2 in the spinal dorsal horn in rats.

Materials and methods

Animals

The experiments were performed on male adult Sprague Dawley rats (6–8 weeks; 180–220 g). They were housed in the animal facility under a 12-h light–dark cycle with food and water freely available. In addition, the room temperature and humidity were well controlled. All procedures were approved by the Animal Care Committee of Nanjing Medical University (IACUC protocol number: NJMU08-092) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No 80-23) revised 1996; in addition, efforts were made to minimize the number of animals used for the experiments and their suffering.

Experimental design

Part 1: we explored the role of the BDNF/TrkB-KCC2 signal in SMIR-evoked PPSP in the spinal dorsal horn

In section 1, 129 rats were equally randomized into three groups: normal, sham, and SMIR. Animal pain behavior was assessed at postoperative days (PODs) 1, 3, 7, 12, 22, and 32 after SMIR (n=8/group, randomly chosen from each group and fixed for the evaluation). At each time point after the behavioral assessment, rats were randomly sacrificed to isolate the L4-6 spinal cords for Western blotting (n=5/group/time point), while five rats from each group were sacrificed to remove the L4-6 spinal cords for immunofluorescence assay at POD 12.

In section 2, 72 rats were equally randomized into four groups: sham + saline, sham + TrkB/Fc, SMIR + saline, and SMIR + TrkB/Fc. The sham + TrkB/Fc and SMIR + TrkB/Fc groups were intrathecally injected with 5 μg TrkB/Fc (Sigma-Aldrich Co., St Louis, MO, USA) (dissolved in 10 μL saline), while the sham + saline and SMIR + saline groups were intrathecally injected with the same volume of saline. Animal pain behaviors were assessed at PODs 1, 3, 7, 12, 22, and 32 after SMIR surgery (n=8/group). At POD 12, rats were sacrificed to remove the L4-6 spinal cord for Western blotting (n=5/group) and immunofluorescence assay (n=5/group).

Part 2: we investigated the effect of DEX on SMIR-evoked PPSP in rats and the interaction with KCC2 at a molecular level in the spinal dorsal horn

In section 1, 72 rats were randomly and equally divided into four groups: sham + saline, sham + DEX, SMIR + saline, and SMIR + DEX. The sham + DEX and SMIR + DEX groups were IP injected with 40 μg/kg DEX (Jiangsu Hengrui Medicine Co., Ltd, Lianyungang, China) at 15 min presurgery and at PODs 1–3, while the sham + saline and SMIR + saline groups were IP injected with the same volume of saline. Pain behaviors were evaluated at presurgery day 1 and PODs 1–32 (n=8/group). At POD 12, rats were sacrificed to remove the L4-6 spinal cord for Western blotting (n=5/group) and immunofluorescence assay (n=5/group).

In section 2, 36 rats were equally randomized into two groups: SMIR + DEX + DMSO and SMIR + DEX + VU0240551. The two groups were both IP injected with 40 μg/kg DEX at 15 min presurgery and at PODs 1–3; at the same time, rats in the SMIR + DEX + DMSO group were intrathecally administered 10 μL 0.01% DMSO, while rats in the SMIR + DEX + VU0240551 group were intrathecally administered 0.27 μg VU0240551 (Sigma-Aldrich Co.) (dissolved in 10 μL of 0.01% DMSO).[10] Pain behaviors were evaluated at presurgery day 1 and at PODs 1–32 (n=8/group). At POD 12, rats were sacrificed to remove the L4-6 spinal cord for Western blotting (n=5/group) and immunofluorescence assay (n=5/group).

Paw mechanical withdrawal threshold (PMWT) test

The PMWT in the ipsilateral paw was assessed using the Dynamic Plantar Aesthesiometer (Ugo Basile S.R.L., Gemonio VA, Italy).[25] Rats were placed in the boxes for 30 min

before the test to adapt to the environment. The filament containing a universal tip exerted an upward force on the plantar surface of the hindpaw (maximum 50 g), and the results were automatically showed by the intensity of hypernociception (in grams) following hindpaw withdrawal. The average of five measurements was considered as the PMWT, and the presurgery PMWT was considered to be the baseline value.

SMIR surgery

The SMIR model was built according to the technique described by Flatters.[26] The rats were anesthetized through IP administration of phenobarbital (60 mg/kg). First, at a position ~4 mm medial to the saphenous vein, a 1.5–2 cm incision was made in the skin of the medial thigh, followed by a 7–10 mm incision in the superficial (gracilis) muscle layer of the thigh. The superficial muscle was then parted further with blunt scissors to allow a microdissecting retractor to be inserted. The skin and superficial muscle of the thigh were retracted to reveal 2 cm of the fascia of the underlying adductor for 1 h, after which the incision was closed with 4.0 Vicryl sutures. Rats in the groups receiving sham surgery underwent the same procedure without skin/muscle retraction.

Intrathecal method

The intrathecal injections were made by percutaneous intrathecal puncture technique with a microsyringe. The lumber regions of rats were shaved followed by the preparation of Betadine Solution and placed on a plexiglas tube to make the intervertebral spaces widened. Then, rats were injected at the lumber interspace (L4-6) using a Hamilton microsyringe filled with necessary drugs. The correct subarachnoid position appeared at the falling sense and was verified by the paw-flick or tail test.

The technique not only has a high success rate but also has safe and reliable without spinal cord damage. Moreover, with the advantage of small trauma, rapid recovery after surgery, reduced leakage of cerebrospinal fluid, and reusable, this technique could avoid the intrathecal catheter-induced inflammatory response and the adverse effects on drug function and pain response.[27]

Western blotting analysis

The L4-6 spinal dorsal horn was rapidly isolated and stored at –80°C for subsequent procedures. The frozen ipsilateral dorsal horn was prepared after tissue lysis and centrifugation for SDS-polyacrylamide gel electrophoresis. The protein with the SDS sample buffer was either mixed with β-mercaptoethanol or not,[28] separated under reducing conditions on a 5% SDS-polyacrylamide gel and finally transferred to a nitrocellulose membrane. The membrane was incubated with mouse anti-KCC2 (1:300; Sigma-Aldrich Co.), anti-BDNF (1:250; Sigma-Aldrich Co.), and anti-TrkB (1:500; Abcam, Cambridge, MA, USA). β-Actin (1:1000; Sigma-Aldrich Co.) was used as a standard. After overnight agitation at 4°C, the sample was then incubated with HRP-conjugated goat antimouse antibody (1:1500) for 1 h at room temperature. The bands were acquired by enhanced chemiluminescence (ECL) (GE Healthcare Biosciences, Piscataway, NJ, USA) and analyzed using ImageJ (National Institutes of Health, Bethesda, MA, USA).

Immunofluorescence assay

After being deeply anesthetized, rats were fixed with 4% paraformaldehyde and perfused with saline. The L4-6 spinal cord was harvested, postfixed overnight, and cryopreserved in 30% sucrose. Sections of a 20 μm thickness were cut with cryostat (Leica Microsystems, Wetzlar, Germany) at –20°C. Slices were blocked with 0.2% Triton X-100 and incubated overnight at 4°C with mouse anti-KCC2 (1:300; Sigma-Aldrich Co.). After being washed three times, sections were incubated in Alexa594-conjugated goat antimouse antibody (1:800; Life Sciences, Pittsburgh, PA, USA) for 2 h and washed with PBS before mounting. ImageJ software was used to analyze the average optical density of KCC2 expression in the spinal dorsal horn.

Data analysis and statistics

SPSS, Version 18 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The results were expressed as the mean ± standard error of the mean (SEM). Two-way analysis of variance (ANOVA) followed by an LSD post hoc comparison was used to compare the nociceptive thresholds to mechanical stimuli with group and time. Western blot and immunofluorescence results were compared by one-way ANOVA and two-way ANOVA (for multiple groups comparison) when necessary. The figures were all created with GraphPad Prism software, Version 4.0. $P<0.05$ was considered statistically significant.

Results

Decrease in PMWT following SMIR

As shown in Figure 1A, no statistically significant difference in the PMWT was observed between the normal and sham groups ($P>0.05$). In the SMIR group, the PMWT in the ipsilateral paw to surgery was significantly and persistently lowered at PODs 3–22 ($P_3=0.004$, $P_7=0.005$, $P_{12}=0.003$, $P_{22}=0.000$, $F=22.978$). A comparison of the PMWT in the SMIR group with that of the normal and sham groups

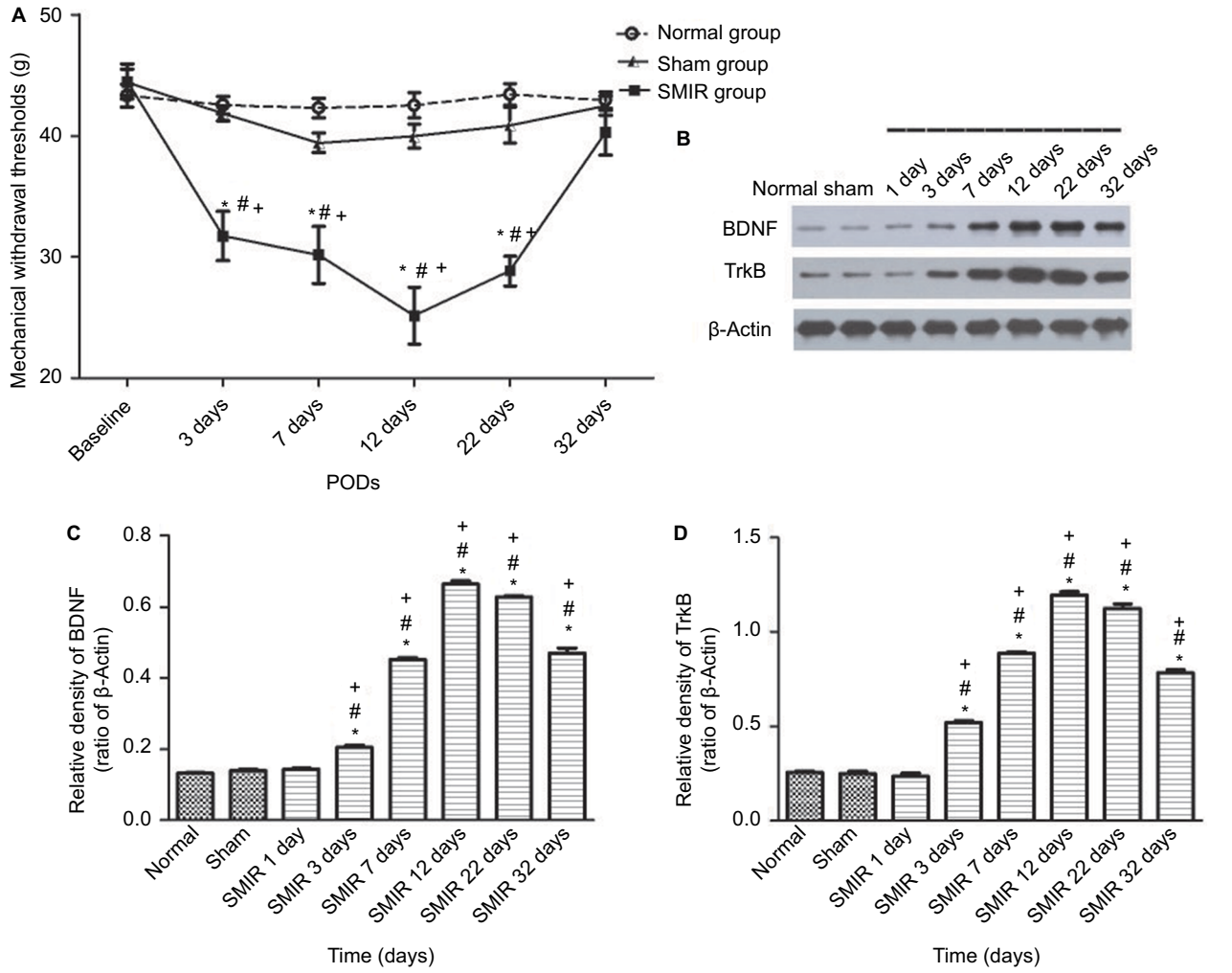

Figure 1 SMIR-evoked changes in rats.
Notes: (**A**) The PMWT in the SMIR group showed a significant and persistent decrease after surgery (n=8). (**B**–**D**) Marked upregulation of the BDNF/TrkB signal in SMIR rats (n=5/group/time point). *P<0.05 vs baseline, #P<0.05 vs normal group, and +P<0.05 vs sham group.
Abbreviations: BDNF, brain-derived neurotrophic factor; PODs, postoperative days; PMWT, paw mechanical withdrawal threshold; SMIR, skin/muscle incision and retraction.

revealed significant differences at PODs 3–22 (P=0.000, F=26.746, P<0.05).

Time course of protein expressions of BDNF and TrkB in the ipsilateral spinal dorsal horn after SMIR

As shown in Figure 1B–D, the results of Western blot indicated that compared with the baseline and the normal and sham groups, SMIR rats appeared to be upregulated in BDNF and TrkB proteins after surgery in the ipsilateral dorsal horn of the L4-6 spinal cord (P=0.001, F=92.903), while no differences were found between the normal and sham groups (P>0.05).

Downregulation of KCC2 expression in the ipsilateral spinal dorsal horn after SMIR

The expression level of KCC2 in the ipsilateral L4-6 spinal dorsal horn of the SMIR rats clearly decreased with the same time course as the BDNF/TrkB signal (P=0.002, F=59.366, Figure 2A and B). To further explore whether SMIR affects KCC2 oligomerization, which was critical for the transport ability of KCC2, we assessed KCC2 expression with a non-sulfhydryl-reducing sample buffer as previously described.[28] The results showed that the KCC2 oligomer (P=0.000, F=277.006) and monomer (P=0.000, F=645.164) and the oligomer/monomer ratio were significantly downregulated after SMIR in the ipsilateral spinal dorsal horn, accompanied by pain behaviors (Figure 2C–F). Immunofluorescence assay confirmed the decrease of KCC2 expression in the spinal dorsal horn after SMIR (Figure 3, P<0.05)

BDNF-sequester TrkB/Fc significantly reversed SMIR-induced changes in rats

To further verify the interaction between the BDNF/TrkB signal and KCC2 in the spinal dorsal horn of PPSP rats, we

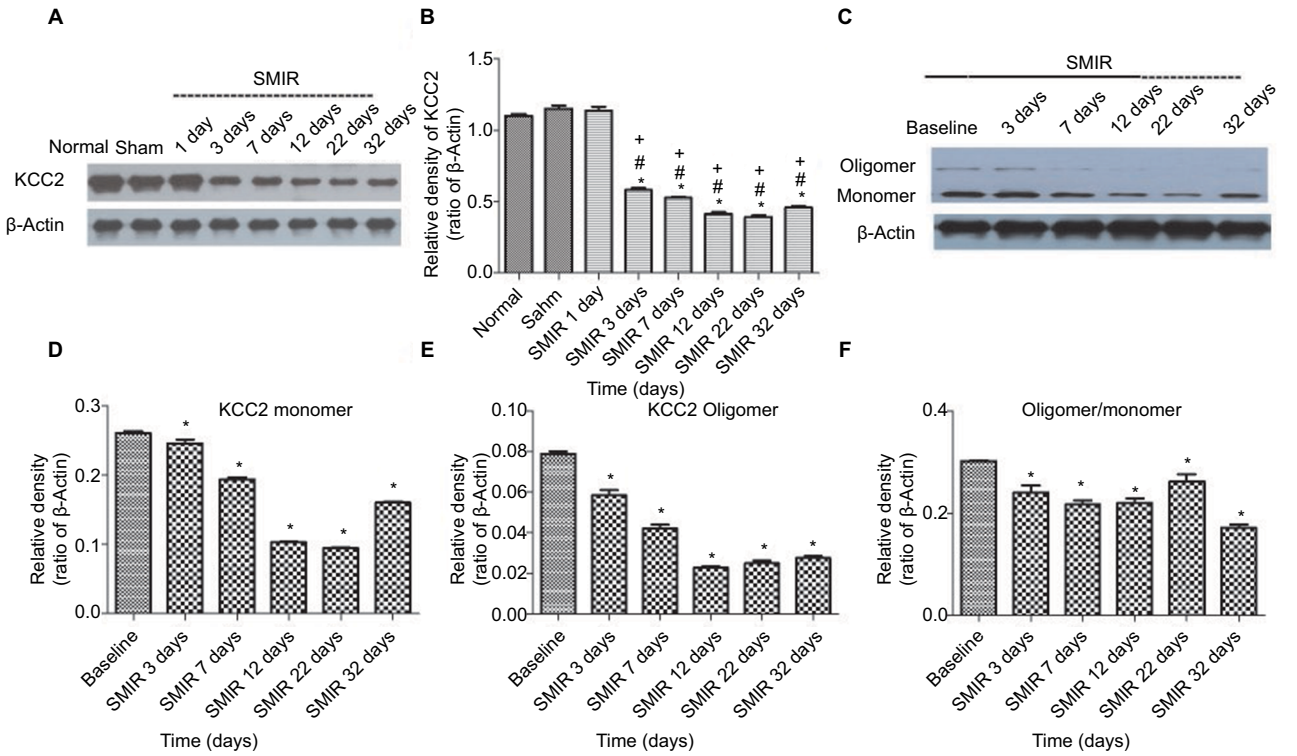

Figure 2 The variations of KCC2 expression in the spinal dorsal horn after SMIR.

Notes: (**A** and **B**) Total KCC2 expression in the ipsilateral spinal dorsal horn significantly and persistently decreased with the same time course as the BDNF/TrkB signal (n=5/group/time point). (**C**–**F**) Western blot with a nonsulfhydryl-reducing sample buffer (n=5/time point). **P*<0.05 vs baseline, #*P*<0.05 vs normal group, and +*P*<0.05 vs sham group.

Abbreviations: BDNF, brain-derived neurotrophic factor; SMIR, skin/muscle incision and retraction.

intrathecally administered 5 μg TrkB/Fc[12] at 15 min presurgery and at PODs 1–3. No differences were detected between the sham + saline group and the sham + TrkB/Fc group (*P*>0.05). The PMWT values of the SMIR + saline group decreased significantly after the SMIR surgery (*P*=0.000, *F*=19.732, Figure 4A). Compared with the SMIR + saline group, the PMWT in the SMIR + TrkB/Fc group significantly increased but was still lower than in the groups that received the sham procedure (*P*=0.045, *F*=3.053, Figure 4A).

The Western blot analysis (*P*=0.011, *F*=93.353) and immunofluorescence assay revealed that KCC2 expression in the ipsilateral spinal dorsal horn was significantly downregulated in the SMIR + saline and SMIR + TrkB/Fc groups compared to the sham + saline and sham + TrkB/Fc groups; but compared with the SMIR + saline group, KCC2 expression was clearly upregulated (Figure 4B and C, *P*<0.05).

IP injections of DEX attenuated the SMIR-evoked PPSP

In our study, DEX was administered 15 min presurgery and at PODs 1–3, which was consistent with clinical multimodal analgesia. After IP injections of 40 μg/kg DEX, rats presented mild drowsiness and reduced locomotor activity but could be easily awaken without significant barrier to activities. Rats without DEX administration did not show any loss of consciousness or movement disorders. A short time later, no obvious differences were observed in all rats with normal activity and righting reflex.

No differences were detected between the sham + saline and sham + DEX groups (*P*>0.05). A comparison of the SMIR + saline group's PMWT values with those of the sham + saline or sham + DEX groups revealed significant differences after the surgery. Compared with the SMIR + saline group, the PMWT in the SMIR + DEX group significantly increased but was still lower than in the groups that received the sham procedure (*P*=0.001, *F*=12.989, Figure 5).

Effects of DEX on the protein expressions of BDNF and TrkB in the ipsilateral spinal dorsal horn in SMIR rats

Western blot analysis revealed that IP administrations of DEX significantly inhibited SMIR-induced upregulation of BDNF and TrkB in the dorsal horn of the ipsilateral L4-6 spinal cord in the SMIR + DEX group versus the SMIR + saline group rats, but the protein expressions were still higher than in the sham + saline and sham + DEX groups (P_{BDNF}=0.001,

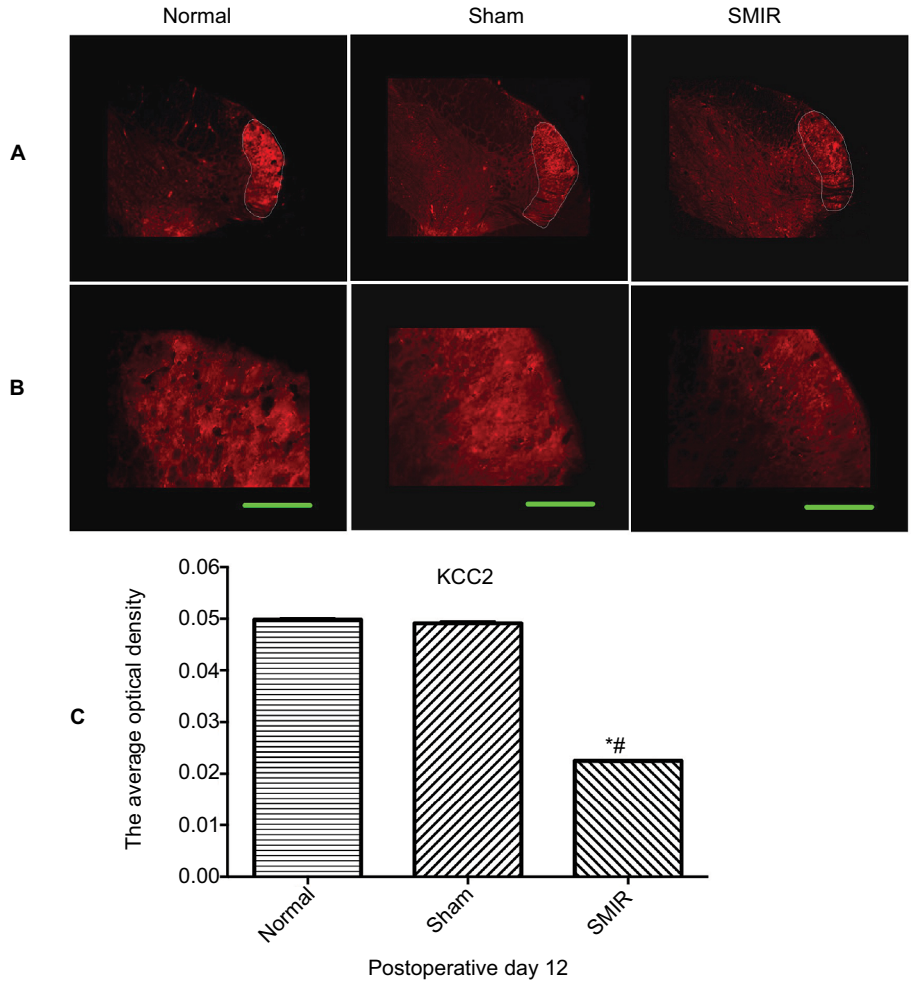

Figure 3 Immunofluorescence assay of KCC2 in the ipsilateral spinal dorsal horn following SMIR.
Notes: KCC2 immunolabeling (red) was significantly downregulated in the ipsilateral spinal dorsal horn (n=5) (**A**) and KCC2 expression in the superficial dorsal horn layers (**B**). Magnification: A =50× and B =400×. Scale bar: 20 mm. (**C**) *P<0.05 vs normal group and #P<0.05 vs sham group.
Abbreviation: SMIR, skin/muscle incision and retraction.

F=1037.952; P_{TrkB}=0.001, F=1818.968). No differences were observed between the sham + saline and sham + DEX groups (Figure 6A–C).

The effect of DEX on KCC2 expression in the ipsilateral spinal dorsal horn in SMIR rats

KCC2 expression in the dorsal horn of the ipsilateral L4-6 spinal cord in the SMIR + DEX group clearly increased compared to that in the SMIR + saline group (P=0.001, F=708.051; Figure 6A and D). Furthermore, compared with the SMIR + saline group, the KCC2 monomer, oligomer, and oligomer/monomer ratio were upregulated in the SMIR + DEX group but were still lower than in the sham + saline and sham + DEX groups (Figure 7A–D, P<0.05). Additionally, we studied the effect of DEX on KCC2 expression in the spinal dorsal horn by immunofluorescence assay. No significant difference between the sham + saline and sham + DEX groups was detected in KCC2 expression in the ipsilateral spinal dorsal horn (P>0.05). Marked upregulation of KCC2 was found in the SMIR + DEX group compared to SMIR + saline group but was still lower than in the sham + saline and sham + DEX groups (Figure 7E and F, P<0.05).

KCC2 inhibitor VU0240551 reduced the analgesic effect of DEX on SMIR-evoked PPSP and downregulated KCC2 in the ipsilateral spinal dorsal horn

To further verify our hypotheses, we intrathecally administered 0.27 μg KCC2 inhibitor VU0240551 15 min presurgery and at PODs 1–3 in DEX-treated rats. The results showed that compared with the SMIR + DEX + DMSO group, the PMWT

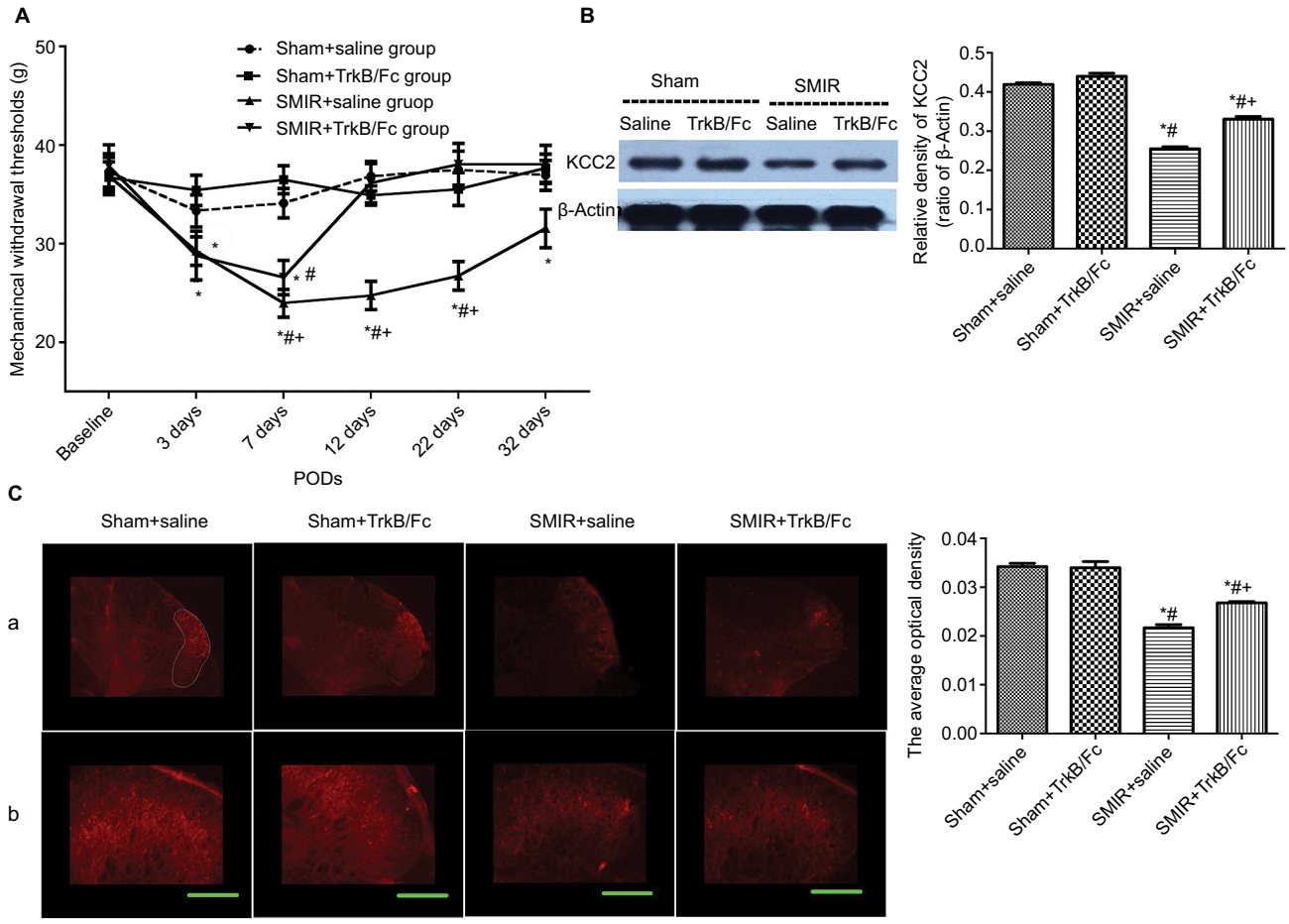

Figure 4 The effects of TrkB/Fc 5 μg intrathecally administered at 15 min presurgery and at PODs 1–3 on SMIR rats.
Notes: (**A**) TrkB/Fc significantly increased the PMWT of SMIR rats after SMIR. (**B**) KCC2 expression in the SMIR + TrkB/Fc group significantly increased compared with the SMIR + saline group. (**C**) KCC2 expression in the ipsilateral of spinal dorsal horn (a) and KCC2 expression in the superficial dorsal horn layers (b). Magnification: a =50× and b =400×. Scale bar: 20 mm. *P<0.05 vs sham + saline group, #P<0.05 vs sham + TrkB/Fc group, and +P<0.05 vs SMIR + saline group.
Abbreviations: PMWT, paw mechanical withdrawal threshold; PODs, postoperative days; SMIR, skin/muscle incision and retraction.

of the SMIR + DEX + VU0240551 group was significantly lower after SMIR surgery (P=0.006, F=11.760, Figure 8A). The Western blot analysis and immunofluorescence assay revealed that KCC2 expression in the ipsilateral spinal dorsal horn in the SMIR + DEX + VU0240551 group clearly decreased compared with the SMIR + DEX + DMSO group (P<0.05, Figure 8B–E).

Discussion

Although a considerable number of drugs are used for clinical analgesia, PPSP still has a high prevalence, particularly in certain surgeries that require essential prolonged tissue retraction, such as thoracotomy and inguinal hernia repair,[3,4] so novel mechanism-based treatments are severely lacking and are undoubtedly required for postsurgical pain management. To better approach this problem, in 2008, Flatters[26] proposed an SMIR-evoked PPSP model to mimic clinical surgeries that require prolonged tissue retraction without damaging the saphenous nerve. In the present study, we observed that the PMWT significantly decreased and remained at a low level throughout the experiment in the ipsilateral hindpaw of rats that received SMIR surgery (Figure 1). It is notable that, unlike the model in which intercostal nerve damage was closely associated with the degree and incidence of postoperative pain described by Buvanendran et al,[29] there were no obviously damaged main nerves in the SMIR model.[26]

One of the main findings of our experiment was the role of KCC2 in the spinal dorsal horn in SMIR-evoked PPSP. The data to date suggest that in pain-signaling pathways, GABAergic inhibition loss plays a critical role in central sensitization, which is indispensable for the acute–chronic pain conversion.[5,12,30–32] One main reason for the transition is the impaired intracellular chloride homeostasis due to the decreased activity and expression of KCC2, a postsynaptically restricted Cl^-/K^+ cotransporter that is crucial for postsynaptic inhibition mediated by GABAA receptors.[33] Previous studies

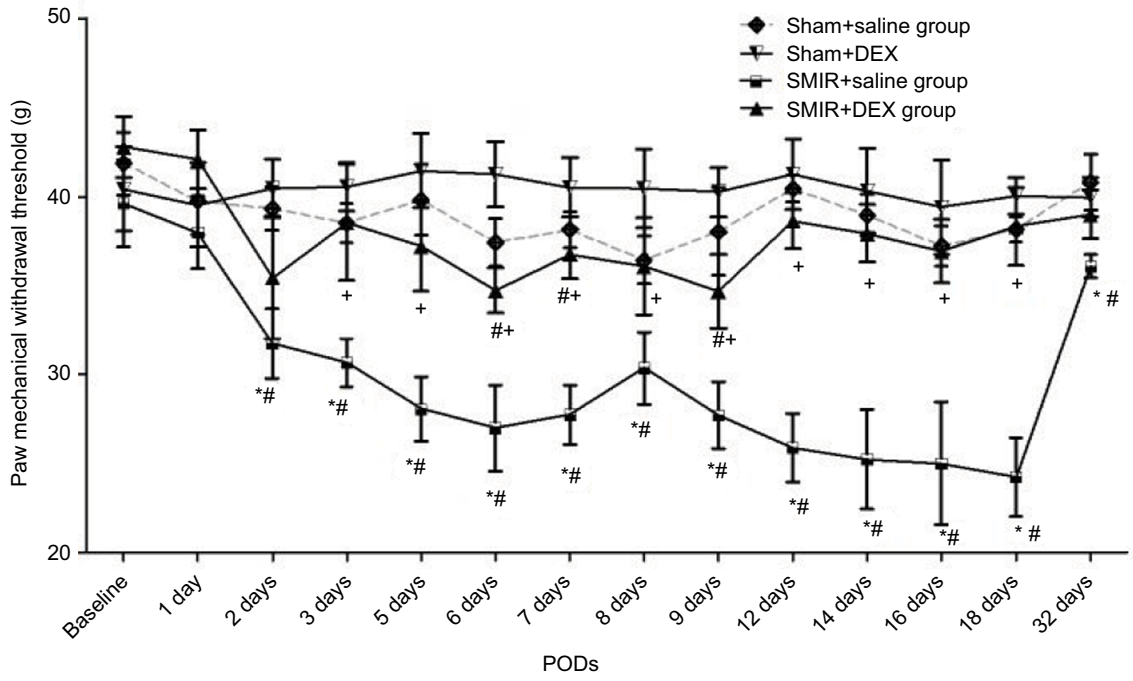

Figure 5 IP administration of 40 μg/kg DEX 15 min presurgery and at PODs 1–3 significantly attenuated the SMIR-evoked PPSP in rats.
Notes: Compared with the SMIR + saline group, the PMWT of the SMIR + DEX rats significantly increased but was still lower than in the sham + saline and sham + DEX groups (n=8). *P<0.05 vs sham + saline group, #P<0.05 vs sham + DEX group, and +P<0.05 vs SMIR + saline group.
Abbreviations: DEX, dexmedetomidine; IP, intraperitoneal; PMWT, paw mechanical withdrawal threshold; PODs, postoperative days; PPSP, persistent postsurgical pain; SMIR, skin/muscle incision and retraction.

have shown that downregulation of KCC2 contributes to the early behavioral signs of hyperalgesia induced by loose ligation of the sciatic nerve,[12] morphine-induced hyperalgesia,[7] and persistent inflammation pain resulting from the activation of the descending pain-facilitating pathway, which may be critical in the process of central sensitization during the acute–chronic pain conversion.[11,31] Pharmacological inhibition of KCC2 by its inhibitor, VU0240551, leads to thermal hypersensitivity in normal rats,[10] while rescuing the plasma membrane expression of KCC2 using CLP257, an analog of CL058 with better chemical stability and overall properties, reduced $[Cl^-]i$ at a maximum of ~40%, arouse KCC2 transport activity by 61% (P<0.01), and substantially increased the Cl^- transport, which could obviously alleviate the hyperalgesia of neuropathic pain in rats, further indicating that KCC2 is necessary and sufficient for nociception modulation. However, the role of KCC2 in SMIR-evoked PPSP is still to be elucidated. In our present experiment, we found a significant and prolonged reduction of KCC2 expression in the spinal dorsal horn following SMIR and the time course of variations was in agreement with the decline of the PMWT. More specifically, functional KCC2 exists mostly in the form of oligomers on the membrane surface of mature neurons and altered KCC2 oligomerization and clusters would lead to a loss of KCC2 transport activity,[28,34] which is closely associated with the modulation of nociception.[7,35] In the SMIR-evoked PPSP model, we also observed significant decrease in KCC2 oligomerization, indicating that KCC2 downregulation was at least partly involved in the process of central sensitization during the acute–chronic pain conversion in SMIR-evoked PPSP, which may provide a novel guidance for the treatment of postoperative chronic pain.

Another important finding was the modulation of the BDNF/TrkB signal on KCC2 in the spinal dorsal horn of SMIR-evoked PPSP rats. We observed a simultaneous time course of variations in KCC2 and the BDNF/TrkB signal in the spinal dorsal horn following SMIR. It has been well documented that BDNF is an important regulator for pain transmission in the spinal dorsal horn, which mainly functions through its high-affinity TrkB, specifically the BDNF/TrkB signal.[36,37] The signal was originally found to downregulate KCC2 in the hippocampal culture,[38] but later, more direct supporting evidence verified that downregulation of KCC2 induced by the activation of the BDNF/TrkB signal is responsible for the disinhibition of GABAergic neurons under sensitized pain conditions, and blocking the signal between

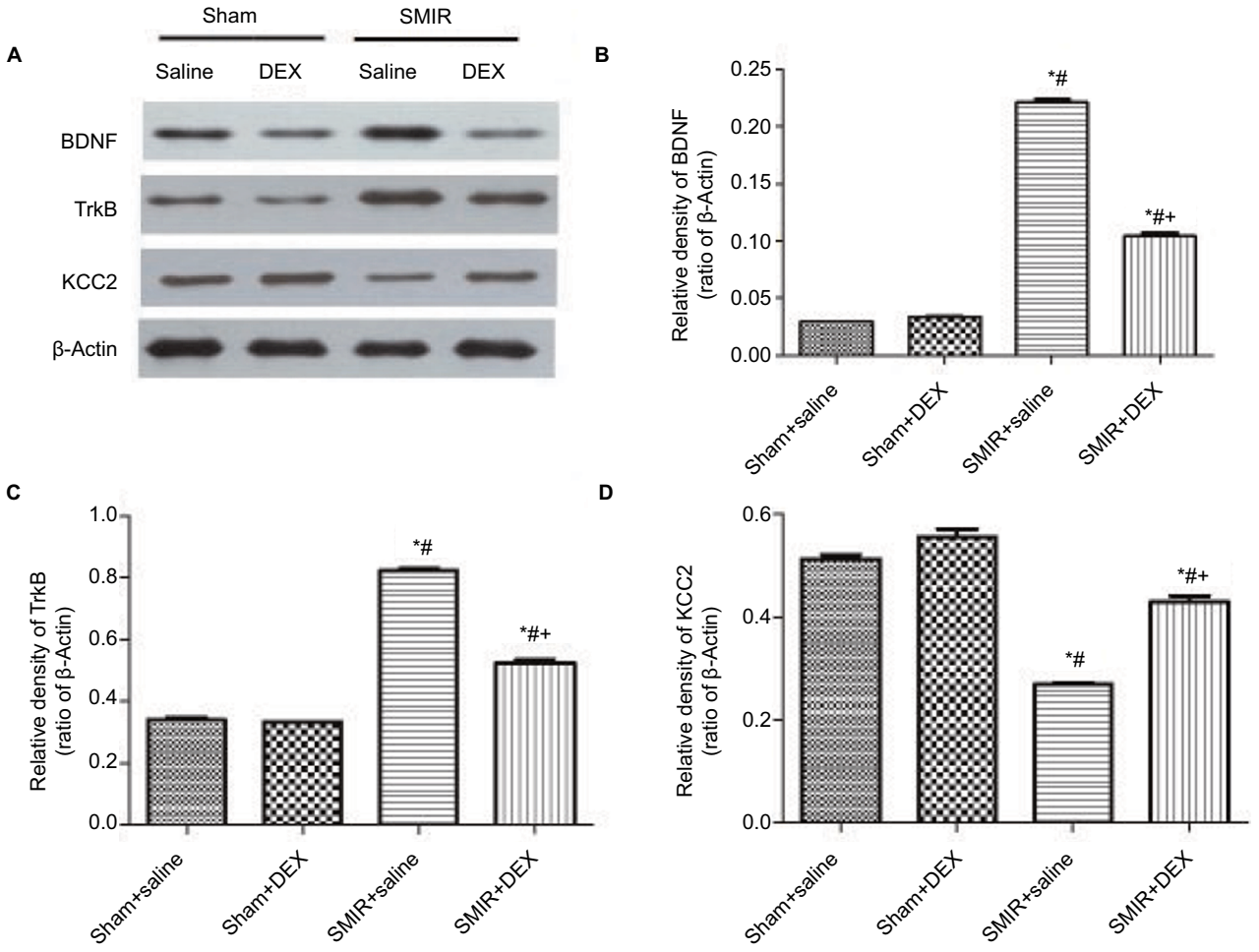

Figure 6 Effect of DEX IP administration 15 min presurgery and at PODs 1–3 on the expressions of BDNF, TrkB, and KCC2 in the spinal dorsal horn.
Notes: Western blot displayed that DEX treatment inhibited BDNF/TrkB signal expression (**A**–**C**) and upregulated KCC2 expression (**A** and **D**) in the spinal dorsal horn of the SMIR + DEX group (n=5/time point). **P*<0.05 vs sham + saline, #*P*<0.05 vs sham + DEX, and +*P*<0.05 vs SMIR + saline.
Abbreviations: BDNF, brain-derived neurotrophic factor; DEX, dexmedetomidine; IP, intraperitoneal; PODs, postoperative days; SMIR, skin/muscle incision and retraction.

BDNF and TrkB reversed the E (anion) shift and hyperalgesia/allodynia.[7,11,12,35] In our experiment, blocking the BDNF/TrkB signal by intrathecal administrations of BDNF-sequester TrkB/Fc significantly attenuated the SMIR-evoked PPSP and upregulated KCC2 expression in the spinal dorsal horn, to some extent indicating that the BDNF/TrkB-KCC2 signal plays a key role in the development of SMIR-evoked PPSP.

Previous studies have reported that the main site in which α2-adrenoceptors' agonists function is the spinal cord,[39] where the activation of α2-adrenoceptors directly blocks the nociceptive neurotransmission.[40,41] Functional α2-adrenoceptors' alterations and actions of BDNF on cholinergic terminals after peripheral nerve injury play a vital role in the function of analgesics commonly used to treat neuropathic pain.[42] Recently, DEX, a highly selective agonist of α2-adrenergic receptors, has attracted researchers' interest because of its good analgesic properties, which are comparable to opioids without the side effects of the latter, such as dependence and tolerance.[19,20,22,23] To date, the mechanisms of analgesic effects in neuropathic pain reportedly involve in the downregulation of P2×4Rs, p-p38, and BDNF in the microglia of the spinal dorsal horn;[22] the activation of α2-adrenoceptor augmented by increased Ach in the lumbar spinal cord;[20] and the augmentation of inhibitory synaptic transmission in the superficial dorsal horn by activating the descending noradrenergic control.[16] However, the interaction between DEX and KCC2 at a molecular level in the development of PPSP is still to be explored. IP administration was selected in our study because DEX is highly lipid soluble; in addition, after systemic administration, it could readily

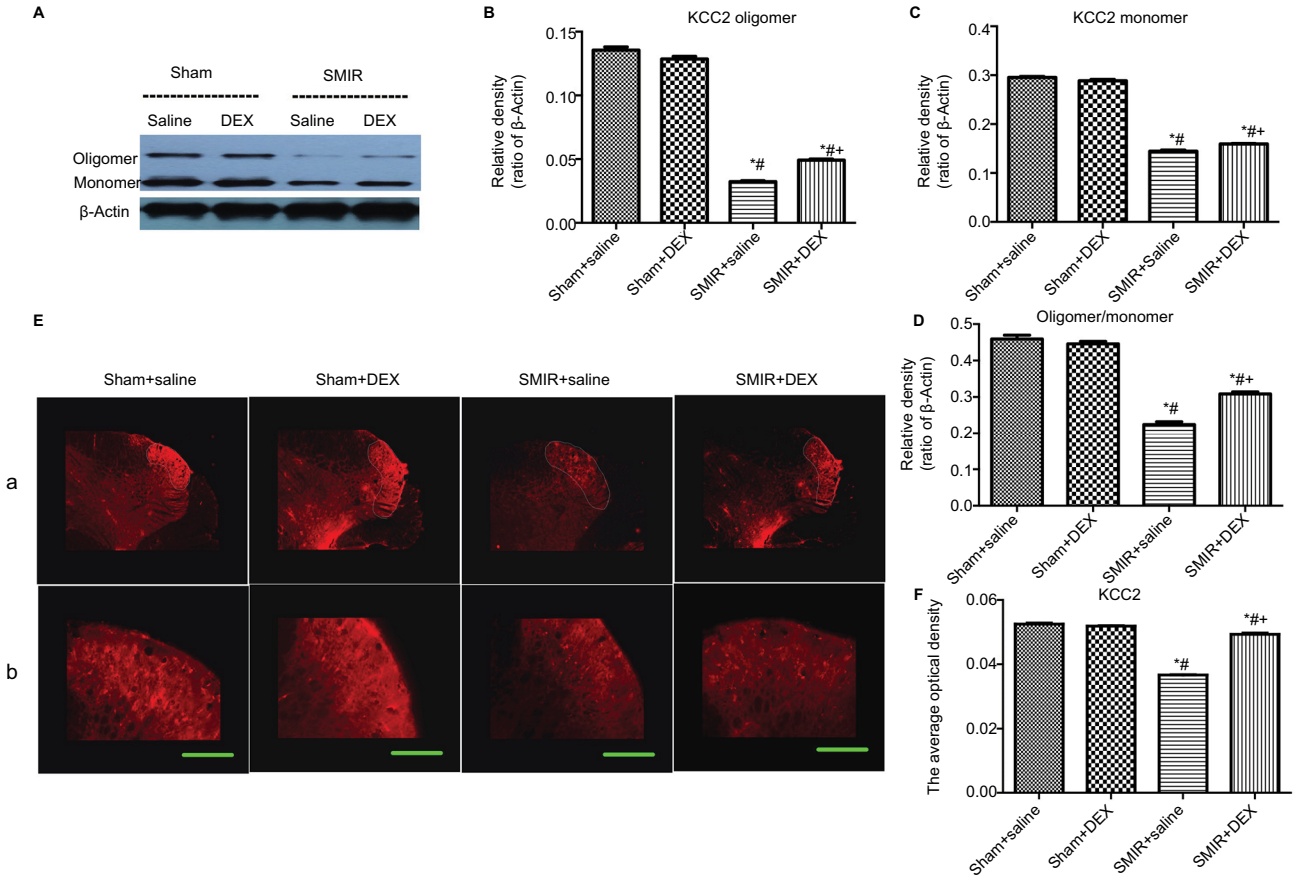

Figure 7 The effect of DEX on KCC2 expression in the spinal dorsal horn.

Notes: (**A**–**D**) Effect of DEX on KCC2 oligomerization in the ipsilateral spinal dorsal horn of rats (n=5/time point). (**E** and **F**) Immunofluorescence analysis indicated that DEX significantly upregulated KCC2 following SMIR in the ipsilateral spinal dorsal horn (a) and KCC2 expression in the superficial dorsal horn layers (b). Magnification: a =50× and b =400×. Scale bar: 20 mm. *P<0.05 vs sham + saline group, #P<0.05 vs sham + DEX group, and +P<0.05 vs SMIR + saline group.

Abbreviations: DEX, dexmedetomidine; SMIR, skin/muscle incision and retraction.

cross the blood–brain barrier. Administrations 40 μg/kg DEX at 15 min presurgery and at PODs 1–3 were carried out in accordance with recommendations for clinical multimodal analgesia and previous reports.[21,22] In our experiment, it was observed that IP administrations of DEX significantly attenuated the postoperative persistent pain and the BDNF/TrkB-KCC2 signal was partly reversed in the spinal dorsal horn. Moreover, KCC2 inhibitor VU0240551 administrations significantly reduced the analgesic effect of DEX on SMIR-evoked PPSP. At the same time, KCC2 expression was also clearly lower than in the DEX-treated group, which further verifies the role of KCC2 in the analgesic effect of DEX on SMIR-evoked PPSP.

Conclusion

In the present study, we explored the role of BDNF/TrkB-KCC2 in the analgesic effect of DEX on SMIR-evoked PPSP. The results suggest that DEX significantly alleviates the development of SMIR-evoked PPSP, while the BDNF/TrkB-KCC2 signal in the spinal dorsal horn might account for the analgesic mechanisms.

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Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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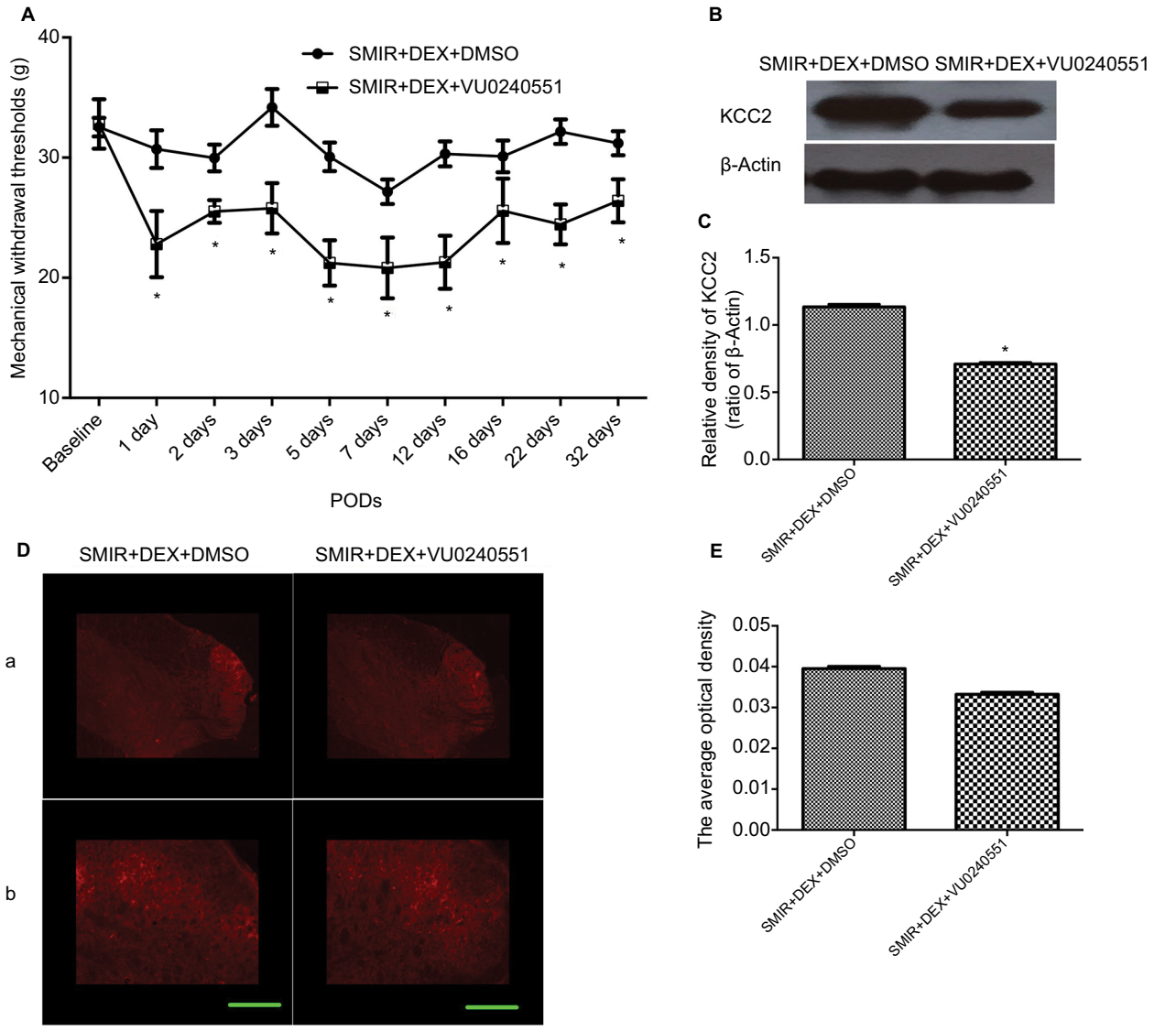

Figure 8 KCC2 inhibitor VU0240551 intrathecally administered at 15 min presurgery and at PODs 1–3 reduced the effect of DEX on SMIR rats.
Notes: (**A**) The influence of VU0240551 on the analgesic effect of DEX in SMIR-induced PPSP rats. (**B** and **C**) KCC2 expression in the SMIR + DEX + VU0240551 group was significantly inhibited compared with the SMIR + DEX + DMSO group. (**D** and **E**) KCC2 expression in the ipsilateral spinal dorsal horn (a) and KCC2 expression in the superficial dorsal horn layers (b). Magnification: a =50× and b =400×. Scale bar: 20 mm. *P<0.05 vs SMIR + DEX + DMSO group.
Abbreviations: DEX, dexmedetomidine; PODs, postoperative days; SMIR, skin/muscle incision and retraction.

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