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Effects of administration of α_2 adrenergic receptor agonist into psoas major muscle on inflammatory pain induced by injection of complete Freund's adjuvant in rats

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Abstract

α_2 adrenergic agonists are widely used in clinical anesthesia and ICU sedation owing to their effective sedative and analgesic effects. Lumbago and leg pain is the most common clinical pain disease. Studies have reported that lumbago and leg pain is associated with dysregulation of paravertebral muscles, especially psoas major muscles. In the present study, a unilateral lower extremity chronic inflammation and pain model was established by subcutaneous administration of low-dose complete Fredrin's adjuvant (CFA) into the posterior paw of rats. α_2 adrenalin receptor agonist was then injected into the psoas major muscle. Behavioral tests were conducted for 21 days. Psoas major muscle tissue was harvested for evaluation of biochemical indexes related to pain. The effect of α_2 adrenergic receptor agonist injected into psoas major muscle on chronic inflammatory pain of lower extremities in rats was explored. The results showed that injection of α_2 adrenergic receptor agonist into the psoas major muscle relieved CFA-induced mechanical hyperalgesia. Administration of α_2 adrenergic receptor antagonist yohimbine reversed the analgesic effect of α_2 adrenergic receptor agonists. Administration of dexmedetomidine into psoas major muscle downregulated the levels of norepinephrine, interleukin-6 and tumor necrosis factor-α in tissues. The findings of the present study show that administration of α_2 adrenoceptor agonists into the psoas major muscle relieves chronic inflammatory pain induced by CFA. Local injection of dexmedetomidine also exerted anti-inflammatory and anti-sympathetic effect by activating α_2-adrenoceptor in the psoas major muscle.

Keywords

Lumbago and leg pain, psoas major, α_2 adrenergic receptor, complete Freund's adjuvant, inflammatory pain

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Introduction

Lumbago and leg pain is one of the most common complaints of patients in clinical work of physicians.[1] Studies report that patients with long-term lumbago and leg pain present with changes in paravertebral muscles, especially psoas major muscles, including muscle atrophy, fat infiltration, and decreased muscle strength.[2,3] Acupuncture method has remarkable curative effect and few side effects, and is often used for treatment of lumbago and leg pain.[4,5] Shenshu Point (BL23) is one of the commonly used acupuncture points,[6] and is located 1.5 inches next to the spinous process of the second lumbar spine in human body, but it is located 10 mm next to the same spinous process in rats according to the *Atlas of*

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Animal Points developed by the Institute of Experimental Acupuncture Research of China. Findings from animal experiments indicate that acupuncture at BL23 acupoint relieves inflammatory symptoms such as paw pain and edema in inflammatory rats and alleviates mechanical ectopic pain caused by spinal nerve ligation.[7,8] A study conducted using human anatomical specimens reported that the psoas major muscle can be reached by acupuncture at the acupoint of BL23. Notably, the psoas major muscle is a key muscle in treatment of lumbago and leg pain.[9]

Reactive oxygen species (ROS) are implicated in occurrence and progression of chronic pain.[10,11] Dysregulation of ROS production causes oxidative stress damage to central and peripheral nociceptors, resulting in pain sensitization.[12] The findings of our previous study showed that subcutaneous injection of Complete Freund's adjuvant (CFA) into the plantar of the posterior paw of rats induced increase in ROS level in ipsilateral psoas major muscle tissue. Administration of ROS scavenging agent to the psoas major muscle alleviated the pain and swelling of the posterior paw of rats.[13] This implies that ROS in psoas major muscle is involved in occurrence and maintenance of inflammatory pain. Dexmedetomidine is a highly selective α_2 adrenergic receptor agonist. Several studies report that dexmedetomidine inhibits ROS synthesis by stimulating α_2 adrenergic receptors, exerts anti-oxidative stress effects, inhibits cell apoptosis, reduces cell damage, and protects organs against ROS damage.[14–16]

Locus coeruleus is the main target for α_2 adrenergic receptor agonists throughout the body.[17] Systemic use of α_2 receptor agonists exhibits good sedation, analgesia, anti-inflammatory, and anti-sympathetic effects as well as protects organ functions.[18–20] α_2 adrenergic receptor agonists excite α_2 receptors before and after synapses in neurons in the spinal dorsal horn, thus inhibit the release of excitatory neurotransmitters and slow action potential conduction velocity and inhibit the transmission of traumatic sensation.[21] Clinical studies report that addition of low dose α_2 adrenergic receptor agonist to local anesthetics during epidural anesthesia and nerve block anesthesia has the advantages of stable circulation, enhanced anesthetic effect, prolonged analgesia time, and reduced anesthetic dosage.[22,23] Peripheral use of clonidine or dexmedetomidine in animal models of pain shows marked analgesic and anti-inflammatory effects.[24,25]

Studies on local analgesia of α_2 receptor agonists report that local analgesia is mediated by α_2 adrenergic receptors in local tissues.[26,27] However, some studies report that local use of α_2 adrenergic receptor agonists does not exhibit analgesic effect. The studies argue that the analgesic effect is attributed to the local action on the α_2 receptors of peripheral nerve or the slow absorption of local anesthetics caused by peripheral vasoconstriction.[28] Findings from our previous study showed that local injection of the ROS scavenger N-tert-Butyl-a-phenylnitrone (PBN) to psoas major muscle significantly ameliorated pain and swelling in the hind paws of inflamed rats.[13] These findings indicate that local injection of α_2 adrenergic receptor agonists into the psoas major muscle of rats may result in analgesic and anti-inflammatory effects. In the present study, low-dose α_2 adrenergic agonists, dexmedetomidine and clonidine, were administered into the psoas major muscle of CFA-induced inflammatory rats to explore the local analgesic effect and mechanism of α_2 adrenergic agonists.

Materials and methods

Animals

This study was approved by the Ethics Committee of Guangdong Provincial People's Hospital and was conducted in strict adherence to the ethical guidelines for experimental pain research in conscious animals. Sprague-Dawley male rats, weighing 150 ± 20 g, were purchased from Guangdong Medical Animal Experimental Center. The rats were housed in a specific pathogen free animal laboratory with 22 ± 1°C temperature, 55 ± 10% humidity, 12 h of natural light cycle. The animals had free access to water and food, and were allowed to adapt to the experimental environment for 1 week. The experiment was performed when the rats attained a body weight of 200 ± 20 g.

Injection preparation

Complete Freund's adjuvant was purchased from Sigma Company, USA. The adjuvant was diluted with PBS to obtain 0.5 mg/mL and mixed evenly before use. Each rat was administered with 50 μL of the adjuvant. Dexmedetomidine hydrochloride (DEX) was purchased from Jiangsu Hengrui Pharmaceutical Co., Ltd. The dosage of DEX was calculated according to the weight of the rats and diluted with normal saline, and 0.2 mL was administered to rats. Clonidine hydrochloride (Selleck, USA) was diluted with normal saline to obtain 30 μg/mL, and 0.2 mL was administered to rats. Yohimbine hydrochloride was (Selleck, USA) diluted with normal saline to obtain 100 μg/mL, and 0.2 mL was administered to rats.

Methods for modeling inflammatory pain induced by CFA

Inflammatory model was established as described previously.[13] Rats were anesthetized and were subjected to 3% sevoflurane by inhalation to maintain mild anesthesia. The skin of the left foot base was disinfected using 75% medical alcohol. A 1 mL syringe was used to suction the mixture of CFA (1 mg/mL) and PBS 50 μL (1:1 dilution). The mixture was injected subcutaneously into the left hind paw of rats. The syringe needle was retrieved after 30 s to avoid drug leakage or entry into the joint cavity. The location and depth of drug injection was the same for all rats. Rats were placed back into the cage after they were completely awake.

Psoas major muscle injection method

The experimental method was the same as described in our previous methods.[13] Rats were anesthetized with sevoflurane and the back skin was disinfected with 75% medical alcohol. The position of the psoas major muscle was determined through anatomical landmarks. The anterior superior iliac spine was leveled against the 6th lumbar spinous process, and was moved cephalally to reach the 4th lumbar spinous process. The insertion point was about 1.5 cm away from the left side of the fourth lumbar spinous process. The tip of the needle was placed at 75°angle with the skin to the midline of the back. The depth of the needle was about 1 cm. The drug was then injected gradually without drawing back blood.

Behavioral tests

Behavioral tests were conducted during the day in a quiet and well-lit laboratory at room temperature of 24 ± 1°C. Rats were placed in transparent square cages with a layer of perforated wire mesh at the bottom such that the plantar of rats could be touched through the mesh. Rats were housed in laboratory cages for three consecutive days for more than 1 h a day before the formal experiment. Rats were allowed to rest for 1 h after acclimatization on the day of the experiment. Paw mechanical withdrawal threshold (PWT) of the left hind paw was determined by mechanical pain stimulation. Rats were after the allowed to rest for 30 min after the PWT test. Further, acetone cold stimulation test was performed. The left and right posterior foot bottom cross-sectional area was determined under sevoflurane mild anesthesia.

Paw mechanical withdrawal threshold was evaluated by stimulating the bottom of the left foot using a series of von Frey filaments (1–15 g) as previously described.[13] At first, 4g von Frey filament (North Coast Medical Inc., Morgan Hill, CA) was slowly applied vertically to the left plantar of the rats at a slightly curved position for 5 s. Each von Frey filament was tested 10 times, with an interval of more than 5 s for each replicate and an interval of more than 10 min between two Frey filaments. Positive reactions included withdrawal of the hind paw, biting and licking of the hind paw, and shaking of the thigh. A lower grade of von Frey filament was used for stimulation if there more than five positive times were obtained, otherwise, a higher grade of von Frey filament was used for stimulation. The lowest value was considered the mechanical stimulation withdrawal threshold (PWT) of the rat left hind paw.

Cold withdrawal response frequency (WRF) was determined by applying acetone to the hind paw plantar surface and observing the reactions of the rats as previously described.[13] A 1 mL blunt needle syringe was used to apply 50 μL acetone to the skin surface of the left plantar of rats. Rats normally showed no response or little response, but after CFA injection, a noticeable response was observed. The observation time was 20 s, and the test was performed in triplicates with an interval of 5 min between the replicates. No response to the stimulation was expressed as a score of "0″, withdrawal of the hind paw once was presented as a score of "1″, more than two times response was expressed as a score of "2″, and biting, licking the hind paw or stamping the foot was expressed as a score of "3". The observation time was increased by 20 s for animals with a score of 1 or 2. The average of the three replicates represented the acetone cold WRF.

The thickness and width of area at the bottom of the hind paw was measured using a Vernier caliper to determine the cross-sectional area. The thickness represented the distance from the lateral malleolus to the heel. Width of the hind paw represented the widest distance of inside and outside the heel. The width and thickness were measured in triplicates and the average value was calculated. The product of thickness and width was the cross-sectional area for the hind paw. The ratio of the cross-sectional area for the left and right sides was calculated with the cross-sectional area of the opposite hind claw as the reference.

The measurement of Norepinephrine and inflammatory cytokines

Rats were anesthetized with sevoflurane. The rats were then sacrificed and 100 mg of the left psoas major muscle tissue was immediately harvested and temporarily frozen under −80°C freezer. Tissue samples were thawed after 1 day and tissue homogenate was prepared under low temperature. The levels of NE, IL-6 and TNF-α in the tissues were determined by Elisa kits according to the manufacturer's instructions.

Experimental design

After establishing the models, rats in the experimental group were injected with 1, 3 and 5 μg/kg (0.2 mL) Dex administered into the psoas major muscle to explore the effects of different doses of Dex. The control group was received 0.2 mL normal saline administered into psoas major muscle. Further analysis was conducted to explore the effect of Dex on lower limb pain in rats after administration through different routes. The rats in the experimental group received Dex 1 μg/kg (0.2 mL) administered through ipsilateral psoas major muscle, erector spine muscle and abdominal cavity after modeling. The control group did not receive any treatment after modeling. Rats in the experimental group were received clonidine 30 μg/kg (0.2 mL) administered through ipsilateral psoas major muscle and abdominal cavity to evaluate the effect of on lower limb pain in rats after clonidine administration through different routes. The CFA group did not receive any treatment after modeling. The control group was received 0.2 mL normal saline administered into the ipsilateral psoas major muscle after modeling. Further experiments were conducted to explore the effect of

α_2 receptor antagonist yohimbine (YOH) on the analgesic effect of Dex. YOH+CFA group received yohimbine 100 μg/kg (0.2 mL) injected into the psoas major muscle before modeling. The CFA group did not receive any treatment after modeling. Complete Freund's adjuvant + Dexmedetomidine hydrochloride group received 1 μg/kg (0.2 mL) Dex administered into psoas major muscle after modeling. The YOH+CFA+DEX group received 100 μg/kg (0.2 mL) yohimbine into psoas major muscle before modeling and 1 μg/kg (0.2 mL) Dex was administered into psoas major muscle after modeling. All experimental rats were randomly assigned to 3 or 4 groups with 6 rats in each group. Observation of the behavior of rats and determination of cross-sectional area of hind paw were carried out continuously on day 1, 3, 7, 14 and 21 after administration of CFA.

Thirty rats were randomly assigned to 3 groups with 10 rats in each group to explore the effects of dexmedetomidine on the content of NE, IL-6 and TNF-α in psoas major muscle tissue. No treatment was administered to the Control group. The CFA+NS group received 0.2 mL normal saline administered into psoas major muscle after modeling. The CFA+DEX group received 1 μg/kg (0.2 mL) Dex injected into psoas major muscle after modeling. The CFA+NS group and CFA+DEX group were received normal saline and Dex administered at the second and fourth day after modeling. Notably, 100 mg of psoas major muscle tissue was harvested after sacrificing the rats on day 7 after CFA injection. The levels of NE, IL-6 and TNF-α in the tissue were then determined.

Statistical analysis

All data were expressed as mean ± standard error mean (Mean ± SEM). SPSS 20.0 and GraphPad Prim 8.0 software were used for statistical analysis. Data on PWT, WRF, cross-sectional area ratio, NE and expression levels of inflammatory cytokineswere were compared by one-way ANOVA followed by Bonferroni post hoc test. $p < 0.05$ was considered statistically significant.

Results

The effect of different doses of Dex injected into psoas major on lower limb pain

Paw mechanical stimulus withdrawal threshold (PWT) of the experimental group (Dex1, Dex3, Dex5) from day 1 to day 21 was significantly higher compared with that of the control group (control) (Figure 1). The results showed no statistical difference in mechanical stimulus withdrawal threshold (PWT) between the three experimental groups (Dex1, Dex3 and Dex5) during the observation time of 21 days.

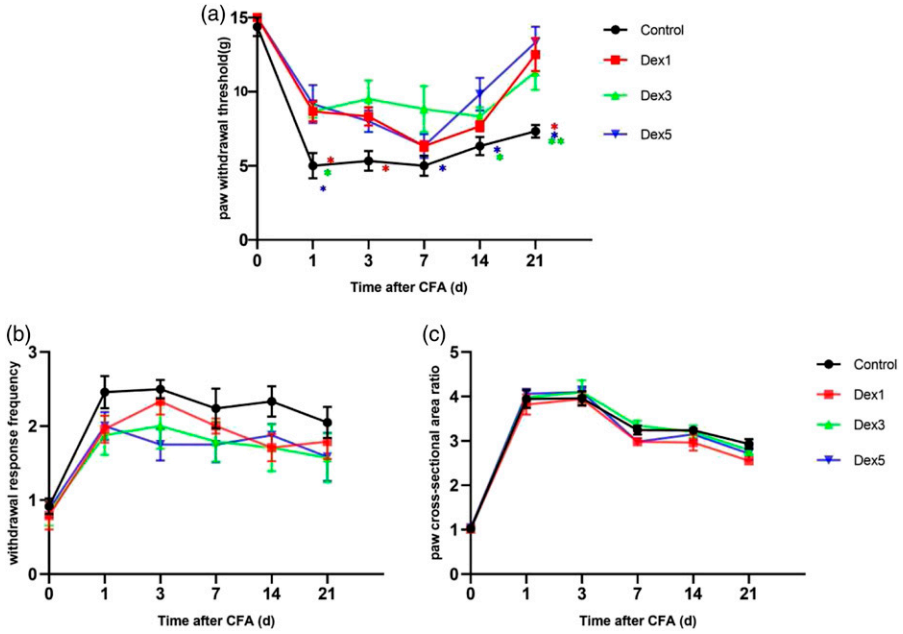

Figure 1. Effects of different doses of dexmedetomidine on inflammatory pain and inflammatory swelling of hind paws of rats after Complete Freund's adjuvant modeling. Dex1: Dex was injected into psoas major muscle at a dose of 1 μg/kg after modeling. Dex3: Dex was administered into psoas major muscle at a dose of 3 μg/kg after modeling. Dex5: Dex was injected into the psoas major muscle at a dose of 5 μg/kg after modeling. Control: not treatment was administered after modeling. * indicates that the difference between the Dex1 group and control group was statistically significant ($p < 0.05$, $n = 6$). * indicates that the difference between the Dex3 group and the control group was statistically significant ($p < 0.05$, $n = 6$). * indicates that the difference between the Dex5 group and control group was statistically significant ($p < 0.05$, $n = 6$). ** indicates that the difference between the Dex3 group and control group was statistically significant ($p < 0.01$, $n = 6$). Effect of administration of Dex injected through different routes on lower limb pain.

The WRF and ratio of plantar cross-sectional area of left and right hind paws of the experimental group (Dex1, Dex3, Dex5) and the control group (control) were not significantly different during the 21 days of observation. These results indicate that administration of different doses of Dex into psoas major muscle exerted analgesic effect on lower limb pain in rats, however, the effect was not dose-dependent. Different doses of Dex administered into psoas major did not relieve cold ectopic pain induced by acetone and did not alleviate the inflammatory swelling on the hind paw.

The PWT of the psoas major group (PM) on day 3, 7 and 14 was significantly higher relative to that of the control group (Figure 2). Paw mechanical withdrawal threshold of the PM group was significantly higher on day 3 compared with that of the intraperitoneal injection (IP) group and erector spinal muscle injection (ES) group. Withdrawal response frequency and ratio of plantar cross-sectional area of left and right hind paws showed no significant differences among the four groups during the 21 days of observation. These results indicate that the analgesic effect of Dex was local rather than systemic, and the site of action was psoas major muscle.

The PWT of PM group was significantly higher relative to that of the control group (CFA+NS group) on day 3 and 7 (Figure 3). Withdrawal response frequency and ratio of plantar cross-sectional area were not significantly different among the four groups during the 21 days of observation. The results showed that the analgesic effect of Clonidine was local effect rather than systemic effect, and the site of action was psoas major muscle.

Antagonistic effect of Yohimbine on analgesic effect of Dex

The PWT of experimental group (YOH+CFA+DEX) on day 7 was significantly lower relative to that of the control group (CFA+DEX) (Figure 4). The PWT of the experimental group (YOH+CFA+DEX) was lower compared with that of the control group (CFA+DEX) from day 14 to day 21, but the difference was not statistically significant ($p > 0.05$). The results showed no statistical difference in PWT between the experimental group (YOH+CFA) and the model group (CFA) ($p > 0.05$, Figure 4). These results indicate that yohimbine itself had no analgesic effects, but it reversed the analgesic effects of Dex. Withdrawal response frequency and ratio of plantar cross-sectional area were not significantly different among the four groups during the 21 days of observation.

Antagonistic effect of yohimbine on analgesic effect of clonidine

Paw mechanical withdrawal threshold of the experimental group (YOH+CFA+CLD) on day 3 and day 7 was

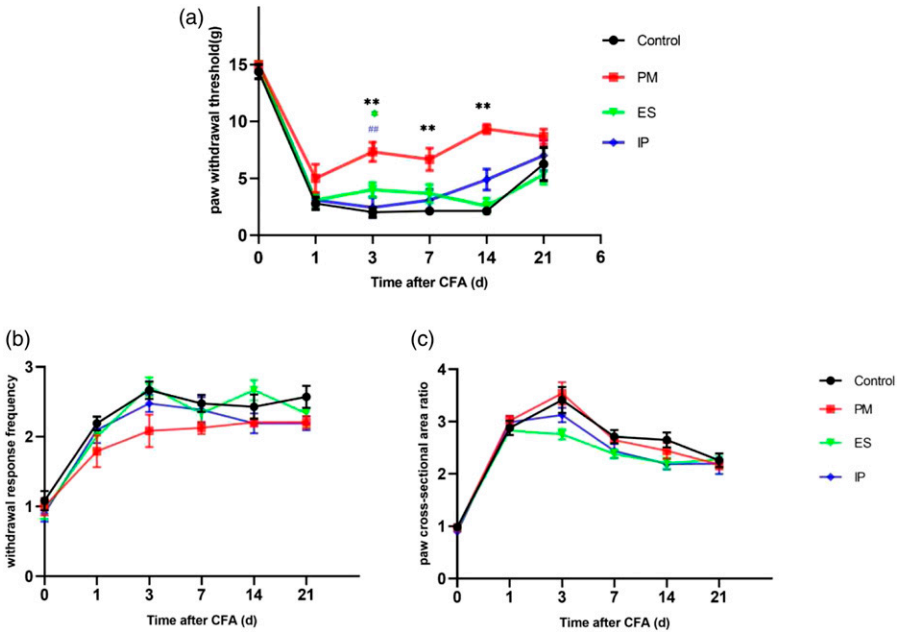

Figure 2. Effects of different administration routes of Dex on plantar inflammatory pain and swelling of rats after Complete Freund's adjuvant modeling. PM group: 1 μg/kg Dex was administered into the psoas major muscle after modeling. ES group: 1 μg/kg Dex was injected into erector spinal muscle after modeling. IP group: 1 μg/kg Dex was injected into the abdominal cavity after modeling. Control group: no treatment was administered after modeling. * indicates that the difference between PM group and ES group was statistically significant ($p < 0.05$, $n = 6$). ** indicates that the difference between PM group and control group was statistically significant ($p < 0.01$, $n = 6$). ## indicates that the difference between PM group and IP group was statistically significant ($p < 0.01$, $n = 6$). Effect of administration of clonidine through different routes on lower limb pain.

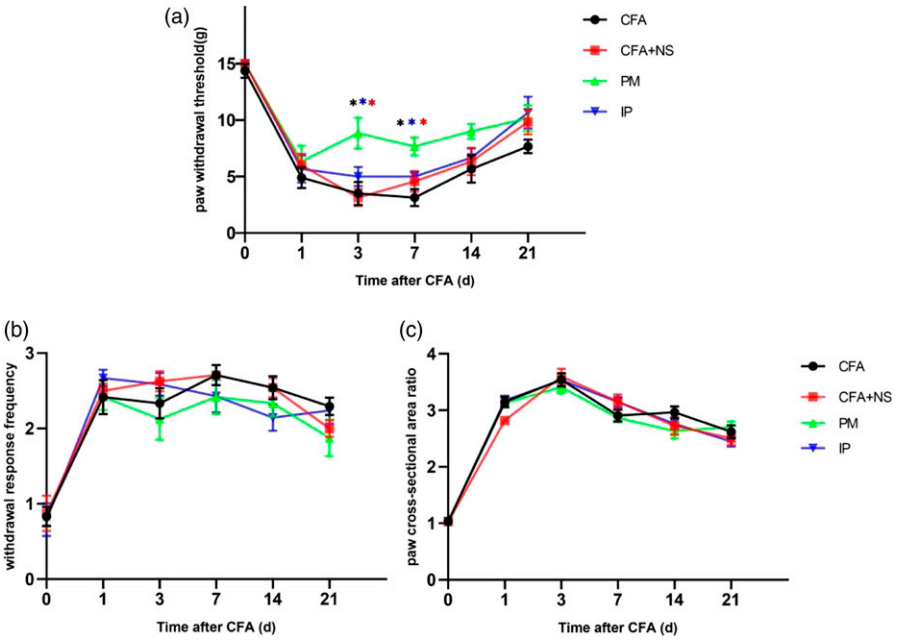

Figure 3. Effects of clonidine administered through different routes on plantar inflammatory pain and swelling of rats after CFA modeling. PM group: 30 μg/kg clonidine was injected into psoas major muscle after modeling. IP group: 30 μg/kg of clonidine was administered intraperitoneally after modeling. CFA+NS group: 0.2 mL normal saline was injected into psoas major muscle after modeling. CFA group: rats in this group did not receive any treatment after modeling. * indicates that the difference between PM group and CFA group was statistically significant (p<0.05, n=6). * indicates that the difference between the PM group and CFA+NS group was statistically significant (p<0.05, n=6). * indicates that the difference between PM group and IP group was statistically significant (p<0.05, n=6). CFA: Complete Freund's adjuvant.

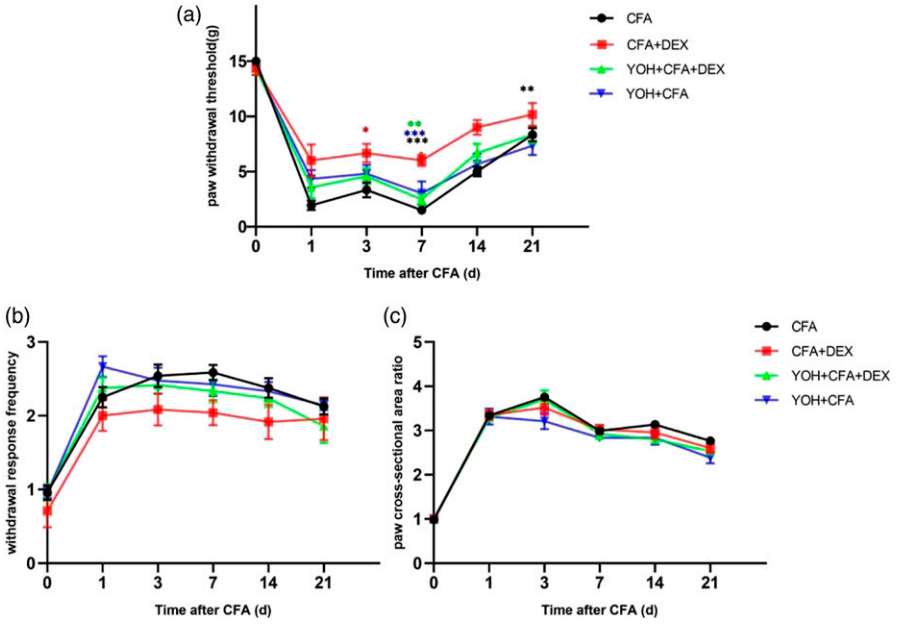

Figure 4. Effects of yohimbine administration into psoas major muscle on plantar inflammatory pain and swelling of rats after CFA modeling. CFA+DEX group: 1 μg/kg Dex was injected into psoas major muscle after modeling. YOH+CFA+DEX group: 100 μg/kg (0.2 mL) yohimbine was administered into psoas major muscle before modeling then 1 μg/kg (0.2 mL) Dex was injected into psoas major muscle after modeling. YOH+CFA group: 100 μg/kg yohimbine was administered into the psoas major muscle before modeling. CFA group: no treatment was performed after CFA modeling. * indicates that the difference between CFA+DEX group and CFA group was statistically significant (p < 0.05, n = 6). ** indicates that the CFA+DEX group showed significant difference compared with the YOH+CFA+DEX group (p < 0.01, n = 6). ** indicates that the CFA+DEX group showed statistically significant difference compared with CFA group (p < 0.01, n = 6). *** indicates that the CFA+DEX group showed significant difference compared with the YOH+CFA group (p < 0.005, n = 6). *** indicates that the CFA+DEX group showed statistically significant difference compared with CFA group (p < 0.005, n = 6). CFA: Complete Freund's adjuvant; DEX: Dexmedetomidine hydrochloride.

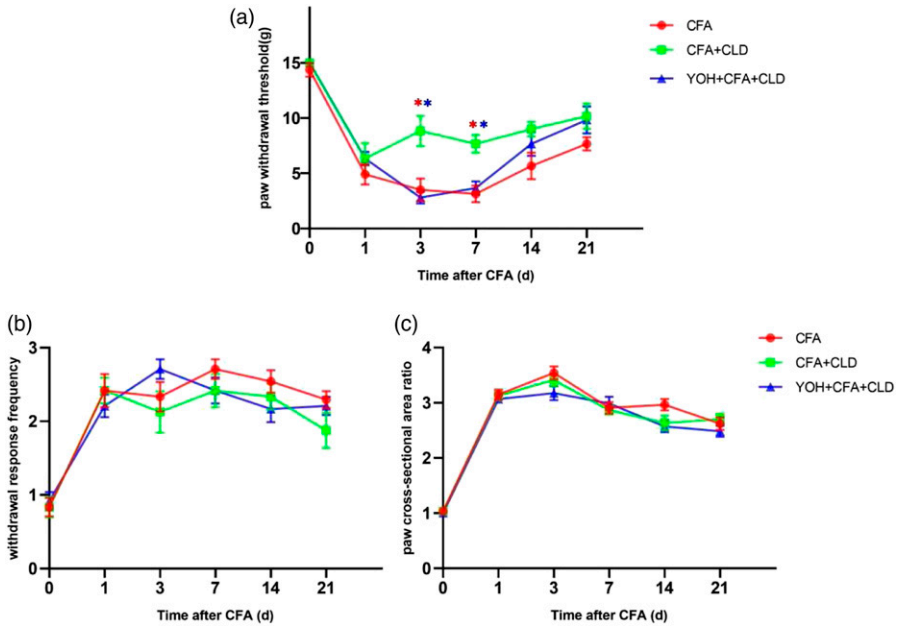

Figure 5. Effects of administration of yohimbine into psoas major muscle on plantar inflammatory pain and swelling of rats after CFA modeling. CFA+CLD group: 30 μg/kg clonidine was injected into psoas major muscle after modeling. YOH+CFA+CLD group: 100 μg/kg (0.2 mL) yohimbine was injected into psoas major muscle before modeling then 30 μg/kg (0.2 mL) clonidine was injected into the psoas major muscle after modeling. CFA group: no treatment was performed after CFA modeling. * indicates that CFA+CLD group showed statistically significant increase in PWT compared with the CFA group ($p < 0.05$, $n = 6$). * indicates that the PWT YOH+CFA+CLD group was significantly lower compared with that of the CFA+CLD group ($p < 0.05$, $n = 6$). CFA: Complete Freund's adjuvant.

significantly lower relative to that of the control group (CFA+DEX) (Figure 5). The findings showed no statistically significant difference in PWT between the experimental group (YOH+CFA+CLD) and the model group (CFA group) ($p > 0.05$, Figure 5). The results indicate that yohimbine antagonized the analgesic effect of clonidine.

Dexmedetomidine downregulates the high levels of NE, IL-6 and TNF-α in psoas major muscle tissue induced by CFA

Levels of NE, IL-6 and TNF-α in CFA+NS group were significantly higher relative to the levels in the control group (Figure 6). The contents of NE, IL-6 and TNF-α in CFA+-DEX group were significantly lower compared with those in CFA+NS group (Figure 6). These results indicated that administration of CFA into the hind paw of rats significantly increased the levels of NE, IL-6 and TNF-α in ipsilateral psoas major muscle tissue. Notably, administration of dexmedetomidine into psoas major muscle downregulated the levels of NE, IL-6 and TNF-α in psoas major muscle induced by CFA.

Discussion

The findings of the present study showed that local administration of α_2 adrenergic receptor agonists, clonidine and Dex into the psoas major muscle of rats successfully alleviated mechanical pain in the posterior paw induced by CFA. Administration of the α_2 receptor antagonist yohimbine reversed the analgesic effect of α_2 receptor agonists and downregulated the levels of NE, IL-6 and TNF-α in psoas major muscle tissue.

Reactive oxygen species are closely implicated in initiation and progression of chronic pain diseases. Elevated levels of ROS have been reported in multiple animal models of chronic inflammatory pain and neuropathic pain.[11,29] A previous study reported that norepinephrine induces increase in the levels of ROS in rat endothelial cells.[30] Notably, α_2 adrenergic agonists inhibit release of norepinephrine and reduce ROS levels by activating α_2 adrenergic receptors in the presynaptic membrane. This process is one of the mechanisms through which α_2 adrenergic agonists alleviate oxidative stress injury.[14] In the present study, CFA was administered subcutaneously into the posterior paw of rats, thus upregulating ROS levels in psoas major muscle.[13] Administration of clonidine and Dex into psoas major muscle significantly ameliorated mechanical hyperalgesia in rat hind paw and decreased the concentration of norepinephrine in psoas major muscle. These findings indicate that clonidine and Dex exhibited analgesic effect through reduction of NE and ROS levels in psoas major muscle tissue and inhibition of oxidative stress.

α_2 adrenergic receptors are widely distributed in central nervous system and peripheral organs. α_2-adrenoceptor are divided into different subtypes namely; α_2-C10, α_2-C4 and

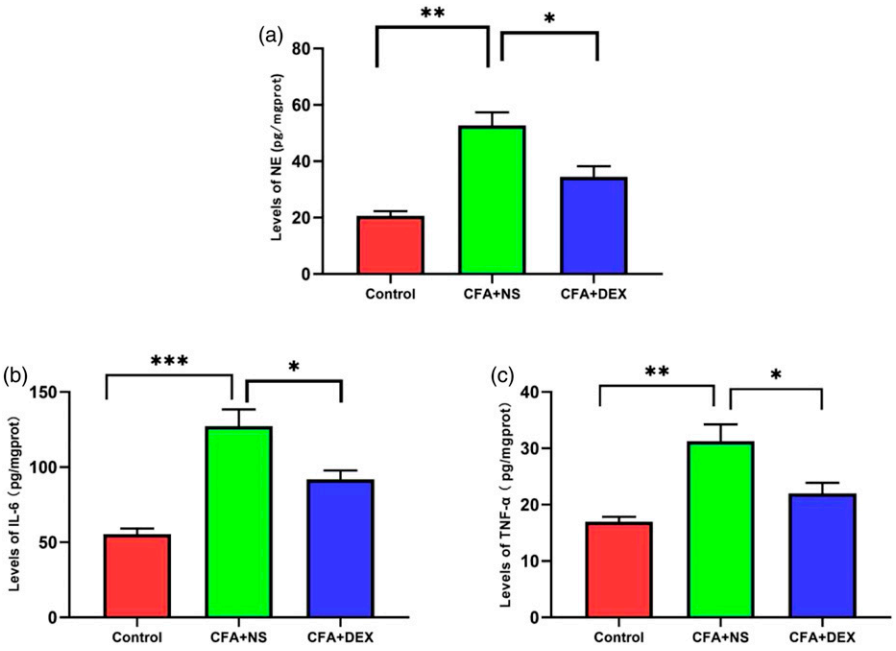

Figure 6. Levels of NE, IL-6 and TNF-α in psoas major muscle tissue. Control group: rats did not receive any treatment. CFA+NS group: 0.2 mL normal saline was administered into the psoas major muscle after CFA injection, then rats received 0.2 mL normal saline repeatedly on day 2 and 4. CFA+DEX group: 1 μg/kg DEX was injected into the psoas major muscle after CFA injection, then rats were repeatedly administered with 1 μg/kg DEX on day 2 and 4. * indicates that the difference between the two groups was statistically significant ($p < 0.05$, $n = 10$). ** indicates that the difference between the two groups was statistically significant ($p < 0.01$, $n = 10$). *** indicates that the difference between the two groups was statistically significant ($p < 0.005$, $n = 10$). CFA: Complete Freund's adjuvant; DEX: Dexmedetomidine hydrochloride.

α_2-C2 according to the location of α_2 receptor gene on the chromosome.[31] α_2 receptor subtypes exhibit differences in distribution among different species and tissues, leading to different physiological functions and pharmacological activities.[32] Aantaa et al. reported that α_2-C2 was mainly expressed in the liver and kidney, α_2-C4 was only expressed in brain but not in peripheral tissues, and α_2-C10 was expressed in central and peripheral tissues.[33] Margaret et al. explored the expression of α_2-adrenaline in 20 parts of the human body including skeletal muscle, and the results showed that α_2-C10, α_2-C2 and α_2-C4 were all expressed in human skeletal muscle.[32] The α_2-C10 receptors distributed in psoas major muscle of rats may be involved in the local analgesic effects of Dex and clonidine observed in the present study.

Local administration of α_2 adrenergic agonists activates α_2 adrenergic receptors in local tissues resulting in analgesic, anti-inflammatory and anti-sympathetic effects. α_2 adrenergic receptor agonists can cause local anesthesia, inhibit nerve signaling in C and A fibers,[34] stimulate release of enkephalin-like substances in peripheral sites and mediate analgesia by regulating opioid analgesic pathways.[35,36] Buerkle et al. Intra-articularly administered clonidine in carrageenan-induced inflammatory pain model rats.[24] The findings showed that clonidine exhibited a dose-dependent analgesic effect, which was reversed by administration of yohimbine indicating that clonidine targets local α_2-adrenergic receptors. Yoshitomi et al. reported that local administration of 10^{-6}M Dex into the back of guinea pigs did not exert any analgesic effect, whereas combination of Dex and lidocaine enhanced the anesthetic effect of lidocaine.[28] This finding implied that α_2 receptor had no analgesic effect in the periphery and only enhanced the effect of local anesthetics. Moroever, Dex enhanced the anesthetic effect of lidocaine because Dex induces peripheral vascular contraction, which slows down absorption of local anesthetics and prolongs the time of action. Our explanation is that there are differences in the distribution and density of α_2 adrenergic receptor subtypes in different peripheral organs and tissues,[33] and the dose of Dex used locally is also different, resulting in different results.

In the present study, local administration of a low-dose α_2 receptor agonist (Dex 1 μg/kg) into the psoas major muscle relieved mechanical pain in the ipsilateral posterior paw which is different from previous studies. Different doses of dexmedetomidine (1, 3, 5 μg/kg) administered into the psoas major muscle relieved CFA induced mechanical pain in the posterior paw, but the analgesic effect was not dose-dependent. Dex showed significant analgesic effect in the psoas major muscle injection group compared with intraperitoneal injection group and erector spinal injection group. The findings showed no significant differences in mechanical

stimulation withdrawal threshold (PWT) between the intraperitoneal injection group and the CFA group. This implies that Dex did not exert systemic effects but only exerted local effects. The results of different injection routes of Clonidine also suggested that the analgesic effect of Clonidine was local. Administration of the α_2 receptor antagonist yohimbine reversed the analgesic effect of Dex and clonidine. This finding indicates that the analgesic effect of α_2 agonists is mediated by α_2 adrenergic receptors in psoas major muscle.

Administration of Dex and clonidine into the psoas major muscle had no effect on cold sensation ectopic pain and posterior paw swelling. This can be attributed to the low dose of drugs used in the present study, and the high threshold of peripheral skin mechanical nociceptors, which rapidly recover after anti-inflammatory therapy. Cold nociceptor has a lower threshold, and its sensitivity is enhanced under inflammatory environment, and it exhibits cold nociceptor ectopic pain under mild stimulation.[37] NE, IL-6 and TNF-α levels in psoas major muscle tissue were determined on day 7 after CFA administration. The findings showed that NE, IL-6 and TNF-α levels in psoas major muscle tissue homogenate in the model group were significantly higher compared with the levels in the blank control group ($p < 0.05$). NE, IL-6 and TNF-α levels in the Dex group were significantly lower relative to the levels in the model group ($p < 0.05$). This implies that administration of Dex into the psoas major muscle downregulates the level of NE, IL-6 and TNF-α, and the peripheral use of Dex exhibits anti-inflammatory and anti-sympathetic effects.

In our previous study, the same procedure was used to inject a reactive oxygen scavenger PBN into the psoas major muscle in a CFA-induced rat model of chronic pain, and the findings showed that it relieved inflammatory pain in the posterior paw.[13] This indicates that it is feasible to treat inflammatory pain in the posterior paw in rats by administration of drugs into the psoas major muscle. The mechanism of alleviation of inflammatory pain has not been fully elucidated. Our explanation is that the psoas major muscle is the main muscle that maintains stability of the lumbar spine and controls activities of the lower limbs.[38] Dysfunction of psoas major muscle can cause spinal instability and may be implicated in lower limb pain.[39] Alleviation of psoas major muscle inflammation reduces chronic pain in lower limbs. The lumbar sympathetic nerve branch extends between the psoas major muscle and the lateral vertebral body, connecting the lumbar sympathetic trunk with the spinal nerve and is implicated in transmission of pain.[40] The main branches of the lumbar plexus and the sciatic nerve are closely associated with the psoas major.[41] The drug penetrates around the nerves after administration into the psoas major muscle. The drug acts on nerves and produces analgesic effects.

In summary, the findings of the present study show that administration of α_2 adrenergic receptor agonists into psoas major muscle alleviates hyperalgesia in CFA-induced inflammatory pain model rats. In addition, local administration α_2 adrenergic agonists exhibits anti-inflammatory and anti-sympathetic effects. α_2 adrenergic agonists exert their effects through α_2 adrenergic receptors of the psoas major.

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