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Intraoperative electroacupuncture relieves remifentanil-induced postoperative hyperalgesia via inhibiting spinal glial activation in rats

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Abstract

Background: Accumulating studies have suggested that remifentanil, the widely-used opioid analgesic in clinical anesthesia, can activate the pronociceptive systems and enhance postoperative pain. Glial cells are thought to be implicated in remifentanil-induced hyperalgesia. Electroacupuncture is a complementary therapy to relieve various pain conditions with few side effects, and glial cells may be involved in its antinociceptive effect. In this study, we investigated whether intraoperative electroacupuncture could relieve remifentanil-induced postoperative hyperalgesia by inhibiting the activation of spinal glial cells, the production of spinal proinflammatory cytokines, and the activation of spinal mitogen-activated protein kinases.

Methods: A rat model of remifentanil-induced postoperative hyperalgesia was used in this study. Electroacupuncture during surgery was conducted at bilateral Zusanli (ST36) acupoints. Behavior tests, including mechanical allodynia and thermal hyperalgesia, were performed at different time points. Astrocytic marker glial fibrillary acidic protein, microglial marker Iba1, proinflammatory cytokines, and phosphorylated mitogen-activated protein kinases in the spinal cord were detected by Western blot and/or immunofluorescence.

Results: Mechanical allodynia and thermal hyperalgesia were induced by both surgical incision and remifentanil infusion, and remifentanil infusion significantly exaggerated and prolonged incision-induced pronociceptive effects. Glial fibrillary acidic protein, Iba1, proinflammatory cytokines (interleukin-1β and tumor necrosis factor-α), and phosphorylated mitogen-activated protein kinases (p-p38, p-JNK, and p-ERK1/2) were upregulated after surgical incision, remifentanil infusion, and especially after their combination. Intraoperative electroacupuncture significantly attenuated incision- and/or remifentanil-induced pronociceptive effects, spinal glial activation, proinflammatory cytokine upregulation, and phosphorylated mitogen-activated protein kinase upregulation.

Conclusions: Our study suggests that remifentanil-induced postoperative hyperalgesia can be relieved by intraoperative electroacupuncture via inhibiting the activation of spinal glial cells, the upregulation of spinal proinflammatory cytokines, and the activation of spinal mitogen-activated protein kinases.

Keywords

Electroacupuncture, postoperative hyperalgesia, remifentanil-induced hyperalgesia, glial cells, proinflammatory cytokines, mitogen-activated protein kinases

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Introduction

Opioids, which serve as the major potent analgesic agents used in clinical settings, can paradoxically activate the pronociceptive systems[1] and cause opioid-induced hyperalgesia (OIH).[2–4] Remifentanil is an ultra-short-acting μ-opioid receptor agonist with rapid onset and predictable rapid recovery profile[5] and has been widely used for pain management during operation. Unfortunately, remifentanil could induce hyperalgesia more rapidly, prominently, and frequently than other opioids.[2,6] Although extensive studies have been conducted and considerable progress has been made concerning the mechanisms underlying remifentanil-induced hyperalgesia (RIH), a clear understanding in this domain is still lacking.

Multiple factors may be involved in RIH, including induction of long-term potentiation at synapses,[3,7] activation of N-methyl-D-aspartate receptors,[3] and activation of μ-opioid receptors.[1] Additionally, studies by us[8] and others[9–11] have shown that activation of spinal glial cells may contribute to the development of OIH/RIH. Activated glial cells can synthesize and release a number of glial mediators including proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, contributing to the development and maintenance of central sensitization.[8,9,12] The mitogen-activated protein kinase (MAPK) family, including p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), have been shown to be involved in the induction and maintenance of various pain conditions.[13,14] Opioid treatment can also activate MAPKs in glial cells, leading to increased synthesis and release of proinflammatory cytokines.[15] The circumstances under which RIH may occur are not yet entirely understood. It is suggested that whether RIH occurs is related to the dose and duration of remifentanil treatment, and large doses and long-term infusion of remifentanil have been shown to induce hyperalgesia more frequently.[16–18]

An increasing number of drugs have been proposed to reduce or prevent OIH, including ketamine,[19] magnesium sulphate,[20] cyclooxygenase inhibitors,[21] dexmedetomidine,[22] and pregabalin.[23] However, as we all know, the above drugs may cause various side effects, which are detrimental to patients during the perioperative period. Acupuncture/electroacupuncture (EA) is a traditional Chinese medical practice, and it is often used as a safe, economical, and effective tool for treating various pain conditions with few side effects.[24] Acupuncture has also been used to prevent various opioid-induced adverse effects, such as addiction, nausea, vomiting, locomotor sensitization, and opioid tolerance.[25–28] Of note, repeated EA does not show analgesic tolerance.[29] With the increasing use of EA, numerous studies have been performed to uncover its potential antinociceptive mechanisms, and a great deal of valuable data have accumulated. Among those, accumulating studies have shown that EA treatment can alleviate pain by inhibiting spinal glial activation, proinflammatory cytokine production, and MAPK activation.[30–33]

In the present study, we hypothesized that intraoperative EA could relieve remifentanil-induced postoperative hyperalgesia, and the underlying mechanisms may be related to its inhibition of the activation of spinal glial cells.

Materials and methods

Laboratory animals

Adult male Sprague-Dawley rats, weighing 200 to 250 g, were supplied by the Laboratory Animal Center of Nanjing Drum Tower Hospital (Nanjing, China). All animals were housed in cages and maintained under a 12-h light/12-h dark cycle (light on at 07:00 a.m.) in a temperature controlled room ($22 \pm 2^{\circ}C$) with food and water available ad libitum. The procedures were approved by the Experimental Animals Welfare and Ethics Committee of Nanjing Drum Tower Hospital. Animals were treated in accordance with the guidelines for the use of laboratory animals,[34] and efforts were made to minimize their number and suffering.

Drugs

Remifentanil (Yichang Humanwell Pharmaceutical Co., Ltd., China) and sevoflurane (Jiangsu Hengrui Pharmaceutical Co., Ltd., China) were used in this study. Remifentanil was dissolved in normal saline (NS). After being anesthetized with sevoflurane delivered via a nose mask (induction, 3.5%; maintenance, 3.0%), rats were subcutaneously administered with remifentanil (0.04 mg/kg, 0.4 ml) at a rate of 0.8 ml/h over a period of 30 min using an infusion pump, and this treatment was shown to induce pronociceptive effects in our previous studies.[8,19] Control rats were subcutaneously administered the same volume of NS (0.8 ml/h, 30 min).

Electroacupuncture

For EA treatment, animals were anesthetized with sevoflurane (induction, 3.5%; maintenance, 3.0%) via a nose mask. After the surrounding skin was sterilized with 75% alcohol, bilateral Zusanli (ST36) acupoints (located approximately 5 mm below and lateral to the anterior tubercle of the tibia)[35] were punctured approximately 5 mm deep with fine stainless steel needles (diameter, 0.18 mm; length, 7 mm). Electrical stimulation (2 Hz in frequency, 1 mA in intensity) was delivered using an electrical stimulation device (HANS 200E, Ji Sheng Medical Technology Co., Ltd., Nanjing, China) for 30 min. EA treatment started at the same time as the infusion of remifentanil or NS.

Surgical incision

We used the rat model of postoperative pain as previously described by Brennan et al.[36] Briefly, after sterilization of the right hind paw with 10% povidone-iodine, a 1-cm longitudinal incision was made with a number 11 blade through the skin and fascia, starting at 0.5 cm from the edge of the heel and extending toward the toes. The underlying flexor muscle was elevated, incised longitudinally, and retracted, leaving the muscle origin and insertion intact. After careful hemostasis with pressure, the skin was closed with 2 sutures of 5-0 silk thread. The wound site was covered with erythromycin ointment. The surgical incision was started approximately 5 min after the infusion of remifentanil or NS. The control group received sham surgery consisting of anesthesia with sevoflurane, sterilization of the hind paw, and erythromycin ointment on the plantar surface, without plantar incision.

Nociceptive behavior tests

To evaluate mechanical allodynia, paw withdrawal mechanical threshold (PWMT) was measured using a biological research apparatus (Ugo Basile 37450, Comerio, Italy). Rats were placed into elevated testing cages with metal mesh floors and allowed to habituate for approximately 15 min. The touch stimulator unit was positioned beneath the right hind paw, and the filament was applied to the area adjacent to the wound. A positive response was defined as lifting, shaking, or licking of the paw following stimulation. We repeated the stimulation 3 times with intervals of approximately 5 min, recorded the thresholds, and calculated the mean value.

To evaluate thermal hyperalgesia, paw withdrawal thermal latency (PWTL) was measured using a biological research apparatus (Ugo Basile 37370, Comerio, Italy). Rats were placed into glass-floored testing cages and allowed to habituate for approximately 15 min. Before starting the experiment, the infrared heat intensity of the apparatus was adjusted to give an average PWTL of approximately 10 s, and the cutoff latency was set at 15 s to avoid tissue damage. The infrared source was positioned directly beneath the area adjacent to the wound of the right hind paw. PWTL was defined as the time from onset of the infrared heat stimulus to withdrawal of the paw from the heat source. We repeated the stimulation three times with intervals of approximately 5 min, recorded the latencies, and calculated the mean value.

Western blot analysis

After being deeply anesthetized with sevoflurane, rats were euthanized. Spinal cord segments (right dorsal part of L4–L5) were rapidly removed and stored in liquid nitrogen. Tissue samples were homogenized in lysis buffer. The homogenate was centrifuged at 13,000 r/min for 10 min at 4°C, and the supernatant was removed. The protein concentration was determined by the BCA Protein Assay Kit, following the manufacturer's instructions. Samples (50 μg) were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 10%) and transferred onto a nitrocellulose membrane. The filter membranes were blocked with 5% skimmed milk for 1 h at room temperature and incubated overnight at 4°C with the following primary antibodies: GFAP (1:800, ab10062, Abcam), Iba1 (1:600, ab15690, Abcam), IL-1β (1:500, ab9722, Abcam), TNF-α (1:500, ab6671, Abcam), p-p38(1:1000, #4511, Cell Signaling Technology), p-JNK(1:1000, #4668, Cell Signaling Technology), and p-ERK1/2 (1:2000, #4370, Cell Signaling Technology). The membrane was washed with Tris-buffered saline with Tween buffer and incubated with the goat anti-mouse secondary antibody (1:5000, ab97040, Abcam) or goat anti-rabbit secondary antibody (1:5000, ab7090, Abcam) conjugated with horseradish peroxidase for 1 h at room temperature. Next, the immune complexes were detected using the electrochemiluminescence system (Millipore Immobilon). β-actin was used as a loading control for total protein. Image-Pro Plus was used to analyze the density of specific bands.

Immunofluorescence analysis

Rats were terminally anesthetized with sevoflurane and perfused transcardially with NS followed by 4% paraformaldehyde in 0.1 M phosphate buffer at pH = 7.4. After perfusion, the L4–L5 spinal cord segments were removed, postfixed in the same fixative, and then transferred to 30% sucrose. Transverse spinal sections (25 μm) were cut in a cryostat, rinsed in phosphate-buffered saline, blocked with 10% goat serum in 0.3% Triton for 1 h at room temperature, and incubated overnight at 4°C with primary antibodies (GFAP, 1:300, Abcam; Iba1, 1:200, Abcam). After washing in phosphate-buffered saline, sections were incubated with secondary antibody (Alexa Fluor 594 for GFAP, 1:500, Invitrogen; Alexa Fluor 488 for Iba1, 1:1500, ThermoFisher) for 1 h at room temperature. The stained sections were mounted on glass slides, air-dried, covered with coverslips, and examined under a Leica multiphoton confocal microscope (Leica Microsystems, Wetzlar, Germany).

Groups of experiments and experimental protocol

According to surgical procedure received, rats were divided into eight groups: Group Sham + NS received

sham surgery and NS infusion; Group Inci + NS received surgical incision and NS infusion; Group Sham + Remi received sham surgery and remifentanil infusion; Group Inci + Remi received surgical incision and remifentanil infusion; Group EA + Sham + NS received EA treatment, sham surgery, and NS infusion; Group EA + Inci + NS received EA treatment, surgical incision, and NS infusion; Group EA + Sham + Remi received EA treatment, sham surgery, and remifentanil infusion; Group EA + Inci + Remi received EA treatment, surgical incision, and remifentanil infusion.

Experiment 1: for behavioral tests, 64 rats were randomly divided into the above 8 groups ($n = 8$) and were tested at 1 d prior to the surgical procedure (baseline value) and at 2 h, 1 d, 2 d, 3 d, 4 d, 5 d, 6 d, 7 d, 8 d, 9 d, and 10 d after the surgical procedure; Experiment 2: for Western blot, 32 rats were randomly divided into the above 8 groups ($n = 4$), and specimens were collected at 1 d after the surgical procedure; Experiment 3: for immunofluorescence, 32 rats were randomly divided into the above 8 groups ($n = 4$), and specimens were collected at 1 d after the surgical procedure. The observers were blinded to the experimental groups in the experiments. The schematic representation of the experimental design and time schedule of the protocol is shown in Figure 1.

Statistical analysis

Data were expressed as mean ± SD. Two-way analysis of variance with repeated measures followed by post hoc Bonferroni multiple comparisons was used to analyze behavioral data. One-way analysis of variance followed by post hoc Bonferroni multiple comparisons was used to analyze Western blot data. Statistical analysis was performed using SPSS 15.0 software. $P < 0.05$ was set as the level of statistical significance.

Results

Effects of remifentanil infusion on mechanical allodynia and thermal hyperalgesia in sham-operated and postoperative rats and the intervention of EA

There were no significant differences in the baseline PWMT and PWTL among all eight groups ($P > 0.05$). In group Sham + NS, treatments (sham surgery, NS infusion, and sevoflurane) did not induce significant changes in nociceptive thresholds after the surgical procedure compared with baseline ($P > 0.05$). In group Inci + NS, surgical incision induced mechanical allodynia and thermal hyperalgesia observed 2 h after the surgical procedure and lasting for 5 d and 6 d, respectively ($P < 0.05$). In group Sham + Remi, remifentanil infusion also induced mechanical allodynia and thermal hyperalgesia observed as early as 2 h after the surgical procedure and lasting for 4 d ($P < 0.05$). Notably, in group Inci + Remi, remifentanil infusion not only exaggerated incision-induced mechanical allodynia and thermal hyperalgesia ($P < 0.05$) but also increased the duration of mechanical allodynia and thermal hyperalgesia (8 d and 8 d vs. 5 d and 6 d, respectively), when compared with group Inci + NS (Figures 2 and 3).

In group EA + Sham + NS, EA did not produce significant changes in PWMT and PWTL after the surgical procedure compared with baseline ($P > 0.05$). In group EA + Inci + NS, EA relieved mechanical allodynia and thermal hyperalgesia induced by surgical incision, compared with group Inci + NS ($P < 0.05$). In group EA + Remi + NS, EA also relieved mechanical allodynia and thermal hyperalgesia induced by remifentanil infusion, compared with group Sham + Remi ($P < 0.05$). Moreover, in group EA + Inci + Remi, EA significantly reduced the enhancement of mechanical allodynia and thermal hyperalgesia ($P < 0.05$) and decreased the duration of mechanical allodynia and

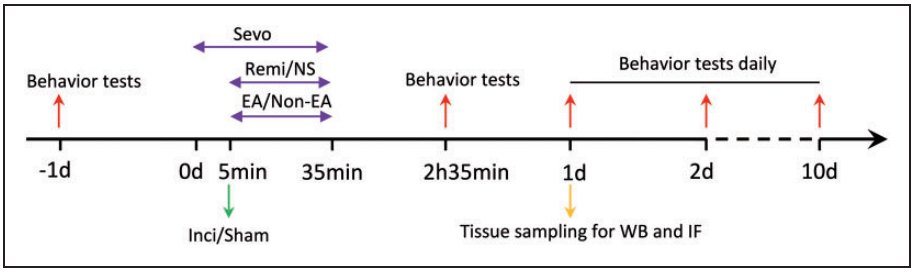

Figure 1. The schematic representation of the experimental design and time schedule of the protocol. All rats were randomly divided into 8 groups: Group Sham + NS, Group Inci + NS, Group Sham + Remi, Group Inci + Remi, Group EA + Sham + NS, Group EA + Inci + NS, Group EA + Sham + Remi, and Group EA + Inci + Remi. Behavior tests ($n = 8$) were conducted at 1 d prior to the surgical procedure (baseline value) and at 2 h, 1 d, 2 d, 3 d, 4 d, 5 d, 6 d, 7 d, 8 d, 9 d, and 10 d after the surgical procedure. Specimens were collected for Western blot analysis ($n = 4$) and immunofluorescence ($n = 4$) at 1 d after the surgical procedure. Sevo: sevoflurane; Remi: remifentanil; NS: normal saline; EA: electroacupuncture; Inci: incision; Sham: sham surgery; WB: Western blot; IF: immunofluorescence.

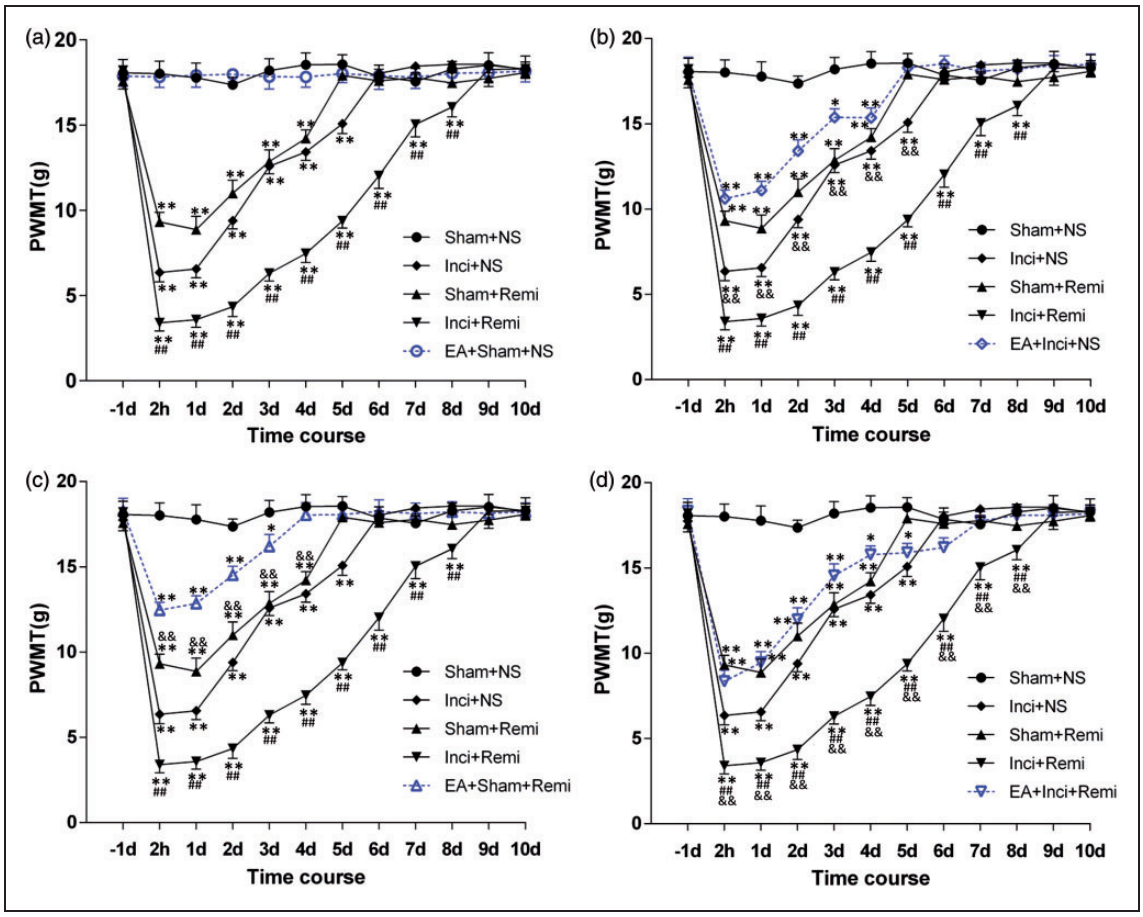

Figure 2. Effects of EA on incision- and/or remifentanil-induced mechanical allodynia ($n = 8$). PWMT was measured at 1 d before (baseline value) and 2 h, 1 d, 2 d, 3 d, 4 d, 5 d, 6 d, 7 d, 8 d, 9 d, and 10 d after the surgical procedure. Both surgical incision and remifentanil infusion induced mechanical allodynia observed at 2 h after the surgical procedure and lasting for 5 d and 4 d, respectively. Remifentanil infusion not only exaggerated incision-induced mechanical allodynia but also increased its duration (8 d vs. 5 d). EA effectively relieved mechanical allodynia induced by surgical incision (b), remifentanil infusion (c), and their combination (d). Data were expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with baseline value; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with Group Inci + NS; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ compared with corresponding EA treatment group. PWMT: paw withdrawal mechanical threshold; EA: electroacupuncture; Inci: incision; Remi: remifentanil; NS: normal saline; Sham: sham surgery.

thermal hyperalgesia (5 d and 6 d vs. 8 d and 8 d, respectively) induced by combination of surgical incision and remifentanil infusion, when compared with group Inci + Remi (Figures 2 and 3).

Effects of EA on incision- and/or remifentanil-induced upregulation of glial markers in the spinal cord

Western blot analyses were used to quantify the expression of GFAP and Iba1 in the spinal cord at 1 d after the surgical procedure. Both surgical incision and remifentanil infusion increased the levels of GFAP and Iba1, when compared with group sham + NS ($P < 0.01$). Furthermore, remifentanil infusion exaggerated the upregulation of GFAP and Iba1 induced by surgical incision ($P < 0.01$). There were no significant changes in the levels of GFAP and Iba1 between group Sham + NS and group EA + Sham + NS ($P > 0.05$). EA during the procedure not only decreased the upregulation of GFAP and Iba1 induced by surgical incision and remifentanil infusion, respectively ($P < 0.01$), but also effectively inhibited the more significant upregulation of GFAP and Iba1 induced by combined surgical incision and remifentanil infusion ($P < 0.01$; Figure 4).

Immunofluorescence techniques were used to examine the expression and location of GFAP and Iba1 in the spinal cord at 1 d after the surgical procedure, and similar results were received. Compared with group Sham + NS rats, the expression of GFAP and Iba1 moderately increased in group Inci + NS and group Sham + Remi rats and dramatically increased in group Inci + Remi rats. EA treatment effectively blocked the

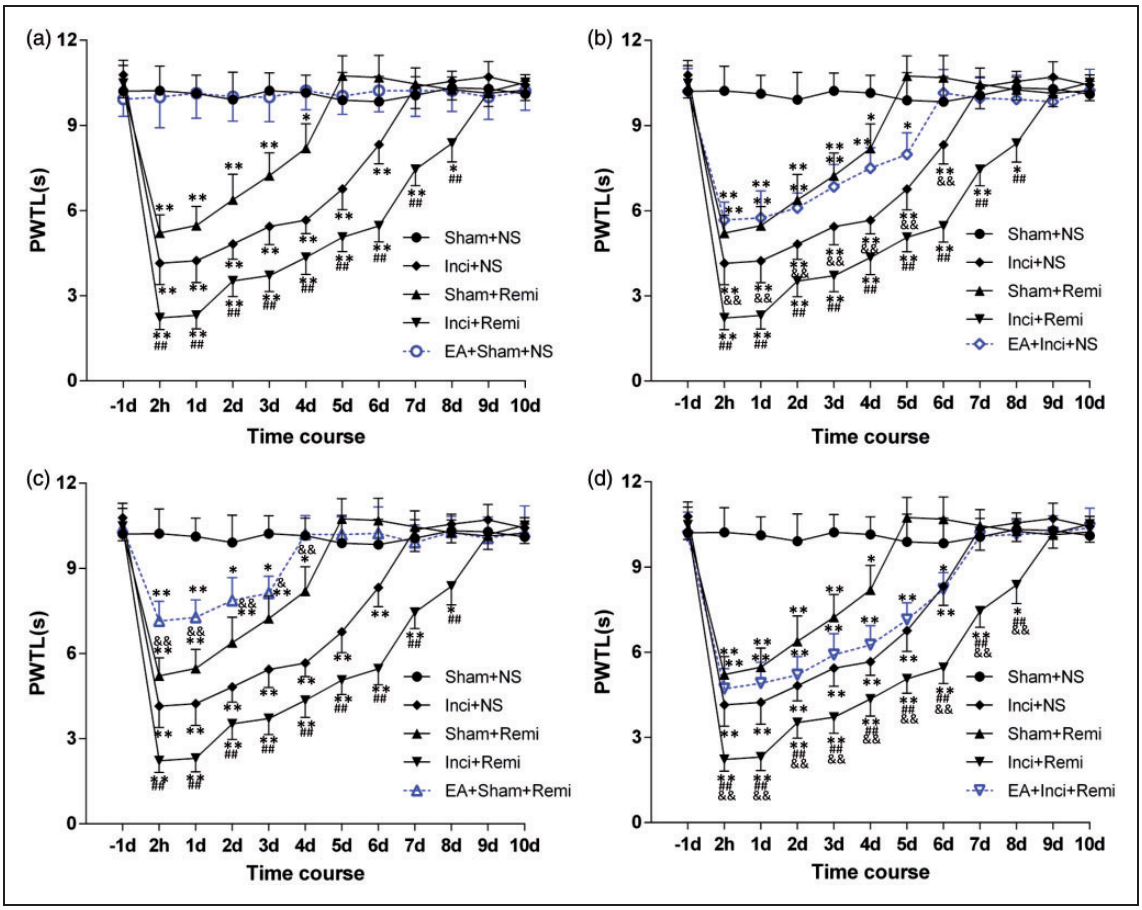

Figure 3. Effects of EA on incision- and/or remifentanil-induced thermal hyperalgesia ($n = 8$). PWTL was measured at 1 d before (baseline value) and 2 h, 1 d, 2 d, 3 d, 4 d, 5 d, 6 d, 7 d, 8 d, 9 d, and 10 d after the surgical procedure. Both surgical incision and remifentanil infusion induced thermal hyperalgesia observed at 2 h after the surgical procedure and lasting for 6 d and 4 d, respectively. Remifentanil infusion not only exaggerated incision-induced thermal hyperalgesia but also increased its duration (8 d vs. 6 d). EA effectively relieved thermal hyperalgesia induced by surgical incision (b), remifentanil infusion (c), and their combination (d). Data were expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with baseline value; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with Group Inci + NS; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ compared with corresponding EA treatment group. PWTL: paw withdrawal thermal latency; EA: electroacupuncture; Inci: incision; Remi: remifentanil; NS: normal saline; Sham: sham surgery.

enhanced expression of GFAP and Iba1 induced by surgical incision, remifentanil infusion, or their combination (Figure 5).

Effects of EA on incision- and/or remifentanil-induced upregulation of proinflammatory cytokines and p-MAPKs in the spinal cord

We also used Western blot analyses to quantify the expression of IL-1β, TNF-α, p-p38, p-JNK, and p-ERK1/2 in the spinal cord at 1 d after the surgical procedure. Consistent with the expression of GFAP and Iba1, surgical incision and remifentanil infusion increased the levels of IL-1β, TNF-α, p-p38, p-JNK, and p-ERK1/2 ($P < 0.01$), and remifentanil infusion exaggerated surgical incision-induced upregulation of IL-1β, TNF-α, p-p38, p-JNK, and p-ERK1/2 ($P < 0.01$). EA significantly suppressed the production of IL-1β, TNF-α, p-p38, p-JNK, and p-ERK1/2 induced by surgical incision, remifentanil infusion, and their combination ($P < 0.01$; Figure 4).

Discussion

With the increasing use of remifentanil as an analgesic in clinical anesthesia, lines of evidence have demonstrated that patients receiving remifentanil to control pain during operation may paradoxically become more sensitive to postoperative pain.[17] In the present study, we simulated the general anesthesia with sevoflurane inhalation and remifentanil infusion commonly used during human surgical procedures. We selected the dosage of remifentanil (0.04 mg/kg) based on our previous studies showing that this treatment could enhance postoperative

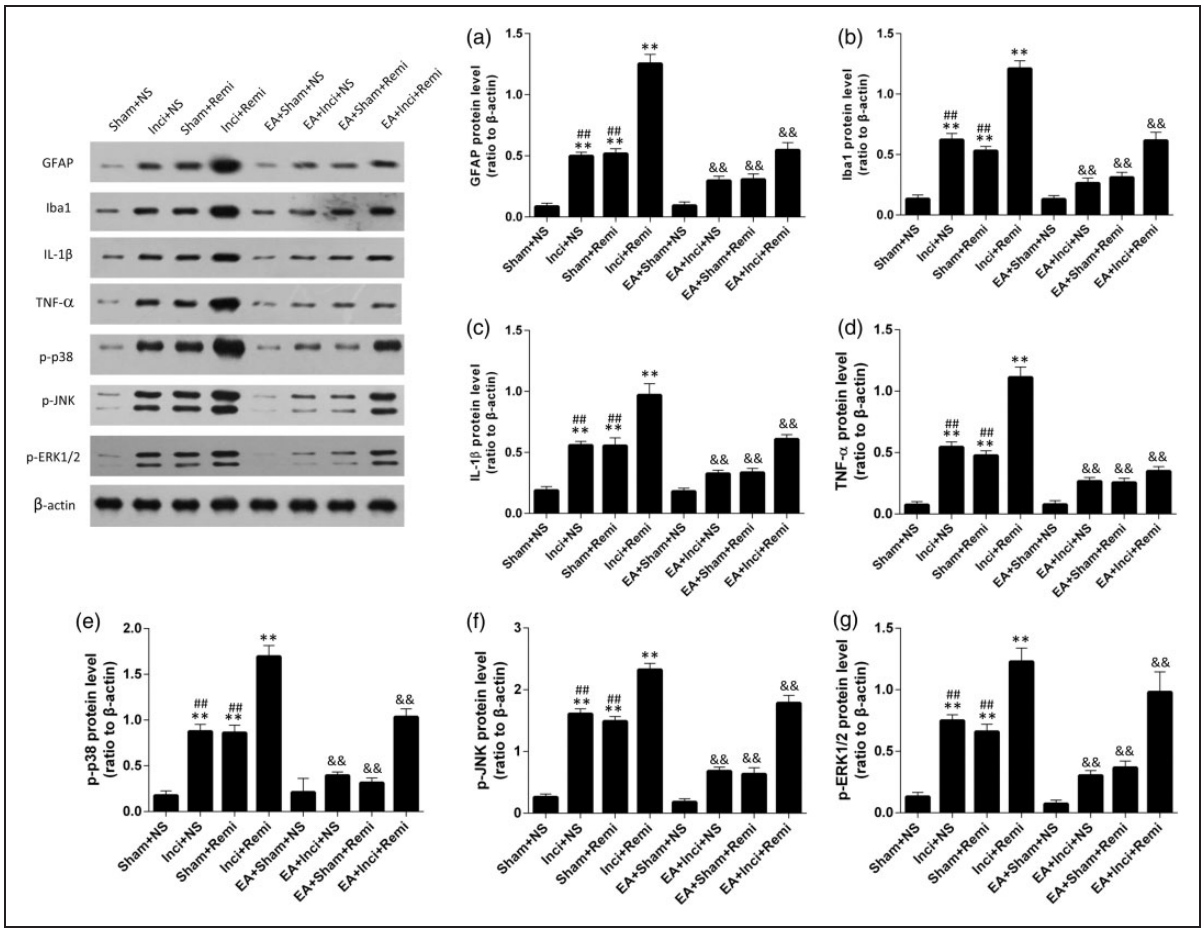

Figure 4. Effects of EA on the upregulation of GFAP, Iba1, IL-1β, TNF-α, p-p38, p-JNK, and p-ERK1/2 in the dorsal horn of L4–L5 spinal cord induced by surgical incision, remifentanil infusion, or their combination at 1 d after the surgical procedure as detected by Western blot ($n = 4$). Compared with group Sham + NS rats, the expression of GFAP, Iba1, IL-1β, TNF-α, p-p38, p-JNK, and p-ERK1/2 moderately increased in group Inci + NS and group Sham + Remi rats and dramatically increased in group Inci + Remi rats. EA effectively blocked the enhanced expression of GFAP, Iba1, IL-1β, TNF-α, p-p38, p-JNK, and p-ERK1/2 induced by surgical incision, remifentanil infusion, or their combination. β-actin was used as the internal control. Data were expressed as mean ± SD. $^{**}P < 0.01$ compared with group Sham + NS; $^{\&\&}P < 0.01$ compared with corresponding non-EA treatment group; $^{\#\#}P < 0.01$ compared with group Inci + Remi. EA: electroacupuncture; Inci: incision; Remi: remifentanil; NS: normal saline; Sham: sham surgery.

hyperalgesia in rats.[8,19] As our results showed, treatments including sevoflurane anesthesia, sham surgery, and NS infusion had no influence on pain behavior, which is consistent with our previous studies[8,37] and other studies.[2,38] Both surgical incision and remifentanil infusion induced significant mechanical allodynia and thermal hyperalgesia. When remifentanil was infused as an analgesic during surgical incision as a part of general anesthesia, it significantly enhanced incisional pain and prolonged mechanical allodynia and thermal hyperalgesia.

It is now well established that glial cells, in addition to neurons, are involved in the initiation and maintenance of various pain conditions.[39] Evidence suggests that glial cells can be activated after painful stimuli and injuries, releasing various glial mediators including proinflammatory cytokines that modulate neuronal activity and synaptic strength.[40–45] In the spinal cord, IL-1β and TNF-α are the main potent proinflammatory cytokines and can be induced under various injury conditions to contribute to pain hypersensitivity.[46] Opioid treatment can also activate glial cells to induce OIH.[47,48] Although studies on the mechanisms underlying OIH have mainly focused on neuronal cells, accumulating evidence has demonstrated that activation of glial cells is closely implicated in the development and maintenance of OIH.[9,48] Our previous study showed that glial cells were activated and proinflammatory cytokines increased during the process of remifentanil-induced postoperative hyperalgesia.[8] Opioid treatment can stimulate MAPKs in glial cells, leading to proinflammatory cytokine upregulation.[15,49] In turn, proinflammatory cytokines can

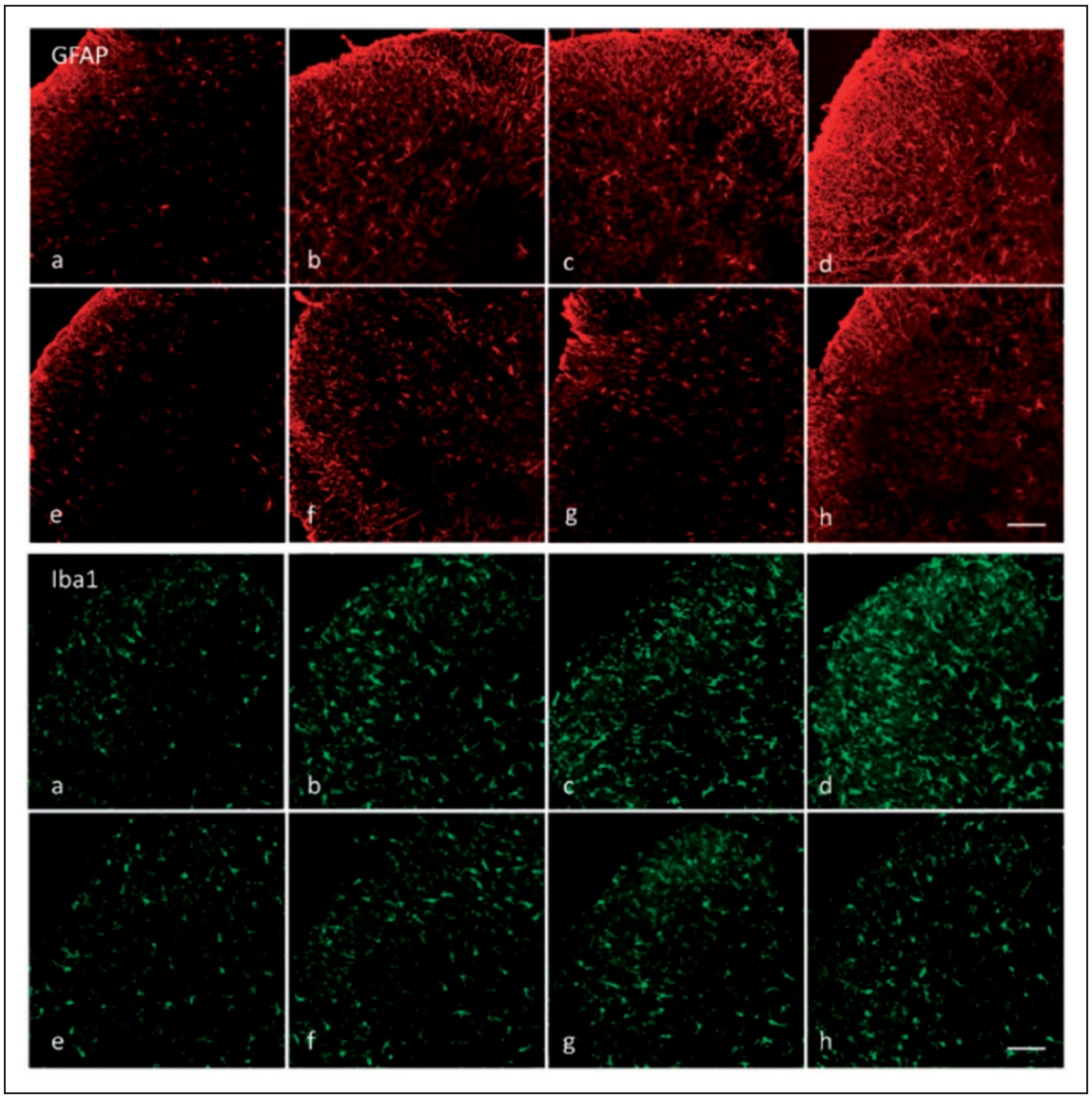

Figure 5. Effects of EA on the enhanced expression of GFAP and Iba1 in the dorsal horn of L4–L5 spinal cord induced by surgical incision, remifentanil infusion, or their combination at 1 d after the surgical procedure as detected by immunofluorescence ($n = 4$). Compared with group Sham + NS (a) rats, the expression of GFAP and Iba1 moderately increased in group Inci + NS (b) and group Sham + Remi (c) rats, and dramatically increased in group Inci + Remi (d) rats. EA effectively blocked the enhanced expression of GFAP and Iba1 induced by surgical incision (f), remifentanil infusion (g), or their combination (h). a: group Sham + NS; b: group Inci + NS; c: group Sham + Remi; d: group Inci + Remi; e: group EA + Sham + NS; f: group EA + Inci + NS; g: group EA + Sham + Remi; h: group EA + Inci + Remi. Scale bar = 100 μm. EA: electroacupuncture; Inci: incision; Remi: remifentanil; NS: normal saline; Sham: sham surgery.

also induce further activation of MAPKs.[50] In our study, surgical incision and remifentanil infusion synergistically increased the expression of GFAP, Iba1, IL-1β, TNF-α, p-p38, p-JNK, and p-ERK1/2 in the spinal cord at 1 d after the surgical procedure when the nociceptive sensitization reached its peak, indicating that significant spinal astrocyte and microglial activation, proinflammatory cytokine upregulation, and MAPK activation may contribute to remifentanil-induced postoperative hyperalgesia.

As an integral part of traditional Chinese medicine, EA has been widely used to relieve multiple types of pain in both clinical and animal studies. The antinociceptive mechanisms of EA are considered multifactorial, including peripheral, spinal, and supraspinal mechanisms.[24,25] Spinal glial cells may be involved in the antinociceptive effect of EA. For example, EA stimulation of Huantiao (GB30) and Yanglingquan (GB34) acupoints significantly relieved complete Freund's adjuvant (CFA)-induced nociceptive behavioral hypersensitivity and

suppressed spinal microglial activation and upregulation of TNF-α, IL-1β, and IL-6 mRNA levels in a rat model of monoarthritis[51]; EA at Zusanli (ST36) and Sanyinjiao (SP6) acupoints once daily for 3 d significantly inhibited CFA-induced inflammatory pain and upregulation of spinal GFAP in rats[31]; EA at bilateral Huantiao (GB30) acupoints once daily for 14 d relieved nerve injury-induced mechanical allodynia and thermal hyperalgesia via the inhibition of spinal P2X7 receptor-positive microglia-mediated IL-1β overexpression in rats[52]; EA at bilateral Zusanli (ST36) and Kunlun (BL60) acupoints inhibited CFA-induced inflammatory pain, paw swelling, and upregulation of spinal p-p38 in rats.[32] Zusanli (ST36) is one of the most commonly selected acupoints in various pain studies.[24] The stimulating parameters (2 Hz in frequency and 1 mA in intensity) are also frequently used in pain studies.[53,54] In the present study, intraoperative EA stimulation at bilateral ST36 with 2 Hz in frequency and 1 mA in intensity for 30 min effectively relieved the mechanical allodynia and thermal hyperalgesia induced by surgical incision, remifentanil infusion, or their combination, as well as suppressed incision- and/or remifentanil-induced activation of spinal glial cells and upregulation of spinal proinflammatory cytokines (IL-1β and TNF-α) and p-MAPKs (p-p38, p-JNK, and p-ERK1/2). With the increasing use of remifentanil to provide intraoperative analgesia during surgery, the development of remifentanil-induced postoperative hyperalgesia has been considered a clinically significant phenomenon requiring appropriate treatment. Although several kinds of drugs (ketamine, magnesium sulphate, cyclooxygenase inhibitors, dexmedetomidine, pregabalin, etc.) have been proposed to treat RIH, these drugs can cause various side effects. We anticipate that EA, as a valuable antinociceptive therapy with few side effects, may be potentially suitable for the treatment of remifentanil-induced postoperative hyperalgesia.

In summary, our results showed that intraoperative EA treatment relieved incision-induced hyperalgesia and/or RIH via inhibiting spinal glial cell activation, proinflammatory cytokine upregulation, and MAPK activation, thereby providing a promising alternative treatment as a part of multimodal perioperative analgesia with few adverse effects for remifentanil-induced postoperative hyperalgesia.

Author contributions

CS and ZM conceived and designed the experiments. CS, YL, WZ, YL, CL, and RS performed the experiments. CS, YS, and MJ analyzed the data. YL and XG prepared the figures. CS drafted the manuscript. ZM and XG edited and revised the manuscript. All authors read and approved the final manuscript. CS and YL contributed equally to this study.

Declaration of Conflicting Interests

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