Dexmedetomidine Attenuates Neuropathic Pain by Inhibiting P2X7R Expression and ERK Phosphorylation in Rats

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α2-Adrenoceptor agonists attenuate hypersensitivity under neuropathic conditions. However, the mechanisms underlying this attenuation remain largely unknown. In the present study, we explored the potential roles of purinergic receptor 7 (P2X7R)/extracellular signal-regulated kinase (ERK) signaling in the anti-nociceptive effect of dexmedetomidine in a rat model of neuropathic pain induced by chronic constriction injury (CCI) of the sciatic nerve. An animal model of CCI was adopted to mimic the clinical neuropathic pain state. Behavioral hypersensitivity to mechanical and thermal stimuli was determined by von Frey filament and Hargreaves’ tests, and the spinal P2X7R expression level and ERK phosphorylation were analyzed using western blot analysis and immunohistochemistry. In parallel with the development of mechanical and thermal hyperalgesia, a significant increase in P2X7R expression was noted in the ipsilateral spinal cord on day 7 after CCI. Intrathecal administration of dexmedetomidine (2.5 μg) for 3 days not only attenuated neuropathic pain but also inhibited the CCI-induced P2X7R upregulation and ERK phosphorylation. Intrathecal dexmedetomidine administration did not produce obvious effects on locomotor function. The present study demonstrated that dexmedetomidine attenuates the neuropathic pain induced by CCI of the sciatic nerve in rats by inhibiting spinal P2X7R expression and ERK phosphorylation, indicating the potential therapeutic implications of dexmedetomidine administration for the treatment of neuropathic pain.

Key words: Dexmedetomidine, Neuropathic pain, Chronic constriction injury, Spinal cord, Purinergic receptor, Extracellular signal-regulated kinase

INTRODUCTION

Neuropathic pain, which affects 7%–8% of the population, is defined as a pathological pain caused by nerve lesions or diseases and is characterized by spontaneous pain, exaggerated responsiveness to noxious stimuli (hyperalgesia), and pain in response to normally non-noxious stimuli (allodynia) [1-3]. Conventional analgesics and treatments cannot satisfactorily alleviate neuropathic pain because of its unknown underlying pathogenesis and mecha-
nisms, and therefore, neuropathic pain remains a vital major clinical and social problem [4].

The α2-adrenoceptor plays an important role in antinociception, and α2-adrenoceptor agonists attenuate hypersensitivity under neuropathic conditions [5-7]. There are three highly homologous subtypes of the α2-adrenoceptor: α2A, α2B, and α2C. Dexmedetomidine, a highly selective α2-adrenoceptor agonist, has been used for conscious sedation and as an adjunct for clinical anesthesia [8-10]. In animal experiments, dexmedetomidine has been shown to produce analgesic effects in models of neuropathic pain, but the mechanisms underlying these effects remain largely unknown [11-13].

Emerging evidence indicates that spinal microglia is activated after peripheral nerve injury and contribute to the development and maintenance of neuropathic pain [14]. Previous studies have indicated that the purinergic receptor 7 (P2X7R), a subtype of the purinergic receptor predominantly expressed on microglia, is upregulated in the spinal cord after nerve injury [15]. In addition, P2X7R seems to play an important role in neuropathic pain [16, 17]. For example, P2X7R-knockout mice fail to develop behavioral hypersensitivity after partial sciatic nerve ligation [18]. Moreover, P2X7 receptors are involved in regulating microglial activation via the release of cytokines [19]. Recently, spinal microglia were shown to be involved in the anti-nociceptive effect of dexametomidine in rats subjected to chronic constriction injury of the sciatic nerve (CCI) [12]. However, no study has examined the effects of dexmedetomidine on P2X7R activation and the related analgesic effects.

Extracellular signal-regulated kinases (ERKs, including ERK1 and ERK2), which are widely expressed in the neuronal cells, play important roles in central plasticity and modulation of pain processing [20, 21]. In the spinal dorsal horn, ERKs are activated by innocuous stimuli in peripheral nerve-injured animals [22]. Furthermore, the inhibition of ERK activation has been shown to reduce pain hypersensitivity and suppress brain-derived neurotrophic factor (BDNF) expression in the spinal cord [23, 24], while BDNF is well known as an important factor for long-term memory [25]. Recent studies have suggested that the ERK signaling pathway is involved in dexmedetomidine analgesia [26]. However, the precise mechanism underlying the anti-nociceptive effects of dexmedetomidine in neuropathic pain remains unknown.

In the present study, we aimed to explore the possible roles of P2X7R in the anti-nociceptive effect of dexmedetomidine in a rat model of neuropathic pain following CCI. The results revealed the underlying mechanism of dexmedetomidine analgesia, indicating the therapeutic implications of dexmedetomidine administration for the treatment of neuropathic pain.

MATERIALS AND METHODS

Animal housing and preparation

Male Wistar rats (weighing ~200 g on arrival) were obtained from the animal center of the Chinese Academy of Sciences and housed two per cage with water and food available ad libitum. A 12-h light/12-h dark cycle was used, with lights on at 08:00. All animal experiments were carried out with the approval of the Animal Care Committee at Zhejiang University and consistent with the ethical guidelines for the investigation of experimental pain in animals [27]. All efforts were made to minimize the number of animals used and their suffering. Animals were acclimated to the housing facility for three days before the experiments began.

The rats were implanted with an intrathecal catheter under intraperitoneal anesthesia (pentobarbital, 60 mg/kg). A polyethylene-10 (Smiths Medical, UK) catheter was inserted into the subarachnoid space at the level of the spinal cord lumbar enlargement according to the method described by Størkson et al. [28]. The animals were allowed to recover for 3 days before being randomly divided into groups. Rats showing neurological deficits postoperatively were discarded from the study.

Induction of neuropathic pain

Animals were randomly divided into naïve, sham, and CCI groups (n=6). To create a neuropathic pain state, we performed a surgical procedure according to the description reported by Bennett and Xie [29] and our recent study [30]. Briefly, under pentobarbital (60 mg/kg, ip) anesthesia, the left sciatic nerve was exposed and freed from the underlying connective tissues. Three ligations were placed around the nerve with 4-0 chromic gut sutures, and a typical twitch of the hind paw was seen when the nerve was constricted. The overlying muscle and skin were closed with the same silk sutures. An identical surgical procedure was performed on sham-operated animals without ligation of the sciatic nerve. All animals received antibiotics (0.5 ml penicillin, 96 mg/ml hypodermic injection) to reduce the possibility of infection. To reduce variability, all animal model procedures were carried out by one investigator who was proficient in modeling.

Drug administration

Animals were divided into naïve, sham, CCI+normal saline (NS), and CCI+dexmedetomidine (DEX) groups (n=8 for each group). Dexmedetomidine (Sigma, Missouri) was diluted in NS. After neuropathic pain was successfully established, a dose of 2.5 μg DEX (in 20 μl volume) or NS (20 μl) was intrathecally administered once daily for three consecutive days from day 8 to 10 after CCI, followed by an injection of 10 μl NS to flush the catheter.
Behavioral test
To minimize the stress induced by handling and measurements, we exposed the rats to the test condition for 3 days before the experiment. The behavioral test was performed 1 h after drug administration.

Mechanical paw withdrawal threshold (MT) testing. Animals were placed in a cage with a wire mesh floor and allowed to explore and groom until they settled. The von Frey hairs were applied to the plantar surface of hindpaws in ascending order (0.4, 0.6, 1, 1.4, 2, 4, 6, 8, 10, 15, and 26 g) for up to 6 s per filament. Once a withdrawal response was established, the paw was re-tested, starting with the next descending von Frey hair until no response occurred. The lowest amount of force required to elicit a response was recorded as the paw withdrawal threshold. The withdrawal threshold was measured three times in each animal, and the values from the three measurements were averaged.

Thermal paw withdrawal latency (TL) testing. Paw withdrawal latency to noxious heat stimuli was evaluated using a Hargreaves' test with the appropriate apparatus (Model 336; IITC/life Science, CA) [32, 33]. Briefly, rats were placed in a Plexiglas chamber on a glass plate under which a light box was located. A radiant heat stimulus was applied using a beam of light aimed through a hole in the glass plate to the heel of each hind paw through the glass plate. The light beam was turned off when the rat lifted the foot, and the interval between the start of the light beam and the lifting of the foot was measured and defined as the paw withdrawal latency. Each heat stimulus was applied three times at 5-min intervals. A cutoff time of 20 s was applied to prevent potential tissue damage.

Locomotor function testing. The effects of medication and surgery on locomotor function were examined using the following methods [34]. The rats were divided randomly into five groups (n=6): naïve, sham, CCI, CCI+NS, and CCI+DEX. Placing reflex: The rat was held with the hind limbs slightly lower than the forelimbs, and the dorsal surfaces of the hind paws were brought into contact with the edge of a table. The experimenter recorded whether the hind paws were reflexively placed on the table surface. Grasping reflex: The rat was placed on a wire grid, and the experimenter recorded whether the hind paws were reflexively placed on the grid surface, and the experimenter noted whether it immediately assumed the normal upright position. Scores for placing, grasping, and righting reflexes were based on counts of each normal reflex shown in five trials.

Western blot analysis
Western blot analysis was conducted as described in our previous reports [35]. Rats were sacrificed immediately under deep anesthesia, followed by isolation of the lumbar 4–5 spinal cords. The spinal cord was divided into ipsilateral and contralateral halves, and then homogenized in ice-cold homogenization buffer (Beyotime Institute of Biotechnology, China). The homogenates were centrifuged at 10,000 × g for 10 min at 4°C, the supernatant was collected, and the protein concentrations were determined by the Micro BCA protein assay reagent kit (Thermo Fisher Scientific Inc., MA). Equivalent protein samples were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred onto polyvinylidene difluoride membranes (Thermo Fisher Scientific Inc.) using a wet transfer apparatus (Bio-Rad Laboratories Inc., CA). Proteins bound to the membrane were stained with Ponceau S solution (Beyotime Institute of Biotechnology) to determine the quality of the transfer. Membranes were blocked with 5% non-fat milk in Tris-buffered saline at room temperature for 2 h and incubated with the primary antibodies against P2X7R (1:1000; Cell Signaling Technology, MA), PERK (1:1000; Millipore, CA), ERK (1:1000; Cell Signaling Technology), and β-tubulin (1:2000; Beyotime Institute of Biotechnology) overnight at 4°C. After the membranes were extensively washed with TBST buffer, a 1:2000 dilution of goat anti-rabbit or goat anti-mouse horseradish peroxidase secondary antibody (Beyotime Institute of Biotechnology) was used as appropriate and incubated for 2 h at room temperature. After extensive washing, signals were detected with enhanced chemiluminescence reagent (Thermo Fisher Scientific Inc.) and captured using a ChemiDoc MP System (Bio-Rad Laboratories Inc.). Immunoreactive density was analyzed using Image Lab software (Bio-Rad Laboratories Inc.). The density of the specific bands was normalized against that of the loading control (β-tubulin). Commercial markers (Thermo Fisher Scientific Inc.) were used as molecular weight standards.

Immunohistochemistry
Immunohistochemical staining was conducted in accordance with our previous reports [35]. Seven days after CCI or sham operation, rats were anesthetized and perfused thoroughly with saline at room temperature for 2 h and incubated with the primary antibodies against P2X7R (1:1000; Cell Signaling Technology, MA), PERK (1:1000; Millipore, CA), and β-tubulin (1:2000; Beyotime Institute of Biotechnology) overnight at 4°C. After the membranes were washed with TBST buffer, a 1:2000 dilution of goat anti-rabbit or goat anti-mouse horseradish peroxidase secondary antibody (Beyotime Institute of Biotechnology) was used as appropriate and incubated for 2 h at room temperature. After extensive washing, signals were detected with enhanced chemiluminescence reagent (Thermo Fisher Scientific Inc.) and captured using a ChemiDoc MP System (Bio-Rad Laboratories Inc.). Immunoreactive density was analyzed using Image Lab software (Bio-Rad Laboratories Inc.). The density of the specific bands was normalized against that of the loading control (β-tubulin). Commercial markers (Thermo Fisher Scientific Inc.) were used as molecular weight standards.
**Statistical analysis**

Data are expressed as the mean±S.E.M and were analyzed using one-way or two-way ANOVA followed by Fisher’s least significant differences (LSD) test or Dunnett’s multiple comparisons as appropriate. p<0.05 was considered statistically significant.

**RESULTS**

CCI induced behavioral hypersensitivity and P2X7R/ERK activation in the spinal cord

In the present study, a model of CCI was used to simulate a chronic neuropathic pain state. Using behavioral measurements, we found that the paw withdrawal thresholds for both mechani-

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**Fig. 1.** CCI induced hyperalgesia and P2X7R upregulation in the spinal dorsal horn. (A) CCI induced mechanical hyperalgesia. The mechanical paw withdrawal threshold on day 7 following CCI surgery was significantly lower than that in the sham group (n=6, mean±SEM, ***p<0.001, ANOVA). (B) CCI induced thermal hyperalgesia. The thermal paw withdrawal latency on day 7 following CCI surgery was significantly lower than that in the sham group (n=6, mean±SEM, p<0.01, ANOVA). (C) Activation of P2X7R/ERK signaling in the spinal dorsal horn after CCI. (D) Upregulation of P2X7R in the spinal dorsal horn after CCI. Western blotting showed that the relative expression of P2X7R was significantly higher in the CCI group than in the sham group (n=4, mean±SEM, *p<0.05, ANOVA). (E) Upregulation of PERK in the spinal dorsal horn after CCI. Western blotting showed that the relative expression of PERK was significantly higher in the CCI group than in the sham group (n=4, mean±SEM, *p<0.05, ANOVA). CCI, chronic constriction injury; Contra, contralateral to CCI; NC, negative control. Scale bar, 100 μm.
cal and thermal stimuli were lower in the CCI group than in the sham-operated group on day 7, the time point at which the CCI group usually reached the maximal behavioral hyperalgesia after nerve injury [36]. As shown in Fig. 1, compared with the sham group, the CCI group exhibited a significantly lower MT on day 7 after nerve injury (n=6, p<0.001; Fig. 1A), with similar results for the TL values (n=6, p<0.01; Fig. 1B), suggesting the development of robust mechanical and thermal hyperalgesia in the CCI rats. We further analyzed whether CCI-induced hyperalgesia is associated with spinal P2X7R activation. On day 7 after CCI, western blot analyses revealed that, in parallel with the behavioral hyperalgesia, P2X7R expression in the ipsilateral spinal cord was significantly higher than that in the sham group (n=4, p<0.05; Fig. 1D), while the expression in the contralateral spinal dorsal horn was not significantly different from that in the sham group (n=4, p=0.131; Fig. 1D). Similarly, 7 days after CCI, PERK expression in the ipsilateral spinal dorsal horn was significantly greater than that in the sham group (n=4, p<0.05; Fig. 1E), while the expression in the contralateral spinal dorsal horn was not significantly different from that in the sham group (n=4, p=0.234; Fig. 1E). The results of immunohistochemical assessments confirmed the activation of spinal P2X7R/ERK signaling after CCI injury (Fig. 1C).

**Dexmedetomidine attenuated the neuropathic pain induced by CCI of the sciatic nerve**

To investigate the effect of α2-adrenoceptor agonists on CCI-associated neuropathic pain, which was reflected by mechanical and thermal hyperalgesia, we administered intrathecal dexmedetomidine (2.5 μg/20 μl) daily from day 8 to day 10 after CCI, when the pain behaviors were well established. Basal MT and TL were not significantly different among the groups before CCI surgery; however, the MT in the CCI+DEX group was significantly higher than that in the NS control (CCI+NS) groups from day 8 to day 10 after CCI (n=8, p<0.001; Fig. 2A), but it was still lower than that in the sham group (n=8, p<0.001; Fig. 2A). In contrast, the TL in the CCI+DEX group was also obviously higher than that in the CCI+NS group from day 8 to day 10 after CCI (n=8, p<0.001; Fig. 2B) and similar to that in the sham group, except on day 10 (p>0.05, from day 8 to 9; p<0.05, day 10, n=8; Fig. 2B).

Importantly, CCI surgery and dexmedetomidine administration did not produce significant effects on locomotor function. As shown in Fig. 3, CCI surgery and intrathecal dexmedetomidine administration at the dosage used in our study did not produce obvious effects on locomotor function. Furthermore, convulsions and hypermobility were not observed in any of the groups.

**Dexmedetomidine inhibited CCI-induced P2X7R upregulation and ERK phosphorylation**

Next, to further explore the involvement of spinal P2X7R and ERK activation in dexmedetomidine analgesia, we examined the expression of P2X7R and ERK and its phosphorylated form (PERK) by western blot analyses. We chose to perform western blot analysis on day 10 after CCI on the basis of the results of behavior tests. After three days of dexmedetomidine or NS administration, the rats subjected to CCI were sacrificed 1 h after drug administration on day 10 after CCI surgery. In parallel with the changes in mechanical and thermal hyperalgesia, western blot analysis revealed that P2X7R expression in the CCI+NS group was highly upregulated compared with the expression in the sham group (n=5, p<0.01; Fig. 4A). However, in contrast to the CCI+NS

Fig. 2. Dexmedetomidine attenuated the mechanical and thermal hyperalgesia caused by CCI in rats. (A) Dexmedetomidine attenuated mechanical hyperalgesia. The mechanical paw withdrawal threshold in the CCI+DEX group was significantly higher than that in the CCI+NS group from day 8 to day 10 following CCI (n=8, mean±SEM, ***p<0.001, ANOVA). (B) Dexmedetomidine attenuated thermal hyperalgesia. The thermal paw withdrawal latency in the CCI+DEX group was significantly higher than that in the CCI+NS group from day 8 to day 10 following CCI (n=8, mean±SEM, **p<0.001, ANOVA). CCI, chronic constriction injury; NS, normal saline; DEX, dexmedetomidine.
group, the CCI+DEX group exhibited significant downregulation of P2X7R expression (n=5, \( p<0.01 \); Fig. 4A). Furthermore, western blot analysis showed that although there was no obvious difference in the total ERK expression in the spinal cord on day 10 following CCI among the naïve group, CCI+NS group, or CCI+DEX group (n=4, \( p>0.05 \); Fig. 4B), PERK expression in the CCI+NS group was significantly upregulated in comparison with that in the sham group (n=5, \( p<0.05 \); Fig. 4C); however, in parallel with the changes in mechanical and thermal hyperalgesia, the expression of PERK in the CCI+DEX group was significantly lower than that in the CCI+NS group (n=5, \( p<0.05 \); Fig. 4C).

**DISCUSSION**

Similar to previous reports, we found that CCI rats showed robust behavioral hypersensitivity to mechanical and thermal stimuli 7 days after nerve injury, and this hypersensitivity lasted beyond the entire experimental period. Using protein analysis, we also found that P2X7R expression was upregulated in the ipsilateral spinal cord in parallel with the CCI-induced behavioral signs of mechanical and thermal hyperalgesia, indicating that spinal P2X7R is involved in the development of neuropathic pain. The results are consistent with those from a previous study conducted by Kobayashi et al. [37], which showed that P2X7R protein expression increased in the spinal cord in a spared nerve injury model of neuropathic pain, peaking at 7 d after injury.

Dexmedetomidine, a selective \( \alpha_2 \)-adrenoceptor agonist, has been used for conscious sedation and as an adjunct for clinical anesthesia in recent years [8-10]. In the present study, we found that intrathecal administration of dexmedetomidine attenuated the behavioral hyperalgesia induced by CCI in rats. In a previous study, Li et al. [38] found that intrathecal dexmedetomidine produced an anti-allodynia effect in a rat pain model. However, in the current study, we demonstrated that dexmedetomidine attenuated not only mechanical allodynia but also thermal hyperalgesia. In another report, Liu et al. [26] found that systemic administration
of dexmedetomidine attenuated thermal and mechanical hyperalgesia in a rat model of partial sciatic nerve ligation (PSNL). Our study further confirmed the previous results that dexmedetomidine attenuates the neuropathic pain induced by nerve injury.

P2X7R, an ATP-gated nonselective cation channel, has been studied as a subtype of purinergic receptors. Spinal P2X7R has been reported to be involved in acute and chronic pain [37, 39, 40]. P2X7R blockade with an antagonist or genetic knockout of P2X7R reduced the mechanical and thermal hyperalgesia induced by nerve injury [41]. He et al. [15] found that spinal P2X7R mediates microglial activation-induced neuropathic pain in a sciatic nerve injury rat model. All of these observations indicate that P2X7R plays a vital role in the development of neuropathic pain induced by multiple causes. Nevertheless, the specific mechanism by which spinal P2X7R mediates neuropathic pain is still largely unknown. Electrophysiological studies have revealed that stimulation of P2X7R leads to reversible Ca$^{2+}$ and Na$^+$ influx and associated membrane voltage changes [42]. Ca$^{2+}$ influx has been widely viewed as the pivotal stimulus to elicit cell reactions and activate intracellular signaling pathways [43, 44], including ERK. As one of the classical mitogen-activated protein kinase (MAPK) signaling pathway activated by nerve injury [26].

In conclusion, our study demonstrated that spinal dexmedetomidine mediates the expression of P2X7R and ERK phosphorylation, suggesting the potential therapeutic implications of dexmedetomidine administration for the treatment of neuropathic pain.

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REFERENCES

1. Jensen TS, Baron R, Haanpää M, Kalso E, Loeser JD, Rice AS, Treede RD (2011) A new definition of neuropathic pain. Pain 152:2204-2205.
2. Torrance N, Ferguson JA, Afolabi E, Bennett MI, Serpell MG, Dunn KM, Smith BH (2013) Neuropathic pain in the community: more under-treated than refractory? Pain 154:690-699.
3. Sandkühler J (2009) Models and mechanisms of hyperalgesia and allodynia. Physiol Rev 89:707-758.
4. Shohayeb B, Diab M, Ahmed M, Ng DC (2018) Factors that influence adult neurogenesis as potential therapy. Transl Neurodegener 7:4.
5. Wei H, Jyväsjärvi E, Niissalo S, Hukkanen M, Waris E, Konttinen YT, Pertovaara A (2002) The influence of chemical
sympathectomy on pain responsivity and α2-adrenergic antinociception in neuropathic animals. Neuroscience 114:655-668.
6. Lavand'homme PM, Ma W, De Kock M, Eisenach JC (2002) Perineural α2-adrenoceptor activation inhibits spinal cord neuroplasticity and tactile allodynia after nerve injury. Anesthesiology 97:972-980.
7. Pertovaara A, Wei H (2000) Attenuation of ascending nociceptive signals to the rostroventromedial medulla induced by a novel α2-adrenoceptor agonist, MPV-2426, following intrathecal application in neuropathic rats. Anesthesiology 92:1082-1092.
8. Wunsch H, Kahn JM, Kramer AA, Wagener G, Li G, Sladen RN, Rubenfeld GD (2010) Dexmedetomidine in the care of critically ill patients from 2001 to 2007: an observational cohort study. Anesthesiology 113:386-394.
9. Paris A, Tonner PH (2005) Dexmedetomidine in anaesthesia. Curr Opin Anaesthesiol 18:412-418.
10. Bae HB (2017) Dexmedetomidine: an attractive adjunct to anaesthesia. Korean J Anesthesiol 70:375-376.
11. Zhou TT, Wu JR, Chen ZY, Liu ZX, Miao B (2014) Effects of dexmedetomidine on P2X4Rs, p38-MAPK and BDNF in spinal microglia in rats with spared nerve injury. Brain Res 1568:21-30.
12. Li SS, Zhang WS, Ji D, Zhou YL, Li H, Yang JL, Xiong YC, Zhang YQ, Xu H (2014) Involvement of spinal microglia and interleukin-18 in the anti-nociceptive effect of dexmedetomidine in rats subjected to CCI. Neurosci Lett 560:21-25.
13. Huang YQ, Guo SH, Liu R, Zhu SM, Sun JL, Yao YX (2018) Additive analgesic effect of dexmedetomidine and dezocine administered intrathecally in a mouse pain model. Oncotarget 9:24391-24397.
14. Inoue K, Tsuda M (2009) Microglia and neuropathic pain. Glia 57:1469-1479.
15. He WJ, Cui J, Du L, Zhao YD, Burnstock G, Zhou HD, Ruan HZ (2012) Spinal P2X, receptor mediates microglia activation-induced neuropathic pain in the sciatic nerve injury rat model. Behav Brain Res 226:163-170.
16. Kambur O, Kaunisto MA, Winsvold BS, Wilsgaard T, Stubhaug A, Zwart JA, Kalso E, Nielsen CS (2018) Genetic variation in P2RX7 and pain tolerance. Pain 159:1064-1073.
17. Sorge RE, Trang T, Dorfman R, Smith SB, Beggs S, Ritchie J, Austin JS, Zaykin DV, Vander Meulen H, Costigan M, Herbert TA, Yarkoni-Abitbul M, Tichauer D, Livneh J, Gershon E, Zheng M, Tan K, John SL, Slade GD, Jordan I, Woolf CJ, Peltz G, Maixner W, Diatchenko L, Seltzer Z, Salter MW, Mogil JS (2012) Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity. Nat Med 18:595-599.
18. Chessell IP, Hatcher JP, Bouutra C, Michel AD, Hughes JP, Green P, Egerton J, Murfin M, Richardson J, Peck WL, Grahames CB, Casula MA, Yiayngou Y, Birch R, Anand P, Buell GN (2005) Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 114:386-396.
19. Chu YX, Zhang Y, Zhang YQ, Zhao ZQ (2010) Involvement of microglial P2X7 receptors and downstream signaling pathways in long-term potentiation of spinal nociceptive responses. Brain Behav Immun 24:1176-1189.
20. Xu Q, Garraway SM, Weyerbacher AR, Shin SJ, Inturrisi CE (2008) Activation of the neuronal extracellular signal-regulated kinase 2 in the spinal cord dorsal horn is required for complete Freund’s adjuvant-induced pain hypersensitivity. J Neurosci 28:14087-14096.
21. Ji RR, Befort K, Brenner GJ, Woolf CJ (2002) ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 upregulation and contributes to persistent inflammatory pain hypersensitivity. J Neurosci 22:478-485.
22. Gao YJ, Ji RR (2010) Light touch induces ERK activation in superficial dorsal horn neurons after inflammation: involvement of spinal astrocytes and JNK signaling in touch-evoked central sensitization and mechanical allodynia. J Neurochem 115:505-514.
23. Obata K, Yamanaka H, Dai Y, Tachibana T, Fukuoka T, Toku-naga A, Yoshikawa H, Noguchi K (2003) Differential activation of extracellular signal-regulated protein kinase in primary afferent neurons regulates brain-derived neurotrophic factor expression after peripheral inflammation and nerve injury. J Neurosci 23:4117-4126.
24. Cao H, Gao YJ, Ren WH, Li TT, Duan KZ, Cui YH, Cao XH, Zhao ZQ, Ji RR, Zhang YQ (2009) Activation of extracellular signal-regulated kinase in the anterior cingulate cortex contributes to the induction and expression of affective pain. J Neurosci 29:3307-3321.
25. Bekinschtein P, Cammarota M, Katche C, Slipczuk L, Rossato JI, Goldin A, Izquierdo I, Medina JH (2008) BDNF is essential to promote persistence of long-term memory storage. Proc Natl Acad Sci U S A 105:2711-2716.
26. Liu L, Ji F, Liang J, He H, Fu Y, Cao M (2012) Inhibition by dexmedetomidine of the activation of spinal dorsal horn glia and the intracellular ERK signaling pathway induced by nerve injury. Brain Res 1427:1-9.
27. Zimmermann M (1983) Ethical guidelines for investigations
of experimental pain in conscious animals. Pain 16:109-110.

28. Storkson RV, Kjorsvik A, Tjølsen A, Hole K (1996) Lumbar catheterization of the spinal subarachnoid space in the rat. J Neurosci Methods 65:167-172.

29. Bennett GJ, Xie YK (1988) A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33:87-107.

30. Huang LE, Guo SH, Thitirassane L, Yang Y, Zhou YF, Yao YX (2018) N-methyl D-aspartate receptor subtype 2B antagonist, Ro 25-6981, attenuates neuropathic pain by inhibiting postsynaptic density 95 expression. Sci Rep 8:7848.

31. Fox A, Medhurst S, Courade JP, Glatt M, Dawson J, Urban L, Bevan S, Gonzalez I (2004) Anti-hyperalgesic activity of the cox-2 inhibitor lumiracoxib in a model of bone cancer pain in the rat. Pain 107:33-40.

32. Hargreaves K, Dubner R, Brown F, Flores C, Joris J (1988) A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32:77-88.

33. Li NN, Huang YQ, Huang LE, Guo SH, Shen MR, Guo CL, Zhu SM, Yao YX (2017) Decozine antagonizes morphine analgesia upon simultaneous administration in rodent models of acute nociception. Pain Physician 20:E401-E409.

34. Tao YX, Johns RA (2001) Effect of the deficiency of spinal PSD-95/SAP90 on the minimum alveolar anesthetic concentration of isoflurane in rats. Anesthesiology 94:1010-1015.

35. Yao YX, Zhang YF, Yang Y, Guo SH, Jiang Z, Zhao ZQ (2014) Spinal synaptic scaffolding protein Homer 1b/c regulates CREB phosphorylation and c-fos activation induced by inflammatory pain in rats. Neurosci Lett 559:88-93.

36. Lee CY, Perez FM, Wang W, Guan X, Zhao X, Fisher JL, Guan Y, Sweitzer SM, Raja SN, Tao YX (2011) Dynamic temporal and spatial regulation of mu opioid receptor expression in primary afferent neurons following spinal nerve injury. Eur J Pain 15:669-675.

37. Kobayashi K, Takahashi E, Miyagawa Y, Yamanaka H, Noguchi K (2011) Induction of the P2X7 receptor in spinal microglia in a neuropathic pain model. Neurosci Lett 504:57-61.

38. Li SS, Zhang WS, Yang IL, Xiong YC, Zhang YQ, Xu H (2013) Involvement of protein kinase B/Akt in analgesic effect of dexmedetomidine on neuropathic pain. CNS Neurosci Ther 19:364-366.

39. Inoue K, Tsuda M (2012) Purinergic systems, neuropathic pain and the role of microglia. Exp Neurol 234:293-301.

40. Yang Y, Li H, Li TT, Luo H, Gu XY, Lu N, Ji RR, Zhang YQ (2015) Delayed activation of spinal microglia contributes to the maintenance of bone cancer pain in female Wistar rats via P2X7 receptor and IL-18. J Neurosci 35:7950-7963.

41. Honnore P, Donnelly-Roberts D, Namovic MT, Hsieh G, Zhu CZ, Mikusa JP, Hernandez G, Zhong C, Gauvin DM, Chandran P, Harris R, Medrano AP, Carroll W, Marsh K, Sullivan JP, Faltynek CR, Jarvis MF (2006) A-740003 [N-1-[(cyanomino)-(5-quinolinylamino) methyl]amino-2,3-dimethylpropyl]-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective P2X7 receptor antagonist, dose-dependently reduces neuropathic pain in the rat. J Pharmacol Exp Ther 319:1376-1385.

42. Hede SE, Amstrup J, Christoffersen BC, Novak I (1999) Purinoceptors evoke different electrophysiological responses in pancreatic ducts. P2Y inhibits K+ conductance, and P2X stimulates cation conductance. J Biol Chem 274:31784-31791.

43. Xu F, Zhao X, Liu L, Song J, Zhu Y, Chu S, Shao X, Li X, Ma Z, Gu X (2017) Perturbing NR2B-PSD-95 interaction relieves neuropathic pain by inactivating CaMKII-CREB signaling. Neuroreport 28:856-863.

44. Liu S, Liu YP, Huang ZL, Zhang YK, Song AA, Ma PC, Song XJ (2015) Wnt/Ryk signaling contributes to neuropathic pain by regulating sensory neuron excitability and spinal synaptic plasticity in rats. Pain 156:2572-2584.

45. Falsig J, Pörzgen P, Lotharius J, Leist M (2004) Specific modulation of astrocyte inflammation by inhibition of mixed lineage kinases with CEP-1347. J Immunol 173:2762-2770.

46. Ma W, Quirion R (2002) Partial sciatic nerve ligation induces increase in the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) in astrocytes in the lumbar spinal dorsal horn and the gracile nucleus. Pain 99:175-184.

47. Ji RR, Chamessian A, Zhang YQ (2016) Pain regulation by non-neuronal cells and inflammation. Science 354:572-577.

48. Wang B, Liu S, Fan B, Xu X, Chen Y, Lu R, Xu Z, Liu X (2018) PKM2 is involved in neuropathic pain by regulating ERK and STAT3 activation in rat spinal cord. J Headache Pain 19:7.

49. Zhang W, Bai Y, Qiao Y, Wang J, Li MY, Wang JW, Jia N, Chen T, Li YQ, Wen AD (2018) 8-O-acetyl shanzhiside methylester from Lamiopholis rotata reduces neuropathic pain by inhibiting the ERK/TNF-α pathway in spinal astrocytes. Front Cell Neurosci 12:54.

50. Chen ML, Cao H, Chu YX, Cheng LZ, Liang LL, Zhang YQ, Zhao ZQ (2012) Role of P2X7 receptor-mediated IL-18/IL-18R signaling in morphine tolerance: multiple glial-neuronal dialogues in the rat spinal cord. J Pain 13:945-958.

51. Song J, Ying Y, Wang W, Liu X, Xu W, Wei X, Ruan X (2018) The role of P2X7/R receptor signaling in dorsal root ganglia satellite glial cells in the development of chronic postsurgical
pain induced by skin/muscle incision and retraction (SMIR). Brain Behav Immun 69:180-189.

52. Amstrup J, Novak I (2003) P2X7 receptor activates extracellular signal-regulated kinases ERK1 and ERK2 independently of Ca2+ influx. Biochem J 374:51-61.

53. Takizuka A, Minami K, Uezono Y, Horishita T, Yokoyama T, Shiraishi M, Sakurai T, Shigematsu A, Ueta Y (2007) Dexmedetomidine inhibits muscarinic type 3 receptors expressed in Xenopus oocytes and muscarine-induced intracellular Ca2+ elevation in cultured rat dorsal root ganglia cells. Naunyn Schmiedebergs Arch Pharmacol 375:293-301.