Article

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Electroacupuncture Treatment Alleviated Intracolonic Capsaicin-Induced Rectal Visceral Pain by Inhibiting the Expressions of TRPV1 and P2X4R and Blocking MAPK Pathway

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Background: Electroacupuncture (EA) treatment can alleviate chronic neuropathic pain, like visceral pain, but the underlying mechanism remains unclear.
Methods: Herein, rats were intracolonically injected with capsaicin to create a visceral (rectal) pain model. Then rats were treated with EA. The number of visceral pain-related behaviors, along with the mechanical pain thresholds of the rats' tail, metapedes and abdomen, were recorded. Meanwhile, the pathology of rectal tissue was observed by hematoxylin-eosin staining. The expressions of inflammatory factors (interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF-α)) and glial fibrillary acidic protein (GFAP) in the rat spinal cord or serum were assessed using enzyme-linked immunosorbent assay or Western blot. Likewise, the expression level of oxycocin-42 (OX42), GFAP, transient receptor potential vanilloid 1 (TRPV1), P2X4 receptor (P2X4R) and mitogen-activated protein kinase (MAPK) pathway-related proteins were determined by immunofluorescence or Western blot in the rats' spinal cord.
Results: The increased visceral behavioral pain, mechanical pain threshold, secretion of inflammatory factors, edema of rectal mucosa and activation of spinal glial cells induced by capsaicin were all reversed by EA treatment. Additionally, the upregulation of TRPV1 and P2X4R as well as the activation of the MAPK pathway caused by capsaicin was offset by EA treatment.
Conclusions: EA treatment ameliorates rats' rectal visceral pain by repressing TRPV1 and P2X4R expression levels as well as blocking MAPK pathway.

Keywords: rectal visceral pain; electroacupuncture; transient receptor potential vanilloid 1; P2X4 receptor

Introduction

Visceral pain is considered all the pain suffered in the internal organs of the body. It is a major symptom of functional gastrointestinal disorders like the irritable bowel syndrome (IBS) [1]. IBS-related visceral pain usually arises from the distal colon and rectum (colorectum) and it is different from cutaneous pain in several key psychophysical characteristics [2]. Therefore, it is not effective to extrapolate results indiscriminately from other types of pain to visceral pain.

Microglia is the first line of defense in the central nervous system, where astrocytes are the most populated nerve cells. A previous research has reported that these two cells are activated during visceral pain and play important roles in the integration and conduction of pain [3]. To this end, treatments for spinal cord glial cells might be the key to alleviate visceral pain.

Acupuncture has been used to relieve various clinical pain in Chinese traditional medicine for thousands of years, with the great merits of definite curative effect, simple operation, economy, practicality and few side effects [4]. It has been reported that electroacupuncture (EA) can inhibit the activation of spinal glial and microglial cells to relieve neuropathic pain [5]. In addition, EA treatment can also downregulate the levels of spinal cord pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α that promote pain [6]. However, whether EA treatment to relieve visceral pain is mechanically related to the inhibition of spinal cord cell activation and pro-inflammatory cytokine secretion remains unclear.

Visceral pain involves multiple ion channels and receptors, where transient receptor potential vanilloid 1 (TRPV1) and P2X receptor (P2XR) have received attention [7]. TRPV1, a non-selective cation channel, acts as an integrator of pain stimuli, which can be activated by noxious

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heat stimuli and capsaicin [8]. P2XR belongs to the family of ligand-gated cation channel, which is mainly activated by extracellular ATP (Adenosine triphosphate), also involving the occurrence and development of inflammatory pain and neuropathic pain. There are 7 subtypes of P2XR, among which P2X4R (P2X4 receptor) in microglia plays an important role in chronic pain [9]. Evidence showed that EA can alleviate neuropathic pain by inhibiting TRPV1 and P2X4R levels [10,11]. Nevertheless, whether the mechanism of EA treatment on visceral pain is associated with TRPV1 and P2X4R needs to be further explored.

MAPKs (mitogen-activated protein kinases) play a central role in many signaling pathways. There are three major subfamilies of MAPKs: The extracellular signal-regulated kinase (ERK 1/2), the c-Jun N-terminal kinase (JNK) and P38 kinase. Activation of ERK signaling is important to maintain nociception and visceral pain [12]. Capsaicin, a TRPV1 agonist, causes transient hyperalgesia in the lower abdomen, and triggers a colonic hyperalgesia, accompanied by spinal ERK pathway activation [13]. EA stimulation inhibits the activation of astrocytes in spinal cord and reduces the release of inflammatory cytokines in IBS-modeled rats with visceral hypersensitivity by repressing P2Y1 receptor-mediated MAPK/ERK signaling pathway [14]. Therefore, we hypothesized that EA stimulation could alleviate rectal visceral pain by inhibiting the expressions of TRPV1 and P2X4R and activating MAPK pathway. A technically simple rectal visceral rat model was constructed by chemical stimulation of the rat colon with capsaicin [15]. Typically, the visceral pain of model rats was evaluated by visceral pain-related behaviors and referred hyperalgesia to the abdominal wall. In this study, we explored the role of EA treatment in rectal visceral pain by the intracolonic injection of capsaicin in rats.

Materials and Methods

Animals and Ethics Statement

A total of 18 Sprague Dawley rats (6–7 weeks, 200–225 g), supplied by Hangzhou Medical College, were reared at the temperature of 21 ± 0.5 °C and the humidity of 45–50% with lights on at 8:00 AM and off at 8:00 PM. Animal experiments were performed in Zhejiang Baiyue Biotechnology Co., Ltd. in accordance with the guidelines of the China Council on Animal Care and Use. The rats were equally and randomly divided into three groups. (1) Control group (rectal injection of saline in rats), (2) the CAP (Capsaicin) group (rectal injection of capsaicin in rats), and (3) the EA group (rectal injection of capsaicin in rats followed by EA treatment).

Establishment of Visceral Pain Model

The visceral pain model was established as previously described with minor modifications [15]. After, the rats were anesthetized, with 45 mg/kg sodium pentobarbital (P010, Merck, Darmstadt, German) and vaseline was applied to the exposed skin around the anus. Then, a round headed thin tube with a diameter of 1.5 mm was quickly inserted into the rat rectum through the anus. Finally, 0.5 mL of 10^{-4} mmol/L CAP (SC8100, Solarbio, Beijing, China) or saline was injected into the rectum of rats in the CAP group or the control group, respectively, through a thin tube, once every 5 min, for a total of 4 injections.

EA Treatment

Rats have only three pairs of posterior sacral foramina, where the 2nd and 3rd pairs are similar to those of humans: Ci Liao (BL 32), Zhong Liao (BL 33), and Xia Liao (BL 34) [16]. The Ci Liao (BL 32) and Zhong Liao (BL 33) are located 5–10 mm lateral to the superior border of the 2nd and 3rd intervertebral space of the rats' tail. The Xia Liao (BL 34) is located 5–10 mm lateral to the superior border of the 3rd and 4th intervertebral space of the rats' tail. In short, the 0.35 × 25 mm acupuncture needles, connected to the electroacupuncture instrument (SDZ-II, HWATO, Suzhou, China), were inserted into the "Baliao" acupoints of the rat in CAP group as previously described [16]. The EA stimulation frequency was set at 2–15 Hz, and the current intensity range was 1–1.5 mA. Electrical acupuncture stimulation was performed for 30 min a day, lasting for 7 consecutive days.

Visceral Pain-Related Behaviors

Rats' visceral pain-related behaviors (licking abdomen, stretching, contractions of abdomen, etc.) in each group were observed and recorded within 20 min after rectal injection of different solutions as previously described [17].

Measurement of Mechanical Pain Threshold

Rats' mechanical pain threshold was measured as previously described [17]. 1 h after the injection of the different solutions, the rats were placed in a transparent plexiglass box with a wire mesh at the bottom. Electronic Von Frey system (2390 series, IITC, Wood Dale, IL, USA) was used to stimulate the tail, metapedes and abdomen of rats. The Von Frey filament was raised with a constant force of 2 g/s (cut-off force 50 g) until the rat withdrawn paw, arched back and raised the tail.

Hematoxylin-Eosin (H&E) Staining

After finishing observation of behaviors and measurement of mechanical pain threshold, the rats were anesthetized with sodium pentobarbital (45 mg/kg). Subsequently, their chests were opened, and the ascending aorta was intubated with the perfusion needle through the left ventricle, followed by a quickly flushed with 0.9% normal saline. Then, the ascending aorta was perfused and fixed with phosphate buffer (pH 7.4, PML4280, Coolaber, Beijing, China) containing 4% paraformaldehyde (E672002,

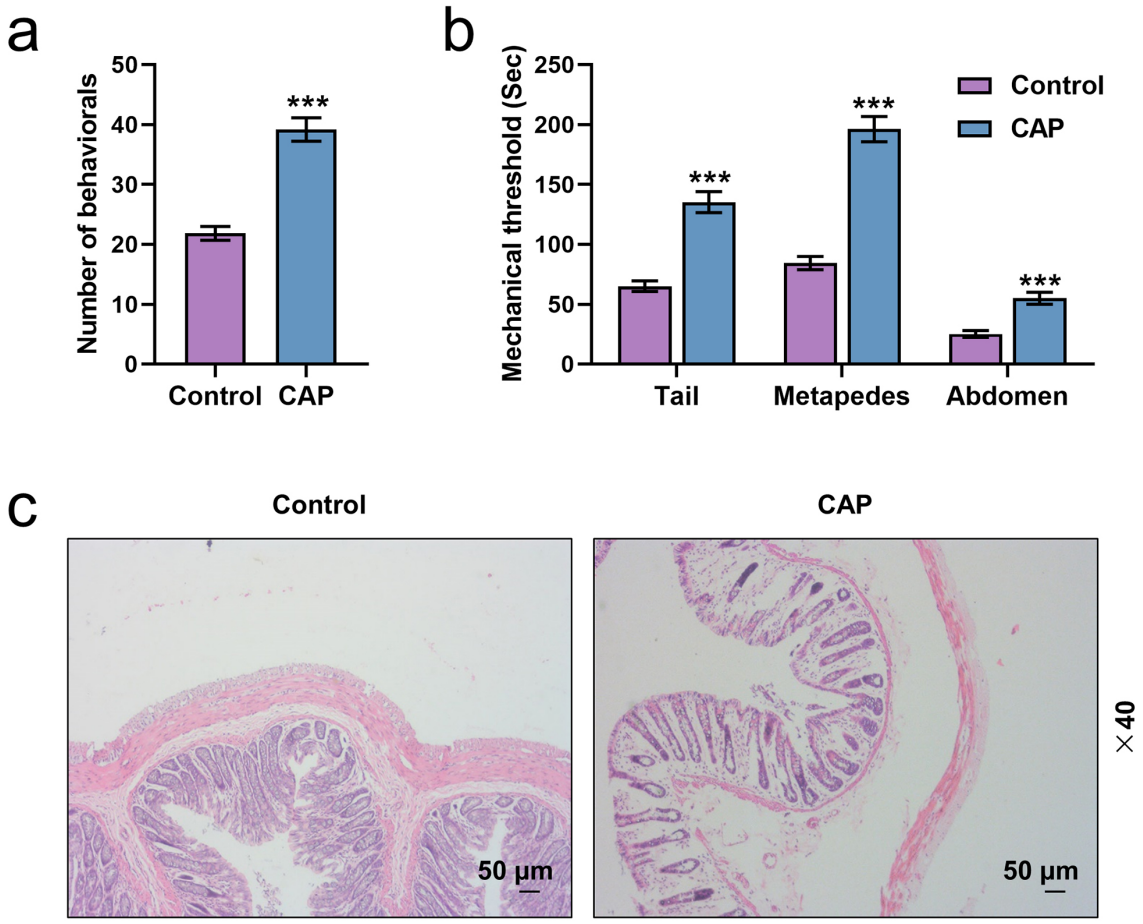

Fig. 1. Establishment of visceral pain model. (a) The number of visceral pain-related behaviors after rectal injection of capsaicin or normal saline in the CAP and control groups. (b) Mechanical pain thresholds of tail, metapedes and abdomen in rats with visceral pain or control rats measured by Von Frey filament in CAP and control groups. (c) Pathological conditions of rectal tissues from rats with visceral pain and control rats determined by H&E staining (magnification, ×40). Scale bar = 50 μm. $^{***}p < 0.001$ vs. control group. Quantified values of at least three independent experiments, reported as mean ± standard deviation. CAP, Capsaicin; H&E, Hematoxylin-Eosin.

Sangon, Shanghai, China) and 0.2% picric acid for 40 min. Next, rectal tissues and S2–S3 spinal cord tissues of rats were collected and immersed in a solution containing 30% sucrose. Then, 30-μm-thick sections of rectal tissue were made using a cryostat microtome (KD-2850, KEHUAI, Shanghai, China). Finally, rectal tissue sections were stained with H&E kit (G1031, Servicebio, Wuhan, China) and their changes were further observed under a microscope (BX53M, Olympus, Tokyo, Japan, ×40).

Immunofluorescence

Spinal cord tissue obtained following the above steps was cut into 40-μm-thick sections using cryostat microtome and then used for immunofluorescence staining. In short, the sections were fixed in 4% paraformaldehyde and then permeabilized with Triton X-100 (A600198, Sangon, Shanghai, China). After being blocked with 5% goat serum (E510009, Sangon, Shanghai, China), the sections were incubated with primary antibodies against Glial fibrillary acidic protein (GFAP, 1:10; ab4648, abcam, Cambridge, UK), oxycocin-42 (OX42; Alexa Fluor® 647, 1:100, ab216524, abcam, Cambridge, UK), TRPV1 (1:1000, ab203103, abcam, Cambridge, UK) and P2X4R (1:50; ab134559, abcam, Cambridge, UK), followed by a reaction with secondary antibody goat anti rabbit IgG (Alexa Fluor® 647, ab150083, abcam, Cambridge, UK). Finally, spinal cord tissue was observed under a fluorescence microscope (IX73, Olympus, Tokyo, Japan).

Enzyme-Linked Immunosorbent Assay (ELISA)

Inflammatory factors content in spinal cord and serum were assessed using the IL-1β ELISA Kit (E-EL-R0012c, Elabscience, Wuhan, China), TNF-α ELISA Kit (E-EL-R2856c, Elabscience, Wuhan, China) and IL-6 ELISA Kit (E-EL-R0015c, Elabscience, Wuhan, China) according to manufacturers' instructions. Briefly, the anti-rat antibody was coated on the ELISA plate, and then the sample or standard was bound to the coated antibody. Then bi-

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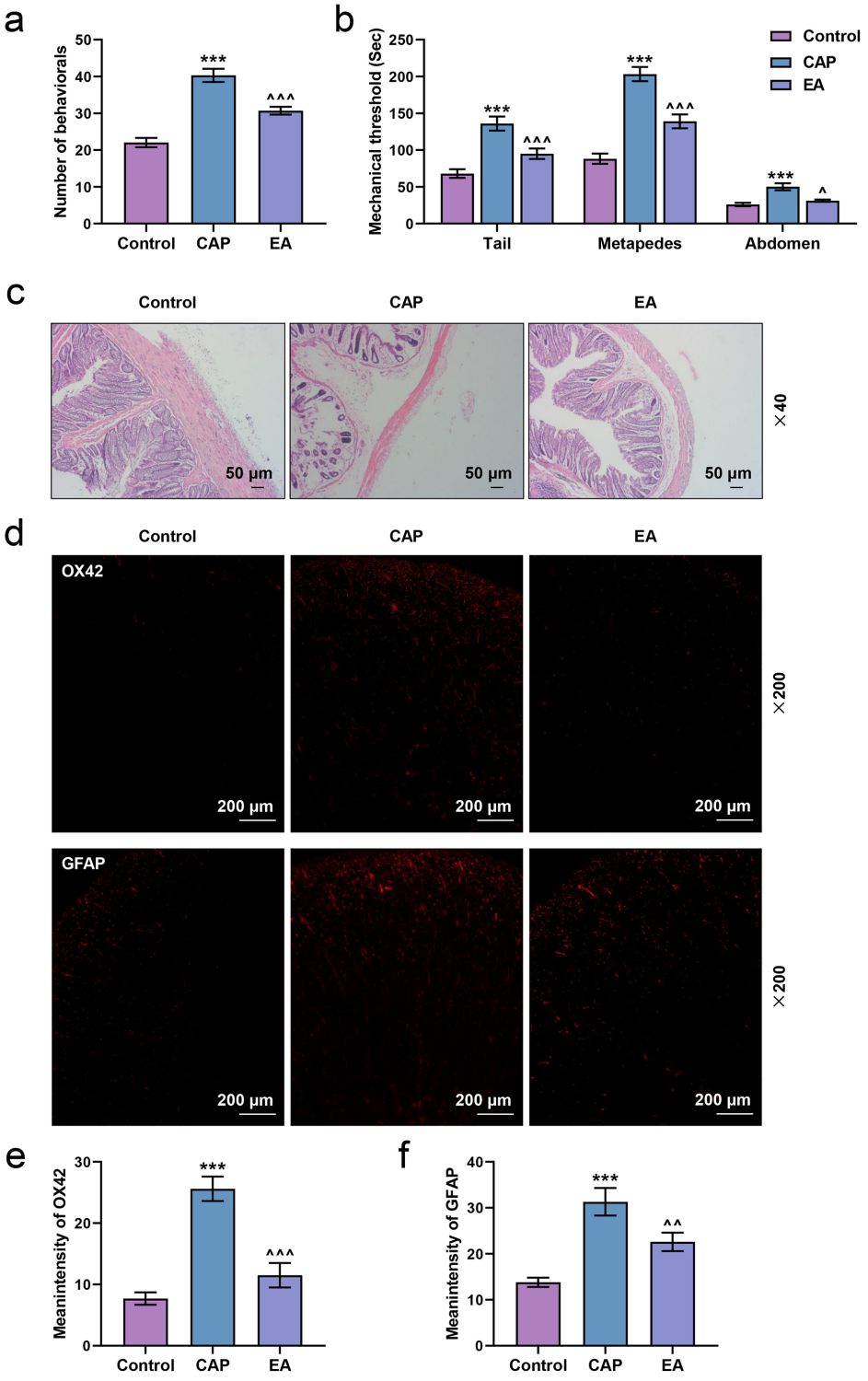

Fig. 2. EA treatment alleviated the pain and inhibited the activation of microglia and astrocytes in rats with visceral pain. (a) Number of visceral pain-associated behaviors of rats in the control, CAP and EA groups. (b) Mechanical pain thresholds of tail, metapedes and abdomen in the control, CAP and EA groups measured by Von Frey filament. (c) Pathological conditions of rat rectal tissues in the control, CAP and EA groups determined by H&E staining (magnification, ×40). Scale bar = 50 μm. (d–f) Immunofluorescence adopted to detect the expressions of OX42 and GFAP in the dorsal horn of the rat spinal cord (magnification, ×200). Scale bar = 200 μm. $^{***}p < 0.001$ vs. control group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ vs. CAP group. Quantified values of at least three independent experiments, reported as mean ± standard deviation. EA, Electroacupuncture; CAP, Capsaicin; GFAP, Glial fibrillary acidic protein; H&E, Hematoxylin-Eosin.

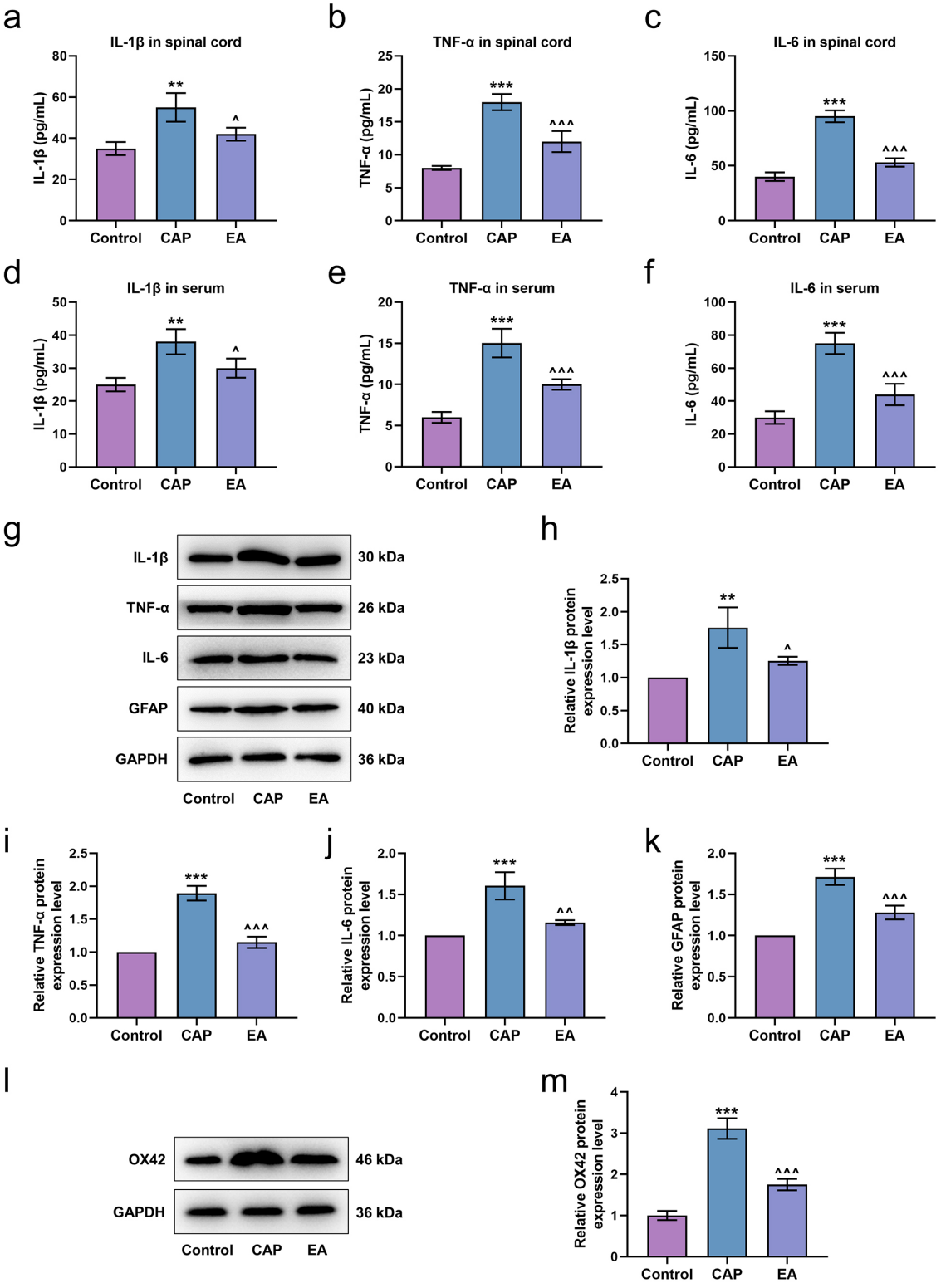

Fig. 3. EA inhibited the expressions of inflammatory factors, GFAP and OX42 in the spinal cord and serum of rats with visceral pain. (a–f) Levels of IL-1β, TNF-α and IL-6 in rat spinal cord and serum detected by ELISA kit in the control, CAP and EA groups. (g–m) The protein expressions of IL-1β, TNF-α, IL-6, GFAP and OX42 in rat spinal cord tissue determined by Western blot in the control, CAP and EA groups. GAPDH was used as internal control. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ vs. CAP group. Quantified values of at least three independent experiments, reported as mean ± standard deviation. EA, Electroacupuncture; GFAP, Glial fibrillary acidic protein; CAP, Capsaicin; IL, Interleukin; TNF, Tumor necrosis factor; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

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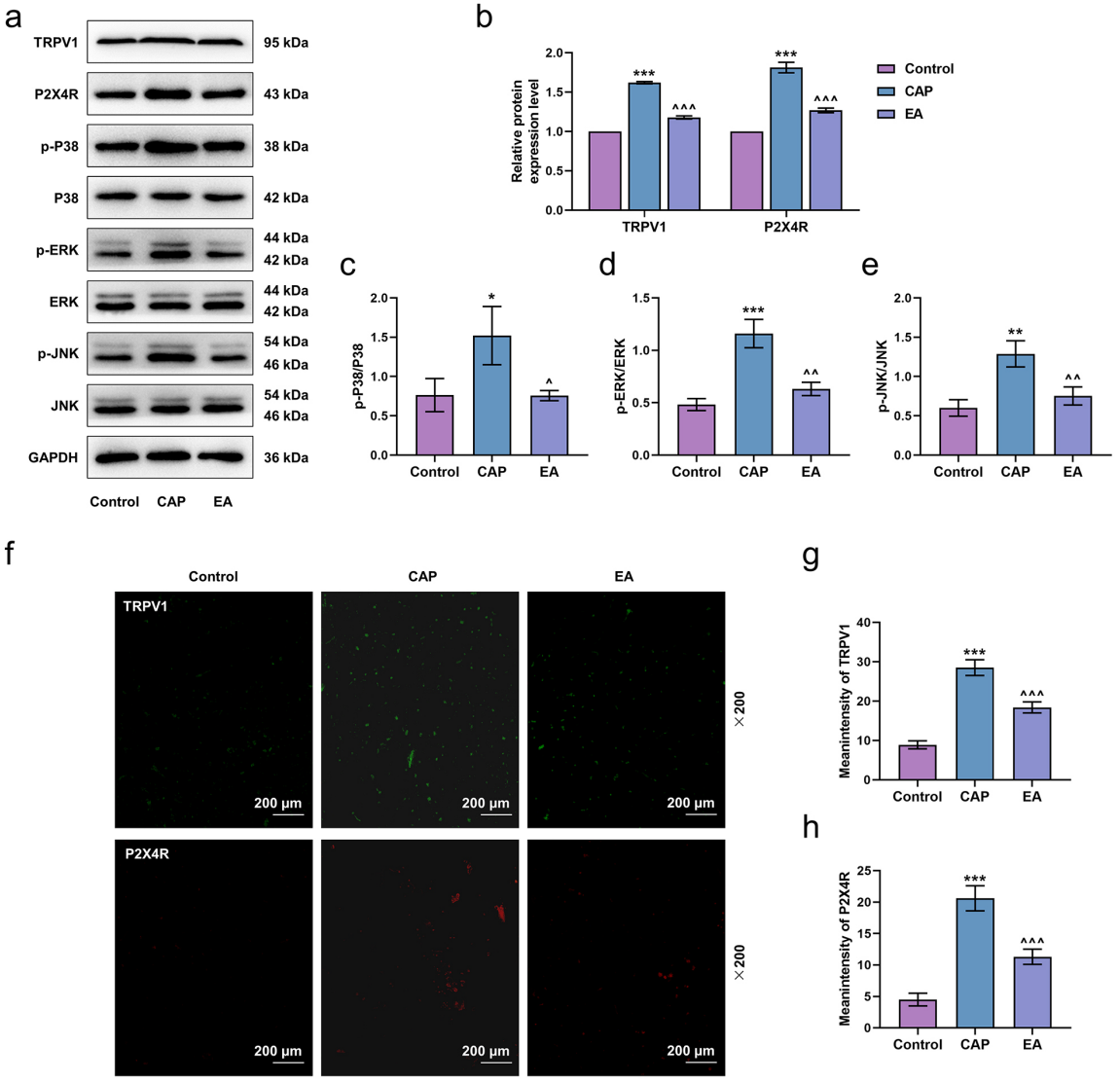

Fig. 4. Reduced the expressions of TRPV1 and P2X4R and inhibition of MAPK pathway in the spinal cord of rats with visceral pain after EA treatment. (a–e) TRPV1, P2X4R, P38, p-P38, ERK, p-ERK, JNK and p-JNK expression in the spinal cord of the control, CAP and EA groups determined by Western blot. GAPDH was employed as internal control. (f–h) Immunofluorescence used to determine TRPV1 and P2X4R expresion in the dorsal horn of the rat spinal cord (magnification, ×200). Scale bar = 200 μm. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ vs. CAP group. Quantified values of at least three independent experiments, reported as mean ± standard deviation. EA, Electroacupuncture; CAP, Capsaicin; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; TRPV1, Transient receptor potential vanilloid 1; P2X4R, P2X4 receptor; p-P38, Phosphor-P38.

otinylated antibody, horseradish peroxidase-labeled avidin, chromogenic substrate and stop solution were added into the plate in turn. Finally, the optical density (OD) value was measured at a wavelength of 450 nm using a multimode microplate reader (VL0000D2, ThermoFisher, Waltham, MA, USA).

Western Blot

Western blot was conducted as described previously [14]. In brief, RIPA lysate (R0010, Solarbio, Beijing, China) was used to extract the protein from the collected rat spinal cord tissue, and then it was quantified by BCA kit (PC0020, Solarbio, Beijing, China). After, the protein was transferred onto a PVDF membrane (IPFL00010, Millipore, Burlington, MA, USA) using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Following blocking and cultured with primary antibodies at 4 °C overnight, the membranes were washed by TBST buffer (T1085, Solarbio, Beijing, China) and then incubated with secondary antibodies. The relative intensity of the protein bands was visualized using West Femto ECL Substrate (PE0030, Solarbio, Beijing, China) on a

gel imaging system (FluorChem FC3, Alpha, San Leandro, CA, USA), with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) serving as the loading control.

The antibodies purchased from abcam were: IL-1β (1:1000; ab254360, 30 kDa), TNF-α (1:1000; ab205587, 26 kDa), IL-6 (1:1000; ab259341, 23 kDa), GFAP (1:5000; ab207165, 40 kDa), TRPV1 (1:1000; ab203103, 95 kDa), P2X4R (1:100; ab134559, 43 kDa), phospho-P38 (p-P38, 1:1000; ab4822, 38 kDa), P38 (1:1000; ab170099, 42 kDa), p-ERK (1:1000; ab201015, 44/42 kDa), ERK (1:10000; ab184699, 44/42 kDa), p-JNK (1:5000; ab76572, 54/46 kDa), JNK (1:1000; ab179461, 54/46 kDa), GAPDH (1:1000; ab8245, 36 kDa), goat anti-rabbit IgG (1:2000, ab6721) and rabbit anti-mouse IgG (1:3000, ab6728). Anti-OX42 antibody (1:1000; GTX76060; 46kDa) was purchased from GeneTex (Irvine, CA, USA).

Statistical Analysis

Data is reported as mean ± standard deviation. Independent sample *t* test was performed to compare the control group against the CAP group. One-way analysis of variance (ANOVA) was used for multiple between groups comparison. The statistical analysis was performed using Graphpad 8.0 software (Graphpad Software, San Diego, CA, USA), where $p < 0.05$ was considered as statistical significance.

Results

Establishment of Visceral Pain Model

A visceral pain model was created by injecting capsaicin into the rectum of rats. Number of rats' visceral pain-related behaviors of the CAP group (number of behavioral = 40) were higher than those in the control group (number of behavioral = 22) (Fig. 1a, $p < 0.001$). In addition, after rectum stimulation with capsaicin, the mechanical thresholds (sec) of the tail (125.2 ± 31.5), metapedes (185.4 ± 45.8) and abdomen (43.8 ± 15.1) of the rats increased in the CAP group compared to the control group (tail (63.4 ± 12.5), metapedes (78.2 ± 16.8) and abdomen (23.1 ± 4.2)) (Fig. 1b, $p < 0.001$). Results of H&E staining showed that there was obvious edema in the rectal submucosal tissue of the CAP group, but not marked edema was observed in rectal submucosal tissue of rats in the control group (Fig. 1c).

EA Reduced Visceral Pain and Mechanical Pain in CAP Rats and Inhibited the Activation of Microglia and Astrocytes in the Spinal Cord

The EA group showed less visceral pain behavior (number of behavioral = 31) (Fig. 2a, $p < 0.001$), lower mechanical thresholds of tail (88.4 ± 21.1), metapedes (122.8 ± 28.2) and abdomen (32.1 ± 11.8) (Fig. 2b, $p < 0.05$), and the edema of the rectal submucosal tissue improved (Fig. 2c) compared to the CAP group. In addition, immunofluorescence uncovered that OX42 (a marker for microglia) and GFAP (a marker for astrocytes) in the rat spinal cord rose sharply after CAP modeling, but these trends were reversed by EA treatment (Fig. 2d–f, $p < 0.01$).

EA Reversed the Upregulation of Inflammatory Factors, GFAP and OX42 Induced by Capsaicin

After ELISA analysis it was observed that CAP induced the upregulation of IL-1β (55 pg/mL; 38 pg/mL), TNF-α (18 pg/mL; 15 pg/mL) and IL-6 (95 pg/mL; 75 pg/mL) in the spinal cord and serum of rats, but EA treatment reduced the levels of these inflammatory factors (IL-1β (42 pg/mL; 30 pg/mL), TNF-α (12 pg/mL; 10 pg/mL) and IL-6 (55 pg/mL; 42 pg/mL)) (Fig. 3a–f, $p < 0.05$). The results of Western blot were consistent with those of ELISA (Fig. 3g–j, $p < 0.05$). Furthermore, we found that GFAP (astrocyte specific protein), and OX42 (microglia specific protein) levels increased in the CAP group, but these trends were reversed by EA treatment (Fig. 3g and Fig. 3k–m, $p < 0.001$).

The Upregulation of TRPV1 and P2X4R and the MAPK Pathway Activation Caused by Capsaicin was Offset by EA

Spinal cord-related proteins expression and the MAPK pathway activation was assessed. The results showed that the TRPV1 and P2X4R expression in the CAP group were significantly higher than those of the control group. P38, ERK and JNK pathways were simultaneously activated in the CAP group as compared to the control group (Fig. 4a–e, $p < 0.05$). After EA treatment, TRPV1 and P2X4R expression, together with the activation of P38, ERK and JNK pathways, were inhibited at different degrees ($p < 0.05$). Similarly, the results of immunofluorescence displayed that EA treatment reversed the increased in TRPV1 and P2X4R expression induced by capsaicin (Fig. 4f–h, $p < 0.001$).

Discussion

There are many animal models of visceral pain, but most of them need to be constructed on specific experimental animals [18]. The establishment of visceral pain models require a great stimulation for animals which may provoke that results are misunderstood with physical pain. A previous study elucidated that capsaicin injection into colon and rectum can elicit inflammation and physical pain in corresponding parts of rats, which is relatively easy to operate and has good repeatability [15]. In view of this, we constructed a visceral pain model by directly injecting capsaicin into the rats' rectum, in order to unveil the effect of EA treatment on visceral pain.

In this study, we conducted EA stimulation at rats' Baliao acupoint. Baliao acupoint is located symmetrically in the four pairs of posterior sacral foramina, and EA applied to Baliao acupoint exerts regulatory effect on nervi sacrales [16]. Generally, EA stimulation of Baliao acu-

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point can affect the sacral innervated effector organs, such as bladder, urethra sphincter and pelvic floor by sacral reflex arc interference. It has been reported that Baliao acupoint stimulation improve intestinal function via balance of the microbiota-gut-brain axis regulation and ameliorate the functional constipation through the intestine-brain axis [16]. As expected, in this study, EA at Baliao acupoint effectively relieved the pain in rats with visceral pain. Mechanically, we speculated that EA stimulation at the Baliao acupoint may affect the domination of the sacral nerves over the pelvic floor muscles, thus positively impacting the coordinated movement of the pelvic floor muscles.

Recently, it was found that regulating the peripheral glia activity emerges as a promising strategy to attenuate colitis-induced visceral pain [19]. Glia is a non-neuronal immune-like cell group, where astrocytes and microglia maintain the steady state of the central nervous system [20]. When stimulated, astrocytes and microglia increase the release of pro-inflammatory factors destroying the steady state of the central nervous system leading to a sustained pain sensation [19]. In this study, EA treatment reversed capsaicin-induced the up-regulation of OX42 and GFAP, and reversed capsaicin-induced the increase in the secretion of inflammatory factors. This means that EA treatment inhibited inflammatory reaction by inhibiting the activation of astrocytes and microglia what led into a relieve of visceral pain in capsaicin-treated rats.

The activation of astrocytes and microglia is related to the activation of ion channels or receptors. A study found that up-regulation of P2X4R could stimulate microglia to cause hyperalgesia [21]. Another study has reported that P2X4R inhibitors prevent the occurrence of remifentanil-induced postoperative hyperalgesia, leading into a reduction in mechanical and thermal pain [22]. Of note, EA treatment can alleviate neuropathic pain in rats by a reduction of P2X4R expression and inhibiting the activation of microglia [11]. In line with this finding, this mechanism has also been verified in our visceral pain model. Besides of the finding which shows that TRPV1 may regulate thermal perception by increasing astrocytes in the dorsal horn of the spinal cord [23]. Li *et al.* [10] in 2019 year found that EA treatment reduces the peripheral neuropathic pain induced by paclitaxel in rats via inhibition of the up-regulation of TRPV1. Similar to the previous report, our research showed that EA treatment suppressed TRPV1 expression in rats with capsaicin-induced visceral pain.

This study also found that the anti-inflammatory and analgesic effects of EA were related to the MAPK signal transduction pathway regulation. This may be due to the fact that MAPK signal transduction pathway is the main valve to regulate multiple signal pathways in the central sensitization process [24]. The sub-pathways of MAPK include P38, ERK and JNK. EA can exert anti-inflammatory and analgesic effects by inhibiting p-P38, p-ERK and p-JNK in spinal cord [25]. A report by Jin *et al.* [26] in 2021 year demonstrated that EA reduces p38 MAPK and TNF-α expression levels, and yet promotes the mechanical pain threshold in rats.

EA inhibits astrocyte activity in the spinal cord dorsal horn of rats with IBS visceral hypersensitivity by inhibiting p-ERK1/2 level [14]. In addition, EA treatment can suppress the activation of p38 MAPK and the release of pain-related inflammatory cytokines in rats with inflammatory pain [27]. In this sense, our study showed that EA inhibited inflammation, decreased the levels of p-P38, p-ERK and p-JNK in the spinal cord and exerted analgesic effects on rats with visceral pain. In addition, it has been reported a regulatory relationship between MAPK pathway and P2X4R or TRPV1. For instance, up-regulation of P2X4R increases calcium influx to promote phosphorylation of P38 and promotes central sensitization by releasing brain-derived neurotrophic factors [21]. TRPV1 activation promotes the phosphorylation of P38 to regulate the microvesicle shedding of microglia via P2X7R activation [28]. However, whether the mechanism of EA alleviating pain in rats with visceral pain is related to the regulation of TRPV1/MAPK or P2X4R/MAPK needs further research.

Jiang *et al.* [29] in 2019 year reported that EA relieves, partially, labor pain through spinal p38 MAPK-mediated prostaglandin E2 (PGE2) release inhibition and uterine prostaglandin E2 receptor (PGER2) expression regulation in rats. Moreover, EA attenuates inflammatory pain through toll-like receptor 2 (TLR2) signaling, and it is also implicated in the regulation of MAPK subfamily members [30]. CX3CL1 intrathecal injection reverses the analgesic effect of EA, re-activates p38 MAPK signaling, and leads into the increased release of pro-inflammatory cytokines in rats [27]. In light of this, PGE2, CX3CL1, TLR2 signaling pathways may be involved in the amelioration of EA treatment on visceral pain via regulating p38 MAPK signaling.

One limitation of the current study is that only the TRPV1/MAPK or P2X4R/MAPK signaling pathway was assessed. Future studies are required to expand the current findings focusing on other mechanisms.

Conclusions

This study illustrates that EA inhibits spinal glial cells activation and ameliorates the inflammatory response by TRPV1, P2X4R and MAPK pathways repression, what improve capsaicin-induced rectal visceral pain and mechanical pain in rats. These findings provide a new insights for EA treatment of rectal visceral pain.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

YX—substantial contributions to conception and design; FL, SH, YJ, XW—data acquisition, data analysis and interpretation; YX—drafting the article or critically revising it for important intellectual content. All authors published final approval of the version. All authors appropriately investigated and resolved agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work.

Ethics Approval and Consent to Participate

All animal experiments were approved by the Experimental Animal Ethics Committee of Zhejiang Baiyue Biotechnology Co., Ltd. (ZJBYLA-IACUC-20220110).

Acknowledgment

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Conflict of Interest

All authors have completed the ICMJE uniform disclosure form. The authors declare no conflict of interest.

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