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Skin/muscle incision and retraction regulates the persistent postoperative pain in rats by the Epac1/PKC-βII pathway

Jiashu Qian[†], Xuezheng Lin[†] and Zhili Zhou[*]

Abstract

Persistent postoperative pain causes influence the life quality of many patients. The Epac/PKC pathway has been indicated to regulate mechanical hyperalgesia. The present study used skin/muscle incision and retraction (SMIR) to induce postoperative pain in rats and evaluated the Epac/PKC pathway in postoperative pain. Mechanical allodynia was assessed by paw withdrawal threshold before and after incision. The levels of Epac, PKC, proinflammatory cytokines, and blood-nerve barrier-related proteins were assessed using Western blotting. We found that SMIR induced the activation of the Epac/PKC pathway, mechanical allodynia, and upregulation of Glut1, VEGF, and PGP9.5 proteins in dorsal root ganglia. Under the influence of agonists of Epac/PKC, normal rats showed mechanical allodynia and increased Glut1, VEGF, and PGP9.5 proteins. After inhibition of Epac1 in rats with SMIR, mechanical allodynia was alleviated, and proinflammatory cytokines and Glut1, VEGF, and PGP9.5 proteins were decreased. Moreover, dorsal root ganglia neurons showed abnormal proliferation under the activation of the Epac/PKC pathway. Using Captopril to protect vascular endothelial cells after SMIR had a positive effect on postoperative pain. In conclusion, SMIR regulates the persistent postoperative pain in rats by the Epac/PKC pathway.

Keywords: Persistent postoperative pain, Skin/muscle incision and retraction, Epac1/PKC-βII

Introduction

Pain refers to a conscious unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. Pain sensation results from complicated mechanisms underlying all the nervous systems, from the periphery, throughout the spinal cord, to the cerebral structures [2]. Patients undergo postoperative pain due to the surgical procedure, preexisted diseases or a combination of the above-related causes [3]. Postoperative pain is deemed as the cause of multiple postoperative complications, and pain relief after surgery remains a major medical challenge [4]. A recent study suggested that 75% of patients who had received major surgery experienced postoperative pain, and 70% of them claimed to have unbearable pain despite taking analgesics [5]. For humanitarian reasons and to decrease postoperative morbidity and mortality, improvement in postoperative analgesia is essential [6]. Methods to control postoperative pain include pharmacologic treatment, anesthesia, cognitive treatment, and behavior methods [7].

Prostaglandin E2 is the best known lipid mediator that contributes to inflammatory pain, which leads to mechanical hyperalgesia by the cAMP/PKCε pathway after inflammation [8]. Under different conditions, cAMP stimulates Epacs to activate small Ras family of G proteins [9, 10], and activates many effector proteins, including PKCε and MAP kinases to facilitate various pathological states, including chronic pain [11–13]. Epac

[†]Jiashu Qian and Xuezheng Lin contributed equally to this work.

[*]Correspondence: Zhouzhl@tzzxyy.com

Department of Anesthesiology, Taizhou Central Hospital (Affiliated Hospital of Taizhou University), No. 999 Donghai Avenue, Jiaojiang Economic Development Zone, Taizhou City 318000, Zhejiang Province, China

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can mediate the activation of PKCε to cause mechanical hyperalgesia.

Maximum plasma levels of proinflammatory cytokines including TNFα, IL-1β, and IL-6 were immediately found after surgery [14]. Moreover, alterations of the blood-nerve barrier (BNB) functions are associated with nerve injury [15]. Besides, this homeostatic barrier disrupts the healing process after injury [16] and also regulates nerve sensitization [17]. A recent study revealed the implication of vascular endothelial growth factor (VEGF) in BNB opening [18].

In the present study, we used a rat model of persistent postoperative pain to evaluate the role of the Epac/PKC pathway in postoperative pain. Effects of this pathway in the expression of inflammation and BNB-related proteins and mechanical hyperalgesia were assessed, which can provide a new insight into the pathogenesis of postoperative pain.

Materials and methods

Animals
Adult male Sprague–Dawley rats weighing at 200–240 g were housed on sawdust bedding in plastic cages with available food and water *ad libitum*. Animals were kept under 50% humidity at $25 \pm 2°C$ in a 12 h light and 12 h dark cycle. All the animal studies were performed following the guidelines of the Association for the Study of Pain and were approved by the Institutional Animal Care and Use Committee of Taizhou Central Hospital. Skin/muscle incision and retraction (SMIR) was operated in the thigh to establish a rat model of persistent postoperative pain, akin to a clinical procedure. Under anaesthesia, skin and superficial muscle of the medial thigh were incised and a small pair of retractors inserted. This tissue was retracted for 1h causing potential stretch of the saphenous nerve [19]. The SMIR model can evaluate pain behavior by hind paw stimulation and prolonging tissue retraction. Persistent mechanical hypersensitivity was evoked in more than 80% of SMIR-operated rats.

Animal grouping
Rats were assigned into eleven groups: (i) the control group: rats received no treatment; (ii) the sham group: rats were sham-operated; (iii) the 0.25% DMSO group: rats were intraperitoneally injected with 0.25% DMSO; (iv) the PMA group: rats were intraperitoneally injected with 100 ng of PMA, the PKC-βII agonist; (v) the 8-pCPT group: rats were intraperitoneally injected with 500 ng of 8-pCPT, agonist for Epac1; (vi) the SMIR group: rats received SMIR surgery; (vii) the SMIR + Ad-sh-NC group: rats received SMIR surgery after injection of adenovirus expressing negative control short hairpin RNA; (viii) the SMIR + Ad-sh-Epac1 group: rats received SMIR

surgery after injection of adenovirus expressing short hairpin RNA against Epac1; (ix) the SMIR + LY333531 group: rats received SMIR surgery and injection of 1 mg/kg LY333531 (Eli Lilly, Indianapolis, IN). LY333531 is selective inhibitors of conventional protein kinase C; (x) the SMIR + saline group: rats were intraperitoneally injected with 20 mg/kg/day normal saline for 14 days before the SMIR surgery; (xi) the SMIR + Captopril group: rats were intraperitoneally injected with 20 mg/kg/day Captopril for 14 days before the SMIR surgery. Captopril is an angiotensin-converting enzyme (ACE) inhibitor with antioxidant and anti-inflammatory properties which has been suggested as the preferred agents for treatment on pain modulation [20]. Here we used Captopril to protect vascular endothelial cells after SMIR and have a positive effect on postoperative pain

Von Frey filament testing
Hind paw allodynia was assessed by von Frey filaments (Bioseb, Chaville, France). Rats were accommodated in a small plastic cage ($35 \times 20 \times 15$ cm) for 1 h. Starting with the lowest force, each filament was applied 3 times with intervals of 3 seconds. The stimulation was terminated upon either a brisk paw withdrawal and/or an escape attempt, and the force was recorded as the mechanical response threshold. The cut-off threshold was set as 30 g.

Isolation and culture of the DRG (DRG) neurons
Dorsal root ganglia (L3-L5) were harvested 1 hr, removed from rats, and dissected in an oxygenated ice-cold dissecting solution. After incubation in the dissecting solution supplemented with 1 mg/mL trypsin and collagenase D at room temperature for 1 h, dorsal root ganglia were removed from the solution, washed, and dissociated by trituration. Cells were cultured in Neuro Basal-A medium with 2% B27 supplement, 2 mM GlutaMAX, and antibiotics at the ambient temperature of 5% CO_2 and 80% humidity. The next day after incubation, cells were treated with 20 mM 5-fluorodeoxyuridine and uridine for 3 days to remove non-neural cells.

Cell treatment
DRG neurons were assigned into six groups: (i) the control group; (ii) cells were treated with 0.25% DMSO; (iii) cells were treated with 100 μM 8-pCPT; (iv) cells were treated with 50 ng/mL PMA; (v) cells were transfected with sh-Epac1 (GenePharma) using the lipofectamine 3000 (Invitrogen) for 48 h and then treated with 100 μM 8-pCPT; (vi) cells were cotreated with 50 ng/mL PMA and 0.5 μM LY333531.

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Reverse transcription-quantitative PCR

Total RNA was extracted from DRG cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and the RNA purity was determined by the SmartSpec Plus Spectrophotometer (Bio-Rad Laboratories). A Transcriptor First Strand cDNA synthesis kit (Roche) was used for the synthesis of the cDNA. PCR was conducted on an ABI 7500 realtime PCR system using SYBR Green PCR Master Mix (both from Applied Biosystems) following the manufacturer's protocol. NF200 and NSE mRNA expression levels were calculated using the 2(-Delta Delta C(T)) method [21] and were normalized to internal control Actin. Primer sequences used in PCR analysis are listed below:

NF200: forward, 5'-GTTCCGAGTGAGATTGGAC-3'; reverse, 5'-GTTATCTCCTCTTGGGCAG-3'.
NSE: forward, 5'-CGGAACTATCCTGTGGTCTC-3'; reverse, 5'-GACATTGGCTGTGAACTTGG-3'.
TNF-α: forward, 5'-CTTCTCATTCCTGCTCGTG-3'; reverse, 5'-TTTGGGAACTTCTCCTCCT-3'.
IL-1β: forward, 5'-TTCATCTTTGAAGAAGAGCCC-3'; reverse, 5'-CTGTCTAATGGGAACATCACAC-3'.
MCP-1: forward, 5'-GAAGCTGTAGTATTTGTCACCA-3'; reverse, 5'-TCTAATGTACTTCTGGACCCA-3'.
Actin: forward, 5'-CTTCCTGGGTATGGAATCCT-3'; reverse, 5'-TCTTTACGGATGTCAACGTC-3'.

Western blotting

After culturing for 48 h, Western blotting was performed on DRG neurons. Proteins (60 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a polyvinylidene difluoride membrane. The membrane was blocked with 5% skim milk powder and incubated with primary antibodies to Epac1 (1:1000, ab124162), p-PKCβII (1:1000, #9375), PKCβII (1:3000, ab32026), Glut1 (1:100000, ab115730), VEGF (1:1000, ab1316), EPO (1:5000, ab226956) or PGP9.5 (1:1000, ab108986), PCNA (1:1000, ab92552), Cyclin D1 (1:200, ab16663), and the loading control β-actin (1:5000, ab8227) at 4°C overnight. On the next day, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:2000, ab6721). Antibodies were purchased from Abcam (Shanghai, China) or Cell Signaling TECHNOLOGY. Finally, membranes were exposed to an Enhanced Chemiluminescence Detection Kit (Thermo, Waltham, MA) for protein visualization. The gray density of the protein bands was quantified with the ImageJ software.

Cell viability assay

Cell viability was assessed by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma) assay. DRG neurons were seeded into a 96-well plate at the density of 1×10^4 cells/well for 12, 24, and 48 h. Next, the media was replaced with the media containing 0.5 mg/mL MTT and cells were incubated at ambient temperature for another 4 h. Formazan crystals were solubilized by dimethyl sulfoxide, and cell absorbance was measured at 570 nm.

Statistical analysis

Data from three technical repeats are presented as the mean ± SEM. Differences between 2 mean values were analyzed with the Student unpaired *t*-test. Comparisons between multiple mean values were performed with one-way ANOVA and the Tukey's *post hoc* test or two-way ANOVA. *P* values less than 0.05 were statistically significant.

Results

Effects of SMIR surgery on the Epac/PKCβII pathway, mechanical hypersensitivity in rats
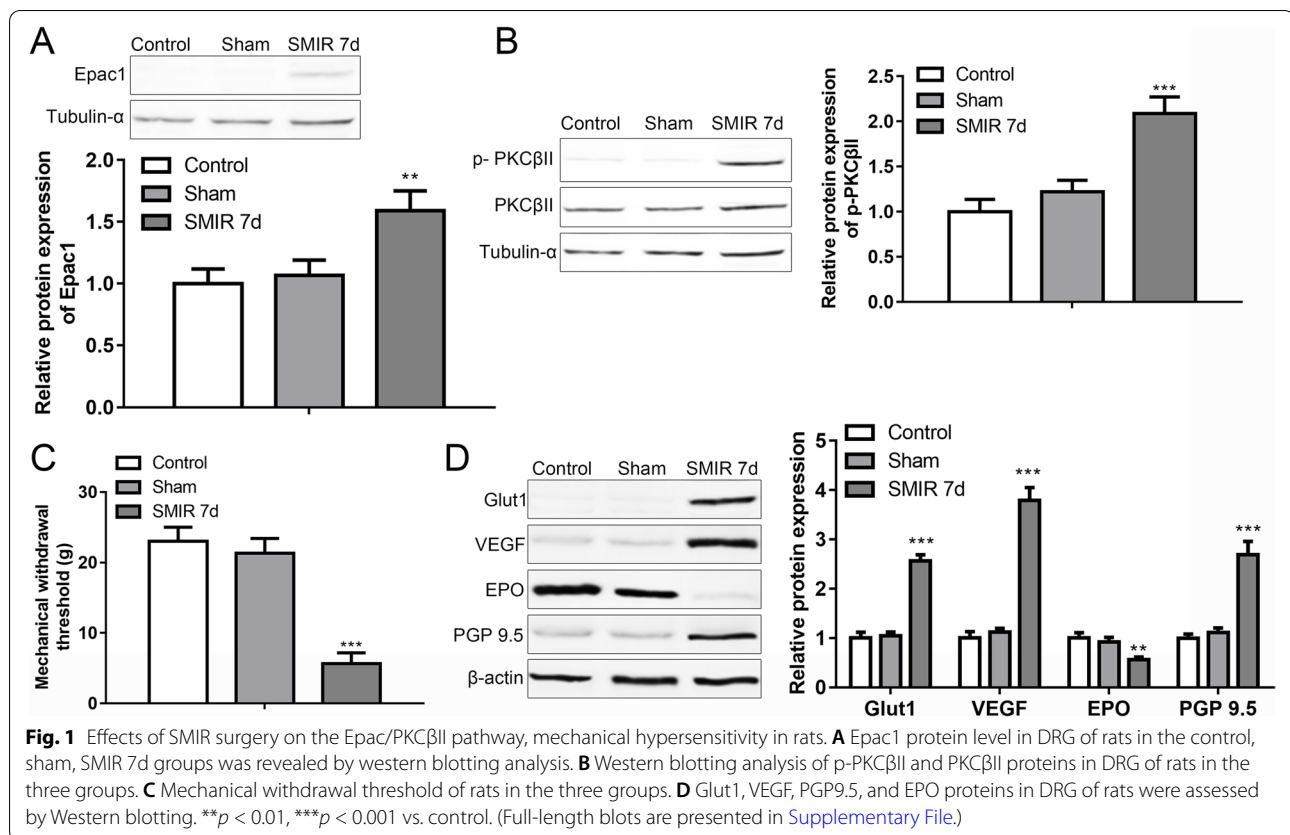
Relative protein expression of Epac1 was higher in the DRGs from rats after SMIR for seven days than DRGs from control rats (Fig. 1A). Expression of p-PKCβII was also higher in the DRGs from rats after SMIR for seven days than control DRGs (Fig. 1B). SMIR surgery significantly evoked the mechanical hypersensitivity in the hind paw of rats (Fig. 1C). Moreover, Glut1, VEGF, and PGP9.5 proteins in DRGs were increased, while EPO protein was decreased by SMIR after 7 days (Fig. 1D).

The Epac/PKCβII pathway mediates inflammatory microenvironment and mechanical hypersensitivity in rats after SMIR

After the injection of 8-pCPT (agonist for Epac1) (Fig. 2A) or PMA (agonist for PKCβII) (Fig. 2B), SMIR-operated rats exhibited the decreased mechanical withdrawal threshold. 8-pCPT treatment caused the upregulation of Epac1, Glut1, VEGF, and PGP9.5 proteins as well as the downregulation of EPO protein in the SMIR-operated rats (Fig. 2C). Furthermore, PMA treatment caused the increase of p-PKCβII, Glut1, VEGF, PGP9.5 proteins and the decrease of EPO protein in the SMIR-operated rats (Fig. 2D).

Suppression of the Epac/PKCβII pathway rescues the SMIR-induced inflammatory microenvironment and mechanical hypersensitivity

After injection of Ad-sh-Epac1, SMIR-operated rats showed decreased Epac1 expression in the DRG

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Fig. 1 Effects of SMIR surgery on the Epac/PKCβII pathway, mechanical hypersensitivity in rats. **A** Epac1 protein level in DRG of rats in the control, sham, SMIR 7d groups was revealed by western blotting analysis. **B** Western blotting analysis of p-PKCβII and PKCβII proteins in DRG of rats in the three groups. **C** Mechanical withdrawal threshold of rats in the three groups. **D** Glut1, VEGF, PGP9.5, and EPO proteins in DRG of rats were assessed by Western blotting. ***p* < 0.01, ****p* < 0.001 vs. control. (Full-length blots are presented in Supplementary File.)

(Fig. 3A). Compared with the rats with only SMIR operation, SMIR-operated rats with adenovirus-mediated Epac1 deficiency showed the lower mRNA expression of proinflammatory factors including TNF-α, IL-1β, and MCP-1 in the DRG (Fig. 3B). Administration of LY333531 caused the inhibition of p-PKCβII protein (Fig. 3C) and induced the decrease of TNF-α, IL-1β, and MCP-1 levels (Fig. 3D) in the DRG of SMIR-operated rats. Administration of Ad-sh-Epac1 or LY333531 decreased mechanical withdrawal threshold in rats after SMIR operation for seven days (Fig. 3E). Figure 3F revealed that suppression of Epac1 and PKCβII decreased the Glut1, VEGF, PGP9.5 proteins and increased the EPO protein in the DRG of rats after SMIR operation for seven days.

The Epac/PKCβII pathway promotes the abnormal proliferation of the DRG cells

Primary DRG cells were isolated and confirmed by RT-qPCR, as evidenced by the presence of NF200 and NSE (Fig. 4A). After treatment with 8-pCPT and PMA, the cell viability of DRG cells was increased, while sh-Epac1 rescued the effect of 8-pCPT, and LY333531 rescued the

effect of PMA on cell viability (Fig. 4B). 8-pCPT or PMA treatment also enhanced the protein levels of PCNA and CyclinD1, and sh-Epac1 and LY333531 rescued the effect of 8-pCPT and PMA on these proteins, respectively (Fig. 4C).

Protection of vascular endothelial cells on mechanical hypersensitivity and MCP-1 expression

Pretreatment with Captopril enhanced mechanical withdrawal threshold (Fig. 5A) and decreased MCP-1 expression in DRG (Fig. 5B) in rats after SMIR operation for seven days. Figure 5C, D indicated that treatment of Captopril after SMIR operation for seven days also increased mechanical withdrawal threshold (Fig. 5C) and reduced MCP-1 expression in DRG (Fig. 5D) in rats.

Discussion

Management of postoperative pain is hindered by the poor understanding of the pathogenesis underlying postoperative pain [22]. Surgery-induced increase of proinflammatory factors in DRG exerts an essential role in generating neuronal hypersensitivity and thus contributing to postoperative pain [23]. In the present
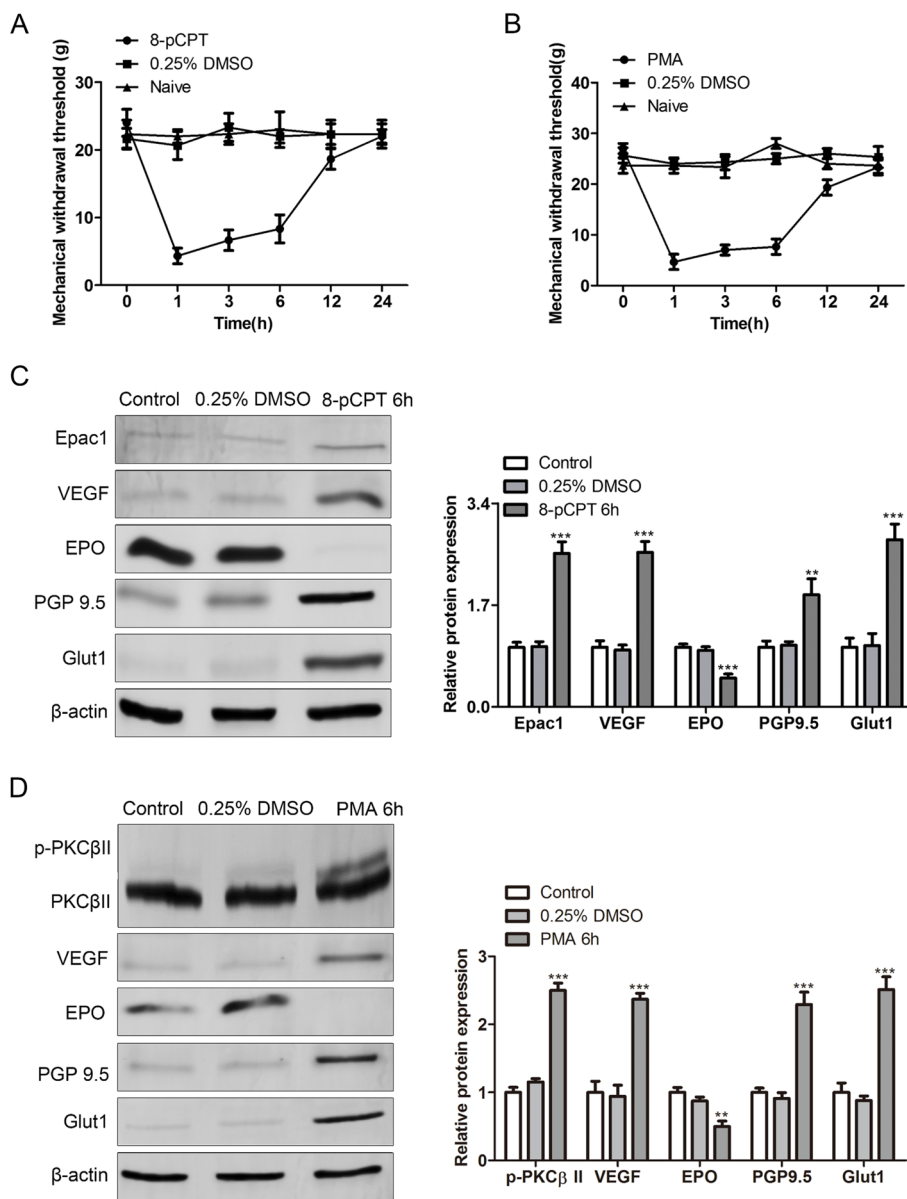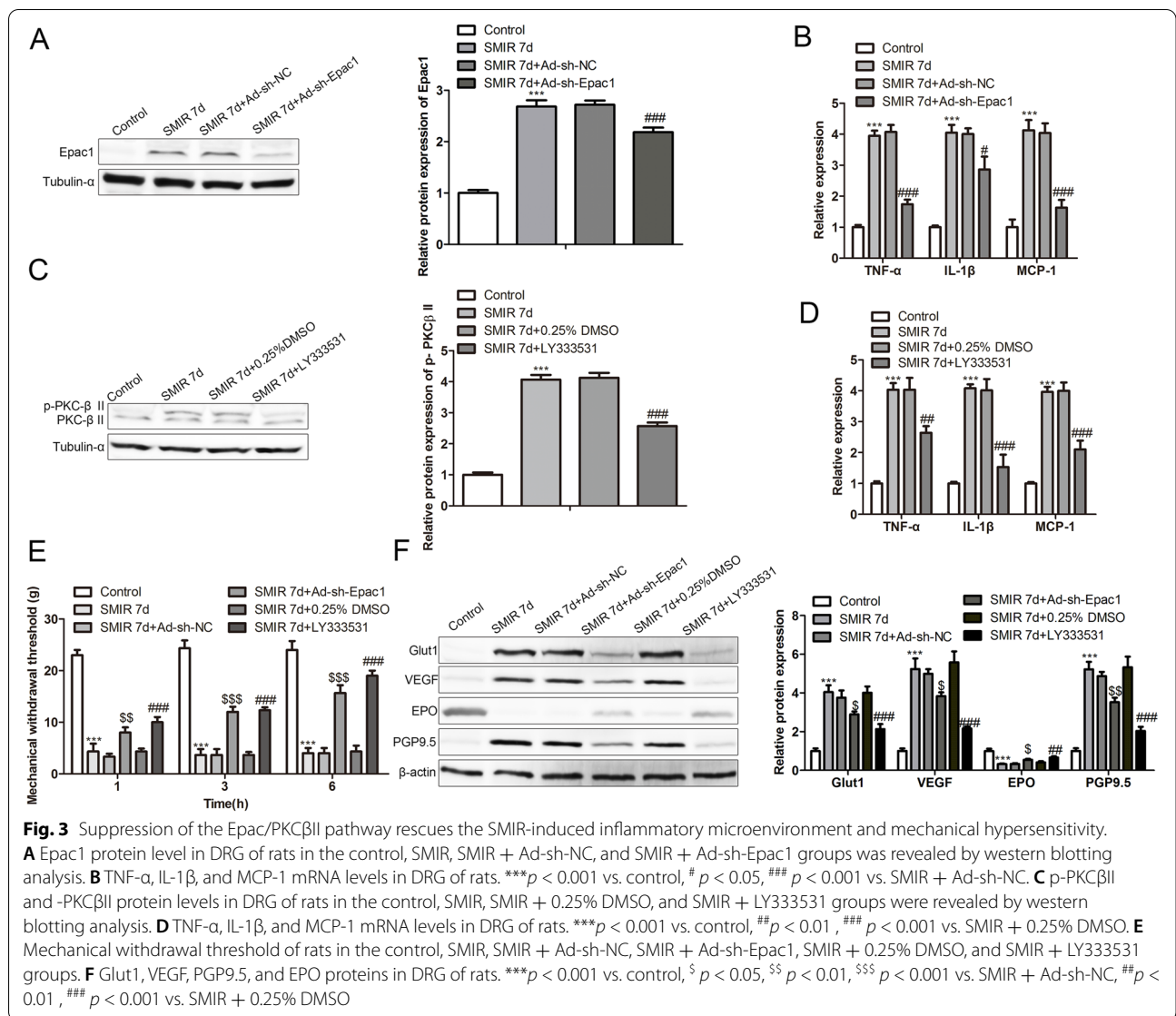
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Fig. 2 The Epac/PKCβII pathway mediates inflammatory microenvironment and mechanical hypersensitivity in rats after SMIR. **A** Mechanical withdrawal threshold of rats of the control, 0.25% DMSO, and 8-pCPT groups. **B** Mechanical withdrawal threshold of rats of the control, 0.25% DMSO, and PMA groups. **C** Epac1, VEGF, EPO, PGP9.5, and Glut1 proteins in DRG of rats of the control, 0.25% DMSO, and 8-pCPT groups. **D** p-PKCβII, PKCβII, VEGF, EPO, PGP9.5, and Glut1 proteins in DRG of rats of the control, 0.25% DMSO, and PMA groups. **$p < 0.01$, ***$p < 0.001$ vs. control

study, we found that SMIR induced the upregulation of TNF-α, IL-1β, and MCP-1 in the DRG in rats, which is consistent with the previous studies. Spinal Epac1 expression was found to increase in an animal model of postoperative pain which might be caused by central sensitization in the spinal cord [24]. In our study, we proved the molecular changes in the DRG, indicating peripheral sensitization, which is not totally
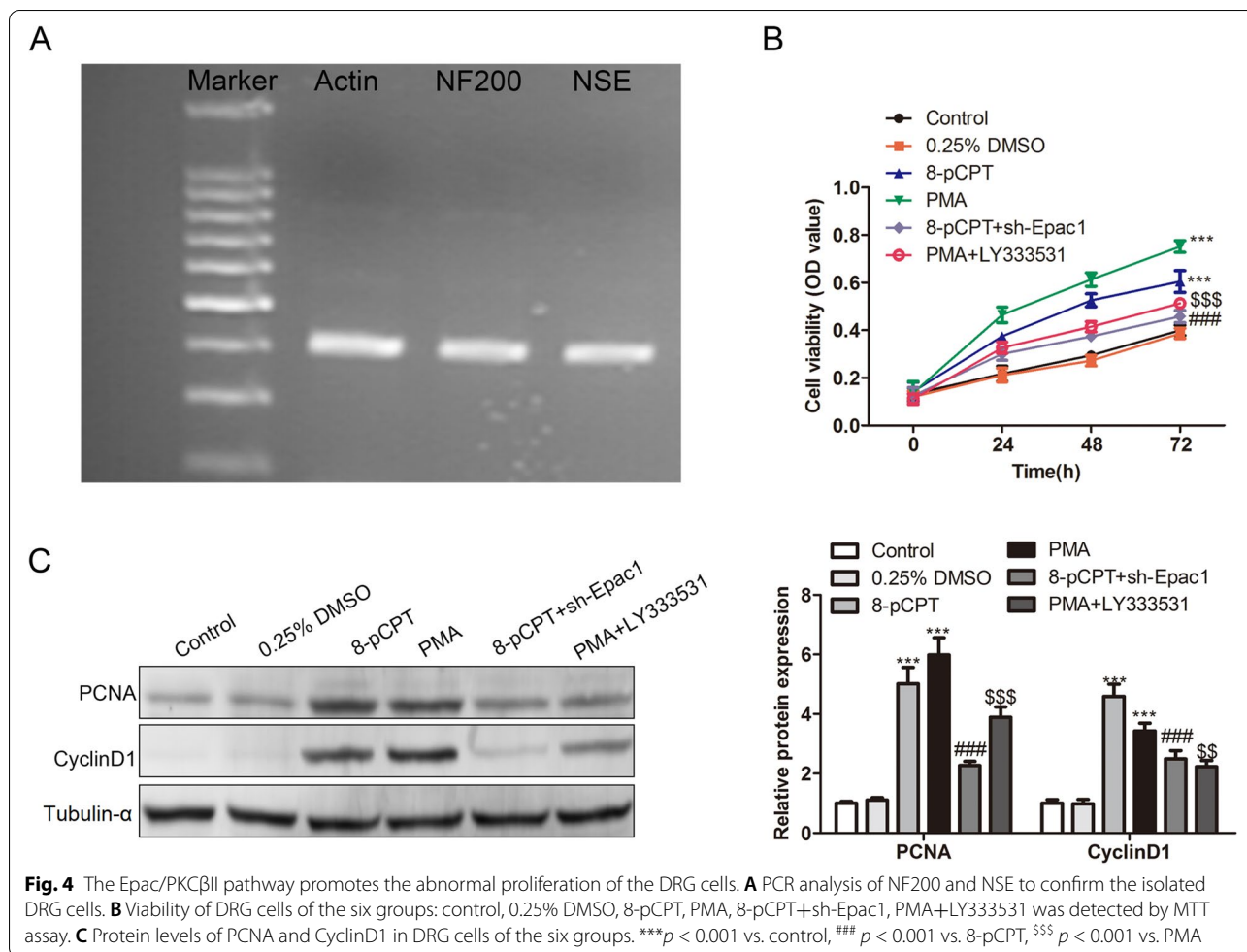
the same as central sensitization. The NMDAR/AC1/cAMP/Epac pathway in the dorsal horn maintains latent pain sensitization in both sexes [25]. Increased PKC-dependent phosphorylation of GluN1 contributes to mechanical allodynia in a chronic constriction injury-induced mouse model of neuropathic pain [26]. Increased PKC was found in postoperative DRG, and PKC, but not PKA, promotes postoperative

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Fig. 3 Suppression of the Epac/PKCβII pathway rescues the SMIR-induced inflammatory microenvironment and mechanical hypersensitivity. **A** Epac1 protein level in DRG of rats in the control, SMIR, SMIR + Ad-sh-NC, and SMIR + Ad-sh-Epac1 groups was revealed by western blotting analysis. **B** TNF-α, IL-1β, and MCP-1 mRNA levels in DRG of rats. ***$p < 0.001$ vs. control, # $p < 0.05$, ### $p < 0.001$ vs. SMIR + Ad-sh-NC. **C** p-PKCβII and -PKCβII protein levels in DRG of rats in the control, SMIR, SMIR + 0.25% DMSO, and SMIR + LY333531 groups were revealed by western blotting analysis. **D** TNF-α, IL-1β, and MCP-1 mRNA levels in DRG of rats. ***$p < 0.001$ vs. control, ## $p < 0.01$, ### $p < 0.001$ vs. SMIR + 0.25% DMSO. **E** Mechanical withdrawal threshold of rats in the control, SMIR, SMIR + Ad-sh-NC, SMIR + Ad-sh-Epac1, SMIR + 0.25% DMSO, and SMIR + LY333531 groups. **F** Glut1, VEGF, PGP9.5, and EPO proteins in DRG of rats. ***$p < 0.001$ vs. control, $ $p < 0.05$, $$ $p < 0.01$, $$$ $p < 0.001$ vs. SMIR + Ad-sh-NC, ## $p < 0.01$, ### $p < 0.001$ vs. SMIR + 0.25% DMSO

hyperalgesia [27]. In the present study, after SMIR for seven days, the Epac1 and PKCβII proteins were increased in DRG in rats, confirming the pivital role of the Epac1/PKC pathway in postoperative pain.

Glut1 expression is positively correlated with microvessel density in postoperative pain [28, 29]. SMIR surgery induces the increased Glut1 protein around the incision site in rats [30]. PKCβII promotes PGP9.5 and VEGF proteins in DRG of the SMIR-operated rats [31]. PGP9.5 is a nerve-specific marker, and an increase or decrease of its expression is associated with nerve repair or damage, respectively [32]. The PGP9.5 expression is increased during the inflammation and pain process [33, 34]. VEGF can increase vascular permeability [35] and glucose

levels in the cell by PKC [36], and protect nerve cells [37]. Increased VEGF induces an inflammatory response and facilitates the release of algogenic substances [38]. EPO is a hematopoietic hormone, exerting anti-inflammatory and neuroprotective effects [39], and has been indicated to prevent postoperative cognitive dysfunction [40]. The present study revealed that SMIR promoted Glut1, VEGF, PGP9.5 protein levels, and decreased EPO protein expression in DRG of rats with postoperative pain, which was in accordance with the previous studies. Pretreatment with Captopril in SMIR rats reduced mechanical hypersensitivity and MCP-1 expression, indicating the positive effect of vascular protection in postoperative pain.

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Fig. 4 The Epac/PKCβII pathway promotes the abnormal proliferation of the DRG cells. **A** PCR analysis of NF200 and NSE to confirm the isolated DRG cells. **B** Viability of DRG cells of the six groups: control, 0.25% DMSO, 8-pCPT, PMA, 8-pCPT+sh-Epac1, PMA+LY333531 was detected by MTT assay. **C** Protein levels of PCNA and CyclinD1 in DRG cells of the six groups. ***$p < 0.001$ vs. control, ### $p < 0.001$ vs. 8-pCPT, ^{SSS} $p < 0.001$ vs. PMA

In some animal studies, the Epac1 agonist and PKCβII agonist were used to treat the normal rats. These agonists induced hyperalgesia and increased Glut1, VEGF, PGP9.5 protein levels, as well as decreased EPO protein level in DRG. After the suppression of the Epac/PKCβ pathway, SMIR rats showed alleviated hyperalgesia, decreased TNF-α, IL-1β, MCP-1, Glut1, VEGF, PGP9.5 levels and increased EPO level. These findings revealed that the Epac/PKCβ pathway plays a significant role in postoperative pain, and its effects were associated with inflammation and BNB function.

However, the detailed mechanism by which the Epac/PKCβ pathway regulates postoperative pain was not explored in the present study. A previous study has indicated the calcium influx after activation of the Epac/PKC pathway [41], which encourages us to study calcium channel in the future. Besides, a study showed that NMDAR→AC1→cAMP→Epac signaling

pathways in the dorsal horn of the spinal cord maintain latent sensitization in both males and females, which presents a novel mechanism for the promotion of chronic postoperative pain [25]. Whether the Epac/PKCβ pathway is activated in other tissues during the SMIR-induced postoperative pain remains unknown and requires further investigation. Moreover, inflammatory communication between neurons and gliocyte as well as the polarization of glial cells are essential in SMIR-induced post-surgical pain [42]. Our further research will focus on neuron-glial communication and glial activation in post-surgical pain.

To sum up, our findings provided evidence that SMIR induces the Epac/PKCβ pathway in the DRG. This pathway influences inflammation and BNB function and regulates mechanical hypersensitivity in rats with post-surgical pain, indicating that targeting the Epac/PKCβ pathway can help treat postoperative pain.
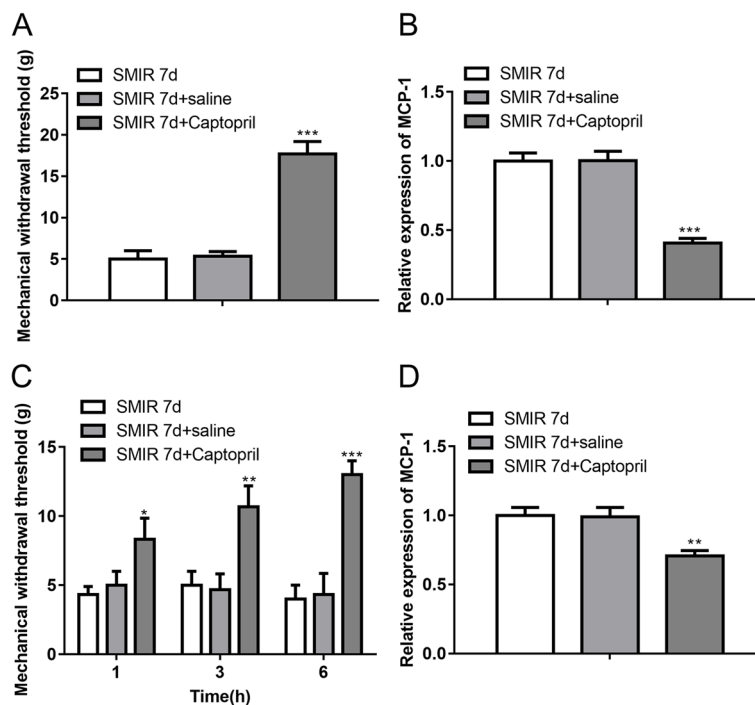
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Fig. 5 Protection of vascular endothelial cells on mechanical hypersensitivity and MCP-1 expression. **A** Mechanical withdrawal threshold of rats with SMIR operation for 7 days, with SMIR operation for 7 days plus pretreatment of saline, or with SMIR operation for 7 days plus pretreatment of Captopril. **B** Relative mRNA expression of MCP-1 in the DRG of rats. **C** Mechanical withdrawal threshold of rats with SMIR operation for 7 days, with SMIR operation for 7 days followed by saline administration, or with SMIR operation for 7 days followed by Captopril administration. **D** Relative mRNA expression of MCP-1 in the DRG of rats. **$p < 0.01$, ***$p < 0.001$ vs. SMIR 7d+saline

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12871-022-01771-w.

Additional file 1.

Authors' contributions
ZLZ conceived and designed the study. JSQ and XZL performed the literature search and data extraction. ZLZ, JSQ and XZL drafted the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Declarations

Ethics approval and consent to participate
The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration, all the animal studies were in accordance with ARRIVE guidelines and were approved by the Institutional Animal Care and Use Committee of Taizhou Central Hospital.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest.

References
1. Gai N, et al. A practical guide to acute pain management in children. J Anesth. 2020;34(3):421–33.
2. Malow RM. The effects of induced anxiety on pain perception: a signal detection analysis. Pain. 1981;11(3):397–405.
3. Woolf CJ. Recent advances in the pathophysiology of acute pain. Br J Anaesth. 1989;63(2):139–46.
4. Michel MZ, Sanders MK. Effectiveness of acute postoperative pain management. Br J Anaesth. 2003;91(3):448–9 author reply 449.
5. Apfelbaum JL, et al. Postoperative pain experience: results from a national survey suggest postoperative pain continues to be undermanaged. Anesth Analg. 2003;97(2):534–40.
6. Rodgers A, Walker N, Schug S, McKee A, Kehlet H, Van Zundert A, et al. Reduction of postoperative mortality and morbidity with epidural or spinal anaesthesia: results from overview of randomised trials. BMJ. 2000;321(7275):1493.
7. Leykin Y, Pellis T, Ambrosio C. Highlights in postoperative pain treatment. Expert Rev Neurother. 2007;7(5):533–45.

8. Parada CA, Reichling DB, Levine JD. Chronic hyperalgesic priming in the rat involves a novel interaction between cAMP and PKCepsilon second messenger pathways. Pain. 2005;113(1-2):185–90.

9. Kawasaki H, et al. A family of cAMP-binding proteins that directly activate Rap1. Science. 1998;282(5397):2275–9.

10. de Rooij J, et al. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. Nature. 1998;396(6710):474–7.

11. Eijkelkamp N, et al. Low nociceptor GRK2 prolongs prostaglandin E2 hyperalgesia via biased cAMP signaling to Epac/Rap1, protein kinase Cepsilon, and MEK/ERK. J Neurosci. 2010;30(38):12806–15.

12. Schmidt M, Dekker FJ, Maarsingh H. Exchange protein directly activated by cAMP (epac): a multidomain cAMP mediator in the regulation of diverse biological functions. Pharmacol Rev. 2013;65(2):670–709.

13. Wang H, et al. Balancing GRK2 and EPAC1 levels prevents and relieves chronic pain. J Clin Invest. 2013;123(12):5023–34.

14. Roth-Isigkeit A, et al. Perioperative cytokine release during coronary artery bypass grafting in patients of different ages. Clin Exp Immunol. 1998;114(1):26–32.

15. Kanda T. Biology of the blood-nerve barrier and its alteration in immune mediated neuropathies. J Neurol Neurosurg Psychiatry. 2013;84(2):208–12.

16. Hirakawa H, et al. Loss and recovery of the blood-nerve barrier in the rat sciatic nerve after crush injury are associated with expression of intercellular junctional proteins. Exp Cell Res. 2003;284(2):196–210.

17. Kubíček L, et al. Alterations in the vascular architecture of the dorsal root ganglia in a rat neuropathic pain model. Ann Anat. 2010;192(2):101–6.

18. Lim TK, et al. Peripheral nerve injury induces persistent vascular dysfunction and endoneurial hypoxia, contributing to the genesis of neuropathic pain. J Neurosci. 2015;35(8):3346–59.

19. Flatters SJ. Characterization of a model of persistent postoperative pain evoked by skin/muscle incision and retraction (SMIR). Pain. 2008;135(1-2):119–30.

20. Miguel-Carrasco JL, et al. Captopril reduces cardiac inflammatory markers in spontaneously hypertensive rats by inactivation of NF-kB. J Inflamm (Lond). 2010;7:21.

21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8.

22. Pogatzki-Zahn EM, Segelcke D, Schug SA. Postoperative pain-from mechanisms to treatment. Pain Rep. 2017;2(2):e588.

23. Richebé P, Capdevila X, Rivat C. Persistent Postsurgical Pain: Pathophysiology and Preventative Pharmacologic Considerations. Anesthesiology. 2018;129(3):590–607.

24. Wong SS, et al. Propofol attenuates postoperative hyperalgesia via regulating spinal GluN2B-p38MAPK/EPAC1 pathway in an animal model of postoperative pain. Eur J Pain. 2019;23(4):812–22.

25. Basu P, et al. Sex differences in protein kinase A signaling of the latent postoperative pain sensitization that is masked by kappa opioid receptors in the spinal cord. J Neurosci. 2021.

26. Choi SR, et al. Spinal D-Serine Increases PKC-Dependent GluN1 Phosphorylation Contributing to the Sigma-1 Receptor-Induced Development of Mechanical Allodynia in a Mouse Model of Neuropathic Pain. J Pain. 2017;18(4):415–27.

27. Song C, et al. TRPV1 channel contributes to remifentanil-induced postoperative hyperalgesia via regulation of NMDA receptor trafficking in dorsal root ganglion. J Pain Res. 2019;12:667–77.

28. Sawayama H, et al. High expression of glucose transporter 1 on primary lesions of esophageal squamous cell carcinoma is associated with hematogenous recurrence. Ann Surg Oncol. 2014;21(5):1756–62.

29. Semaan A, et al. Expression of GLUT-1 in epithelial ovarian carcinoma: correlation with tumor cell proliferation, angiogenesis, survival and ability to predict optimal cytoreduction. Gynecol Oncol. 2011;121(1):181–6.

30. Cao S, et al. Effects of pinacidil on changes to the microenvironment around the incision site, of a skin/muscle incision and retraction, in a rat model of postoperative pain. Mol Med Rep. 2015;12(1):829–36.

31. Zhu X, et al. PKCβII-induced upregulation of PGP9.5 and VEGF in postoperative persistent pain in rats. J Pain Res. 2018;11:2095–106.

32. Shirai C, et al. The pattern of distribution of PGP 9.5 and TNF-alpha immunoreactive sensory nerve fibers in the labrum and synovium of the human hip joint. Neurosci Lett. 2009;450(1):18–22.

33. Kosacka J, et al. Increased autophagy in peripheral nerves may protect Wistar Ottawa Karlsburg W rats against neuropathy. Exp Neurol. 2013;250:125–35.

34. Giordano C, et al. The galactosylation of N(ω)-nitro-L-arginine enhances its anti-nocifensive or anti-allodynic effects by targeting glia in healthy and neuropathic mice. Eur J Pharmacol. 2011;656(1-3):52–62.

35. Koyama J, et al. The novel VEGF receptor antagonist, VGA1155, reduces edema, decreases infarct and improves neurological function after stroke in rats. Kobe J Med Sci. 2010;56(1):E1–11.

36. Sone H, Deo BK, Kumagai AK. Enhancement of glucose transport by vascular endothelial growth factor in retinal endothelial cells. Invest Ophthalmol Vis Sci. 2000;41(7):1876–84.

37. Bespalov AG, et al. The role of VEGF, HSP-70 and protein S-100B in the potentiation effect of the neuroprotective effect of hypercapnic hypoxia. Patol Fiziol Eksp Ter. 2014;2:24–7.

38. Murakami K, et al. Vein wrapping for chronic nerve constriction injury in a rat model: study showing increases in VEGF and HGF production and prevention of pain-associated behaviors and nerve damage. J Bone Joint Surg Am. 2014;96(10):859–67.

39. Hemani S, et al. Systematic Review of Erythropoietin (EPO) for Neuroprotection in Human Studies. Neurochem Res. 2021;46(4):732–9.

40. Lee JH, et al. Erythropoietin Attenuates Postoperative Cognitive Dysfunction by Shifting Macrophage Activation toward the M2 Phenotype. Front Pharmacol. 2017;8:839.

41. Hucho TB, Dina OA, Levine JD. Epac mediates a cAMP-to-PKC signaling in inflammatory pain: an isolectin B4(+) neuron-specific mechanism. J Neurosci. 2005;25(26):6119–26.

42. Li T, et al. Microglia induce the transformation of A1/A2 reactive astrocytes via the CXCR7/PI3K/Akt pathway in chronic post-surgical pain. J Neuroinflammation. 2020;17(1):211.