Neurobiological Mechanism of Acupuncture Analgesia in Chronic Somatic Pain

Wei Yang, Teng Chen, Wen-Wen Zhang, Jia-He Tian, Ya-Chen Yang, and Yan-Qing Wang

Abstract

Acupuncture reduces pain by activating specific areas called acupoints on the patient's body. When these acupoints are fully activated, sensations of soreness, numbness, fullness, or heaviness called De qi or Te qi are felt by clinicians and patients. There are two kinds of acupuncture, manual acupuncture and electroacupuncture (EA). Additionally, the "acupuncture +" strategy, such as a newly reported acupuncture method, acupoint catgut embedding, can achieve better analgesic effects. Acupuncture alleviates pain mainly by modulating local changes of acupoints and nerve conduction and regulating the levels of endogenous opioids, cannabinoid, and their receptors, serotonin, and norepinephrine and by inhibiting somatic nociceptors, inflammatory cytokines, and CNS activation. The endogenous nociceptive modulation system plays an important role in EA analgesia, including the descending inhibitory system and the descending facilitatory system. The inactivation of microglia and astrocytes mediates the immediate and long-term analgesic effects of EA, respectively. A variety of pain-related substances released by glial cells such as the proinflammatory cytokine tumor necrosis factor α, interleukin-1β, interleukin-6, and prostaglandins such as prostaglandins E2 can also be reduced. The autonomic nervous system (ANS), including sympathetic and parasympathetic nervous systems, also plays an important role in acupuncture anti-inflammatory effects.

Keywords

Electroacupuncture · Chronic pain · Catgut embedding · Peripheral nervous system · Central nervous system · Autonomic nerves system

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Abbreviations

5-HT	5-hydroxy-tryptamine
ACC	Anterior cingulate cortex
ACE	Acupoint catgut embedding
ANS	Autonomic nerves system
ATP	Adenosine triphosphate
CAM	Complementary and alternative medicine
CAMP	Compound muscle action potential
CB	Cannabinoid
CCI	Chronic constriction injury
CFA	Complete Freund's adjuvant
CNS	Central nervous system
COX	Cyclooxygenase
DML	Distal motor latency
DOR	Delta opioid receptor
DPN	Diabetic peripheral neuropathy
DSL	Distal sensory latency
EA	Electroacupuncture
EM	Endomorphin
ENK	Enkephalin
GFAP	Glial fibrillary acidic protein
GPCRs	G protein-coupled receptors
HPA	Hypothalamic-pituitary-adrenal
IFN	Interferon
IL	Interleukins
JAK	Janus kinase
KOR	Kappa opioid receptor
LC	Locus coeruleus
LPS	Lipopolysaccharides
MA	Manual acupuncture
mA	Milliampere
MAPK	Mitogen-activated protein kinase
MBP	Myelin basic protein
MCP-1	Macrophage chemoattractant protein-1
MMP	Matrix metalloproteinase
MNS	Median nerve stimulation
NA	Noradrenaline
NCV	Nerve conduction velocity
NOP	Nociceptin
NPY	Neuropeptide Y
NRM	Nucleus raphes magnus
NS	No significance
NSFC	Natural science foundation of China
NT-3	Neurotrophin-3
OFQ	Orphanin FQ
ORL1	Opioid receptor like-1
PAG	Periaqueductal gray
PDN	Painful diabetic neuropathy
PDYN	Prodynorphin
PKA	Protein kinase A
PNS	Peripheral nervous system
POMC	pro-opiomelanocortin
RCT	Randomized controlled trial
RT-PCR	Reverse transcription polymerase chain reaction
rVLM	Rostral ventrolateral medulla
RVM	Rostral ventromedial medulla
SDH	Spinal dorsal horn
SEA	Sham electroacupuncture
SNAP	Sensory nerve action potential
SNL	Spinal nerve ligation
STAT3	Signal transducer and activator of transcription
TGF	Tumor growth factor
TNF	Tumor necrosis factor
VAS	Visual analogue scale/score

1 Introduction

Acupuncture has been used in China for thousands of years to relieve many different types of pain based on traditional Chinese medicine theories. One of the basic premises of traditional Chinese medicine is that there are hundreds of acupoints distributed throughout the human body and can be activated by acupuncture needles to relieve pain. With the development of modern technology, we have a better understanding of the mechanisms behind these ancient Chinese treatment methods.

Chronic pain, one of the most prevalent health problems, has a serious impact on our society and economy. It is defined as pain which persists at least 3 months (Mills et al. 2019). According to its etiologies, chronic pain can be generally classified into neuropathic pain, inflammatory pain, and dysfunctional pain (Burma et al. 2017). Neuropathic pain is caused by nerve injury or disease. Inflammatory pain arises from persistent or unresolved inflammation. Dysfunctional pain, which have both neuropathic and inflammatory components, does not fall into either of the above two categories. Dysfunctional pain, such as cancer-related pain (pain caused by cancer), visceral pain (pain originated from visceral organ),

diabetes-related pain (pain caused by diabetes), chemotherapy-related pain (pain caused by chemotherapy), and so on, usually has multiple etiologies and differs in presentation of signs and symptoms.

Using animal models, we can capture the human pain experience accurately by understanding how key genes, cells, and circuits mediate the development of chronic pain. Generally, animal models of neuropathic pain are achieved by full or partial nerve injury via ligation, transection, or compression of certain nerves which can be sciatic nerve, trigeminal nerve, and so on. The common model for inflammatory pain is achieved by injecting chemical irritant like the complete Freund's adjuvant and formalin into the paw to cause local inflammatory responses. Model for cancer-related pain including bone cancer pain, in which injected certain cancer cells like Walker 256 mammary gland carcinoma cells are injected into the tibia cavity (Hu et al. 2019). Visceral pain model can be induced by injecting chemical irritants like acetic acid or mustard oil to produce colonic inflammation (Larauche et al. 2012). Diabetes-related pain can be induced by injecting streptozotocin (Jolivalt et al. 2016). Chemotherapy-related pain model is induced by injecting chemotherapy drug like cisplatin (Colvin 2019).

2 Clinical Application of Acupuncture on Somatic Chronic Pain

Conventional medical treatments for pain relief are not always satisfactory with problematic side effects. Acupuncture, which has been used in China for thousands of years to relieve many different types of pain, represents a potentially valuable way for pain relief (Chen et al. 2020). Generally speaking, there are two kinds of acupuncture, the classical acupuncture (manual acupuncture) and the modern one (electroacupuncture). In manual acupuncture, clinicians insert the acupuncture needles through the skin at the acupoint and then move up and down with twirling in different directions to induce mechanical stimulation of the acupoint. Sensations of soreness, numbness, fullness, or heaviness called De qi or Te qi can be felt by clinicians and patients, and this phenomenon is considered the hallmark of activated acupoints (Chen et al. 2020). In electroacupuncture, the acupuncture needles are electrified so that the acupoints are activated by both mechanical stimulation and electrical stimulation. Unlike manual acupuncture whose effect varies from clinicians and De qi is hard to quantify, electroacupuncture can be set by different acupoints and a variety of parameters including wave form, electrical intensity, frequency, interval, and time. Recently, a novel way to stimulate acupoint, called acupoint catgut embedding (ACE), has been reported to achieve better acupuncture analgesia (Cui et al. 2019; Du et al. 2017). Acupoint catgut embedding is a type of acupuncture that seeks to exert long-term effects by injecting sutures made of absorbable materials at acupoints (Chen et al. 2020).

Other methods have also been used to stimulate acupoints like dry needling, warm acupuncture, fire acupuncture, auricular acupuncture, eye acupuncture, and laser acupuncture. Dry needling uses a fine, solid filiform needle to cause intramuscular stimulation (Cagnie et al. 2013). Warm acupuncture and fire acupuncture are methods which cause thermal stimulation at the acupoints. The temperature is moderate in warm acupuncture, while fire acupuncture is very hot. Ear acupuncture and eye acupuncture are methods which insert needles around the ears or the eyes. Laser acupuncture is a method which stimulates the acupoints with low-intensity, nonthermal laser irradiation.

Based on the above animal models, the neurobiological mechanism of acupuncture analgesia was investigated. In total, acupuncture-induced analgesia is a comprehensive effect that starts with the activation of acupoints, which have special anatomic structures. The acupuncture-induced signals are then transmitted to the spinal cord and relevant areas of the brain where they increase or decrease multiple neurotransmitters, modulators, and inflammatory factors in order to relieve pain (Chen et al. 2020).

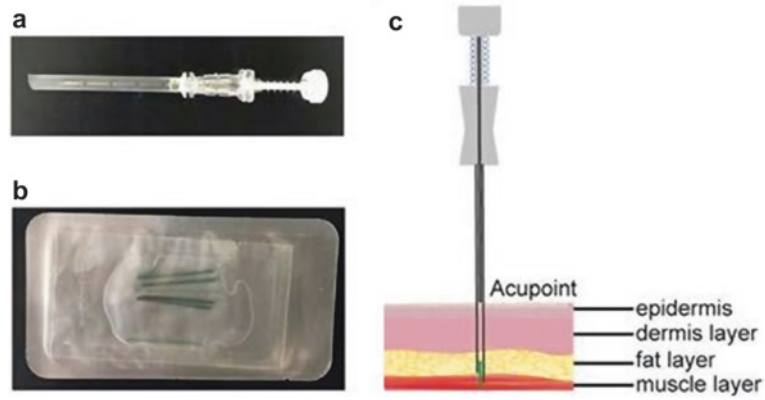

Fig. 1 The needle (**a**), catgut (**b**), and schematic (**c**) of acupoint catgut embedding. (Cui et al. 2019)

ACE refers to injecting sutures made of absorbable materials at acupoints that are associated with different physiological processes or diseases. This treatment is a combination of ancient traditional acupuncture and modern tissue therapy, and, as a variant of acupuncture, it has been practiced along with traditional acupuncture in China for nearly half a century. ACE stimulates the acupoint persistently for a week or longer, until the suture softens, liquifies, and absorbs. Therefore, ACE is more convenient than traditional acupuncture, which needs to be performed daily or every other day. Moreover, ACE is easier to perform than traditional acupuncture and is, thus, widely used to treat various disorders in China, such as obesity and allergic rhinitis. In particular, it has been widely used to manage clinical pain (Fig. 1).

3 Potential Mechanisms of Acupuncture Analgesia on Chronic Somatic Pain

Acupuncture has been used to treat various pain disorders, including somatic pain, and has shown considerable effects on pain relief. Acupuncture alleviates pain mainly by modulating local changes of acupoints and nerve conduction and regulating the levels of endogenous opioids, serotonin, and norepinephrine and by inhibiting somatic nociceptors, inflammatory cytokines, and CNS activation.

3.1 Peripheral Mechanisms

3.1.1 Local Changes of Acupoints in Acupuncture Analgesia

Acupoint is a concept opposite from non-acupoint with anatomical structure and functional effect, which can modulate the physiology of the body after being stimulated by manual acupuncture and electroacupuncture (Li et al. 2015). Anatomically, acupoint is consistent of mast cells, blood vessels, nervous system components, and musculoskeletal tissues (Kuo et al. 2004; Lee et al. 2008; Wu et al. 2015). Functionally, at local acupoint, acupuncture (both MA and EA) can activate those cells and nerve fiber terminals with pain relief by releasing some peptides, immune cytokines, adenosine triphosphate (ATP), and adenosine (Chen et al. 2017, 2018a, b, 2020; He et al. 2020; Li et al. 2019a, b; Wang et al. 2014). During the pain process, there are many inflammatory responses by triggering immune cells to release a series of inflammatory mediators. Similarly, electroacupuncture stimulation at the ST36 acupoint can also enhance the level of immune cytokines interferon-γ (IFN-γ), interleukin (IL)-2, and IL-17 level in the serum (Chen et al. 2017). Peripheral nociceptive afferent fibers include Aδ- and C-fibers, and their peripheral axonal branches are at nociceptor terminals. The acupuncturemediated analgesia stimulates and modulates these peripheral afferent pathways, transmitters, and modulators (Zhang et al. 2014). The cutaneous/subcutaneous mast cells play a

vital role in anti-inflammatory responses, and due to their location, they are sensitive to mechanical stimulation from the external environment. Mast cells contain ATP which may be released as a result of acupuncture needling (He et al. 2020). Meanwhile, an interesting study reported that adenosine, a neuromodulator with antinociceptive properties, was released during acupuncture and induced acupuncture analgesia through adenosine A1 receptor (Goldman et al. 2010).

3.1.2 Effect of Acupuncture on Nerve Conduction

Nerve conduction can be detected by the sensory nerve action potential (SNAP) and the compound muscle action potential (CMAP), which provide information on sensory axon in the skin and motor nerve fiber along the muscle, discerning the underlying nerve physiology and pathophysiology. Various parameters, such as amplitudes, latencies, and other measurements, of the SNAP and CMAP waveforms are used to determine the number of functioning nerve fibers and the speed of conduction (Tavee 2019). Since the branches and terminals of nerve fibers are enriched in the acupoint, nerve conduction changes can also be modulating by acupuncture stimulation. In the clinical trials on diabetic peripheral neuropathy (DPN) and carpal tunnel syndrome, acupuncture produced significant effects on median nerve CMAP amplitude, median nerve distal motor latency (DML), and motor nerve conduction velocity (NCV) of the median, ulnar, and peroneal nerves on motor nerve function, while sensory NCS showed an increase in SNAP amplitude in the median nerve, lowered median nerve distal sensory latency (DSL), and increased median and peroneal nerve NCV (Dimitrova et al. 2017). An interesting study reported that acupuncture treatment of peripheral neuropathy (PN) results in a significant improvement of nerve conduction studies of the sural nerve amplitudes, which was fully correlated with a subjective improvement of symptoms (Schroder et al. 2007). And also an unfinished clinical study aims to characterize the local, nerve-specific effects of acupuncture on the median and ulnar nerves in the forearm, using nerve conduction studies and quantitative sensory testing.

3.1.3 Acupuncture Affects Dorsal Root Ganglion Function

The nociceptive system consists of neurons and their terminals activated by stimuli potentially threatening the integrity of our body. In the pain condition, structural dysfunction causes nociceptive pain by stimulating nerve fibers with nociceptors, such as some kinds of somatic pain. These actions are driven by the terminals and transported to dorsal root ganglia, promoting substance P synthesis and release. Many studies reported that dorsal root ganglia may be one of the targets of acupuncture analgesia. At present, the relatively commonly used somatic pain animal models include the neuropathic pain model, such as the spinal nerve ligation (SNL) model (Wei et al. 2021), and the inflammatory pain model, such as the complete Freund's adjuvant (CFA) model (Table 1).

3.1.4 Acupuncture Affects Endogenous Opioids and Their Receptors

The endogenous opioid system consists of opioid peptides and their receptors (μ, mu (MOR); δ, delta (DOR); κ, kappa (KOR); nociceptin (NOP)). It is reported in the central and peripheral nervous systems (CNS and PNS) and has a central role in inducing potent and clinically measurable analgesia and other physiological functions and pharmacological responses, with unwanted and common side effects. Although only central opioid mechanisms were considered to modulate acupuncture analgesia in the beginning, peripheral opioid mechanisms were investigated in the acupuncture field and increasingly emphasized because of potential avoidance of central opioid side effects such as analgesic tolerance (Table 2).

3.2 Central Mechanisms

3.2.1 Endogenous Opioids, Cannabinoid, and Their Receptors

The endogenous opioid system consists of five opioid peptides, enkephalin, β-endorphin, dynorphin, endomorphin, and orphanin FQ and four

Table 1 Effect of acupuncture on different pain models in the dorsal root ganglion

Year	Model	Acupoints	Conclusion
2021 (Wei et al. 2021)	Neuropathic pain: SNL	"Huantiao" (GB-30) "Yanglingquan" (GB-34)30¬†min daily from day 7 to day 10 after SNL	EA gradually attenuated SNL-induced mechanical allodynia, associated with suppressing the expression of phosphorylated AXL (p-AXL). Combination of AXL inhibitor and EA might be a new strategy for clinic analgesia on neuropathic pain.
2021 (Shen et al. 2021)	Inflammatory pain: CFA (ankle joint)	Zusanli 20-min acupuncture	ATP metabolism of peripheral sensory nerve system was simultaneously regulated during acupuncture analgesia.
2020 (Zhang et al. 2020)	Inflammatory pain: CFA (paw)	Zusanli (ST36), Kunlun (BL60) 0.5–1.5 mA, initial strength of 0.5 mA, increased by 0.5 mA every 10 min, for 30 min per session, one section per day	Regulation of adenosine-mediated substance P secretion. Substance P-mediated pathway may be involved in the analgesia process by electroacupuncture in rats.
2019 (Liang et al. 2019)	SNL	ST36 (Zusanli), BL60 (Kunlun) 7D-14 D; three consecutive square waves of 0.6 ms duration and amplitude of 0.5, 1.0, 1.5 mA for 10 min each (a total 30 min) were applied at 2 Hz frequency	EA stimulation alters P2X3R activity in DRG to produce analgesia under neuropathic pain condition EA effectively reduces injury-induced chronic pain by selectively reducing the expression of P2X3Rs in nerve-uninjured L4 dorsal root ganglion neurons.
2018 (Zhou et al. 2018)	PDN	Zusanli (ST36) Kunlun (BL60) 1 mA for 15 min followed by 2 mA for another 15 min	Analgesic effect of EA in PDN is mediated by suppressing PKC-dependent membrane P2X3 upregulation in DRG. EA at low frequency is a valuable approach for PDN control.
2017 (Yang et al. 2017)	Inflammatory pain CFA	Bilateral Zusanli (ST36) 100-μs square pulses of 1 mA for 15 min at 2 Hz	EA significantly reduced chronic mechanical and thermal hyperalgesia, mechanism of TRPV1 in DRG.

opioid receptors, mu-, kappa-, delta-, and opioid receptor like-1 opioid receptors (MOR, KOR, DOR, and ORL1). Opioid receptors belong to the G protein-coupled receptors (GPCRs) and are mainly Gi/o-coupled (Prather et al. 1995). All endogenous opioid peptides share the enkephalin sequence (Tyr1-Gly2-Gly3-Phe4) at the N terminal. Opioid receptors transmit signals by G protein-dependent or G protein-independent pathways (Ferre et al. 2019).

Endogenous Opioids

Enkephalins are a family of pentapeptide derived from proenkephalin (PENK), which are divided into methionine-enkephalin (met-ENK) and leucine-enkephalin (leu-ENK) according to the difference of the last amino acid. Enkephalins are broadly distributed in the body, such as the diencephalon (Sanchez et al. 2016), the telencephalon, the hypothalamus, and the spinal cord (Vijayalaxmi et al. 2020), and have high selectivity for DOR. They play a role in neurotransmission, pain modulation (Francois et al. 2017), immunomodulation (Liu et al. 2020a, b), and so on.

β-Endorphin is produced mainly in the anterior lobe of the pituitary gland (Andrew 1991) and the pro-opiomelanocortin (POMC) cells located in the arcuate hypothalamic nucleus (Veening et al. 2012). β-Endorphin is a mu-preferring ligand, though it can also band to delta-opioid receptors. β-Endorphin, exclusively β-endorphin1–31 but not shorter β-endorphin1–27, exerts strong analgesic effect. In general, β-endorphin produces an anti-inflammatory effect by suppressing immune

Table 2 Compilation of data from 30 preclinical articles regarding peripheral acupuncture endogenous opioids receptors (Trento et al. 2021)

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation method	Location of the peripheral receptor/ neuromediation	Conclusions
Opioid receptor (Ceccherelli et al. 2002)	Capsaicin in the rat paw Male Sprague-Dawley rat Age not informed	Intraplantar injection of capsaicin (50 µg) and/or naloxone (20 µg) Intraperitoneal injection of naloxone (1 mg/kg)	MA stimulated by left and right rotating movements for 30 s at the beginning of the session and every 5 min for 20 min (GB30 and ST36) EA stimulated with 5 Hz and 5 mA (GB30 and ST36)	Opioids at a peripheral level, on the receptors sited on capsaicin-sensitive fibers	MA and EA have a modulating effect on capsaicin-induced neurogenic edema. The local administration of small dose of naloxone inhibits the modulating effect.
µ, δ, and κ opioid receptors (Taguchi et al. 2010)	Carrageenan in the rat paw Male Sprague-Dawley rat Age not informed	Intraplantar injection (0.1 ml) of naloxone methiodide (125, 250, and 500 µg) Intravenous injection (0.1 ml) of 10 µg CTOP, 100 µg NTI and 200 µg nor-BNI (opioid antagonist receptors µ, δ e κ, respectively)	EA ipsilateral to injured paw (60 min, 3 Hz, 0,1 ms). The intensity of EA was increased of 1–3 mA for 20 min at each intensity ST36	Selective peripheral receptors µ, δ, and κ sited on terminal nerves	EA-induced analgesia may be dose-dependently blocked by intraplantar injection of the nonselective peripheral opioid receptor antagonist and the µ, δ, and κ opioid receptor antagonists.
Opioid receptor/β--endorphin (Zhang et al. 2010)	CFA in the rat paw Male Sprague-Dawley rat Age not informed	Intraplantar injection (0.05 ml) of naloxone methiodide (5 and 50 µg) and α-helical CRF (0.2 mL, 2 ng) Intraplantar injection of anti-β-endorphin, (0,05 mL, 0.2 µg)	EA ipsilateral to injured paw (2.0 mA, 30 Hz, and 0.1 ms pulse for 30 min) GB30 and a non-acupoint 10 mm distant from GB30	Opioid receptors located on peripheral sensory nerve fibers and their terminals	EA induces release of peripheral opioids and activates their opioid receptors. EA-promoted analgesia was blocked by the β-endorphin antibody and the locally administered CRF antagonist.
Opioid receptor (Sekido et al. 2003)	Carrageenan in the rat paw Male Sprague-Dawley rat Age not informed	Intraperitoneal (1.0 mg/kg, 0.5, 1.0 e 2.0 mg/kg) or intraplantar (0.1 mL, 0.6, 1.2 e 2.4 µg) injection of naloxone (nonselective opioid receptor antagonist)	EA ipsilateral to injured paw (3 Hz, 0.1 ms, 60 min.). The intensity ranged from 2 to 3 mA, every 20 min. ST36 and a non-acupoint 10 mm distant from ST36	Opioids are released locally from immune cells during environmental stimuli	Acupuncture-induced analgesia in rats with inflammatory pain involves both central and peripheral receptors.

(continued)

Table 2 (continued)

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation method	Location of the peripheral receptor/ neuromediation	Conclusions
Opioid receptor/β--endorphin (Fang et al. 2013)	CFA in the rat paw Male Wistar rat Age not informed	Intraplantar injection (0.05 mL) of nonselective peripheral opioid receptor antagonist (naloxone methiodide, 50 μg) and CRF (α-helical CRF, 2 ng) Radioimmunoassay for endogenous opioids Local release of exogenous beta-endorphin	EA ipsilateral to injured paw (30 min once a day, 2–100 Hz with pulses of 0.6 ms at 2 Hz and 0.2 ms at 100 Hz) ST36 and BL60	μ and δ opioid receptors located on peripheral sensory nerve fibers and their terminals. Local opioids can be released from immune cells infiltrating the inflamed area	EA may inhibit inflammatory pain and peripheral beta-endorphin (CRF being a mediator) and the three opioid receptor subtypes may also be involved.
Opioid receptor, δ opioid receptor (Wu et al. 2014)	CFA in the rat paw Male Wistar rat Age not informed	Intraplantar injection of a nonselective opioid receptor antagonist (naloxone; NLX, 0.56 ng) and a δ opioid receptor antagonist (NALTRIDOLE; NTI, 50 μg)	EA bilateral (20 min on day 0 and 24 h after CFA, 100 Hz, 2–3 Ma) GB30	Opioid receptors on the periphery of nociceptive neurons	EA caused significant mechanical and thermal antinociception and reduced paw volume and temperature up to 144 h, indicating anti-inflammatory effects. Sustained late mechanical antinociception was mediated by peripheral opioid receptors and was suppressed by intraplantar application of nonselective and a = δ opioid receptors antagonists.
Opioids peptides: β-endorphin, meta-enkephalin, dynorphin A (Wang et al. 2013)	CFA in the hind paw Wistar rats Male Wistar rat Age not informed	Intraplantar injected with anti-opioid peptide antibodies (anti-END, anti-ENK, or anti-DYN) Double immunohistochemistry staining on paw tissue sections for macrophages with coexpression of opioids peptides (beta-endorphin, meta-enkephalin, dynorphin A)	EA (20 min/100 Hz/2–2.5–3 mA pulse width: 0.1 ms) GB30 bilateral	Opioid peptides at the region of the inflamed paw	EA increases of opioid containing macrophages at the inflammatory pain site.

μ and κ opioids receptors (Chai et al. 2018)	Intra-articular MSU injection in rat ankle Male Sprague-Dawley rat Age not informed	Intra-articular injection of μ opioid receptor antagonist (β-Funaltrexamine, 50 μg/ankle), κ (nor-binaltorphimine, 76 μg/ankle), and δ (Naltrindole, 48.7 μg/ankle) Intra-articular injection (40 μg/ankle) and intraperitoneal (2 mg/kg) nonselective opioid receptor antagonist (Naloxone) Intra-articular injection of μ opioid receptor agonist (DAMGO, 4.9 μg/ankle) Intra-articular injection of A1 receptor antagonist (KW-3902, 600 μg/ankle) and κ ((±) U50488, 1 μg/ankle) Intraperitoneal injection of COX antagonist (indomethacin 5 mg/kg) Ankle joint histopathology Immunohistochemistry	EA bilateral (15 min for intensity, total 30 min/2/100 Hz/0.2 ms/1–2 mA) ST36 bilateral and BL60 bilateral	Peripheral sensory nerve system and opioid peptide-containing monocytes/macrophages to the local inflamed site	Our study demonstrated that 2/100 Hz EA effectively alleviates pain response and inflammation in a rat model of acute gout arthritis. EA can produce equivalent analgesic effect compared with the widely used NASIDs indomethacin. The analgesic effect of EA on acute gout arthritis is largely mediated via peripheral μ- and κ-opioid receptors.

responses, but in the brain, it seems to be related to the inflammation in the case of disease. β-Endorphin can also reduce stress-related activity, participate in the reward system, and be related to addictions (Pilozzi et al. 2020).

All dynorphin isoforms such as dynorphin A 1–17, dynorphin A 1–8, dynorphin B 1–13, big dynorphin, and leumorphin are derived from the precursor named prodynorphin (PDYN). Dynorphins show strong affinity with delta-opioid receptors. The primary stress neuropeptide corticotropin-releasing factor (CRF) causes dynorphin release in stressful situations, dynorphin thus involved in the stress-induced analgesia (Bruchas et al. 2010). In discrete subregions of dynorphin-containing cells in the shell of nucleus accumbens aversive behaviors and place preference can both be induced (Al-Hasani et al. 2015).

Endomorphin has two subtypes: Tyr-Pro-Trp-Phe-NH2 (EM1) and Tyr-Pro-Phe-Phe-NH2 (EM2). Endomorphin has strong affinity and selectivity for mu-opioid receptor. In addition, a study found that EM1/2 preferentially activated cAMP signaling which indicates biased opioid ligands can induce specific physiological responses and thus avoid some side effect (LaVigne et al. 2020).

OFQ is a heptadecapeptide derived from prepronociceptin (PNOC) and has a high affinity for ORL1 receptor. OFQ is a highly basic peptide, and the number of Lys and Arg residues of OFQ is similar to dynorphin (Toll et al. 2016).

Acupuncture-Related Release of Endogenous Opioids

When EA is given (1–4 mA, 0.5 ms, 2 Hz) twice at P5–P6 acupoints bilaterally, both relative ratios of preproenkephalin mRNA levels and Met-enkephalin levels were increased in the rostral ventrolateral medulla (rVLM) after 24 h (Li et al. 2012). Another study used low-frequency EA (2–3 mA, 2 Hz), which stimulates Zusanli and Sanyinjiao for 20 min and observed the spinal microglial expression of β-endorphin increased successively in neuropathic rats (Ali et al. 2020).

It is very interesting that EA promotes or inhibits dynorphin release under different conditions. For example, EA (0.1–0.3 mA, 2/100 Hz, alternately) at Sanyinjiao (SP-6) for 20 min and repeated every 2 h could significantly reduce labor pain in rats, and the protein expression of KOR and PDYN and mRNA expression in the lumbar spinal cord was increased after EA treatment (Jiang et al. 2016), while another study found EA (2 mA, 0.4 ms, 10 Hz) at Huantiao (GB-30) for 30 min significantly attenuated bone cancer-induced hyperalgesia and inhibited spinal preprodynorphin expression in a rat model (Zhang et al. 2008).

In our studies of the effects of EA on neuropathic pain, we found that EA at Huantiao (GB-30) and Yanglingquan (GB-34) with dense-sparse frequencies (60 Hz for 1.05 s and 2 Hz for 2.85 s, alternately) could promote OFQ synthesis and OFQ peptide level in the nucleus raphes magnus (NRM) in the sciatic nerve chronic constriction injury (CCI) model (Ma et al. 2004).

Acupuncture and Opioid Receptors

Acupuncture is observed to alter the expression of opioid receptors and increase their density in the brain. A study provided the evidence of short- and long-term effects of acupuncture therapy on MOR binding potential in the thalamus, the cingulate, the insula, the caudate, the putamen, and the temporal pole in chronic pain patients (Harris et al. 2009). Besides, bilateral intra-habenula infusion of naltrexone could reduce the analgesic effect of EA, and as MOR gene is richly expressed in the habenula, it indirectly proved that EA could attenuate hyperalgesia via habenular MORs (Li et al. 2017).

Now we know that different physical states and acupuncture parameter all may affect acupuncture analgesia. There is evidence that intracerebroventricular injection of MOR antagonist or antiserum against EM1 or EM2 antagonized the analgesia induced by EA of 2 Hz, but not 100 Hz at Zusanli (ST-36), in an uninjured animal model (Huang et al. 2000). However, another team that uses complete Freund's adjuvant (CFA)-induced inflammatory pain model found that both 10 Hz and 100 Hz EA at Huantiao (GB-30) produced anti-hyperalgesia, which could be blocked by intra-RVM administration of MOR antagonists (Zhang et al. 2011).

Endogenous Cannabinoid and Their Receptors

The endocannabinoid system (ECS) includes cannabinoid (CB) and its central cannabinoid receptors 1 (CB1 receptors) and peripheral cannabinoid receptor 2 (CB2 receptors). The ECS has increasingly been seen extensively involved in the regulation of various physiological and cognitive processes, such as female reproductive events, pain sensation, and mood. Notably, CB1 and CB2 receptors appear to contribute to the analgesic and anti-inflammatory effects of acupuncture, respectively (MacDonald and Chen 2020).

Antinociceptive effect of EA is related to the activation of the CB1 receptors and partly through the regulation of the spinal CB1-ERK1/2 signaling pathway (Zheng et al. 2019). The CB1 receptors in the ventrolateral area of the periaqueductal gray (vlPAG) and the striatum also proved to be vital for the EA antinociceptive effect (Shou et al. 2013; Ho et al. 2011). A study found that low-frequency median nerve stimulation (MNS) through acupuncture needles at Neiguan (PC-6) (MNS-PC6), which is equivalent to EA, induced an antinociceptive effect in chronic constriction injury (CCI) mice model through orexin 1 receptor (OX1R)-initiated cannabinoid 2-AG signaling in the vlPAG (Chen et al. 2018a, b). These findings were also reinforced by another study which stressed MNS-PC6 treatment remained effective in morphine-tolerant CCI-mice via the above signaling pathway (Lee et al. 2021).

3.2.2 Endogenous Nociceptive Modulation System: Descending Inhibitory System

Chronic pain involves peripheral and central sensitization. The imbalance between the descending facilitatory systems and the descending inhibitory systems could be taken as part of the explanation for central sensitization.

The descending inhibitory system mainly consists of the periaqueductal gray (PAG), rostral ventromedial medulla (RVM), dorsolateral pontine reticular formation, and spinal dorsal horn (SDH). The PAG-RVM-SDH pathways project along the dorsolateral funiculi onto the SDH. RVM contains the nucleus raphes magnus (NRM) and its adjacent ventral reticular structure, and dorsolateral pontine reticular formation contains the locus coeruleus (LC), the subcoeruleus, and the Kolliker-Fuse nucleus (Willis and Westlund 1997). Actually, there are two major monosynaptic descending pathways, the descending serotonergic pathway and the noradrenergic pathway, respectively (Lv et al. 2019).

Descending Serotonergic Pain Inhibitory Pathways

The descending serotonergic pathways originate from the NRM, as it is rich in serotonergic neurons. Serotonergic receptor has multiple subtypes, which contribute different effects to the acupuncture analgesia. Exogenously intracerebroventricular administration of serotonin (5-hydroxy-tryptamine, 5-HT) exhibited an EA-like analgesic effect. Furthermore, the 5-HT1A and 5-HT3 receptor antagonists blocked 2, 10, and 100 Hz EA-induced analgesic effects, but the 5-HT2 receptor antagonist enhanced the antinociceptive effect of 100 Hz EA (Chang et al. 2004). In a neuropathic pain rat model, 5-HT1A and 5-HT3 antagonist, but not 5-HT2A antagonist, blocked the analgesic effects of 2 Hz EA on cold allodynia (Kim et al. 2005). However, another study shows that in pain induced by formalin, intrathecal (i.t.) 5-HT7 receptor agonist induced antinociceptor effect, which could be reversed by i.t. 5-HT3 receptor antagonist (Yang et al. 2014). As opposite results have been found in different studies, the role of 5-HT receptors in pain modulation in normal or neuropathic states still remains controversial.

Descending Noradrenergic Pain Inhibitory Pathways

LC releases noradrenaline (NA) into the SDH and serves as the source of the descending noradrenergic pathways. In a study, EA (60 Hz, 3.2 V) at set of Baihui-Santai acupoints or Housanli acupoints of goats for 30 min induced analgesic effect and increased c-Fos expression in the LC

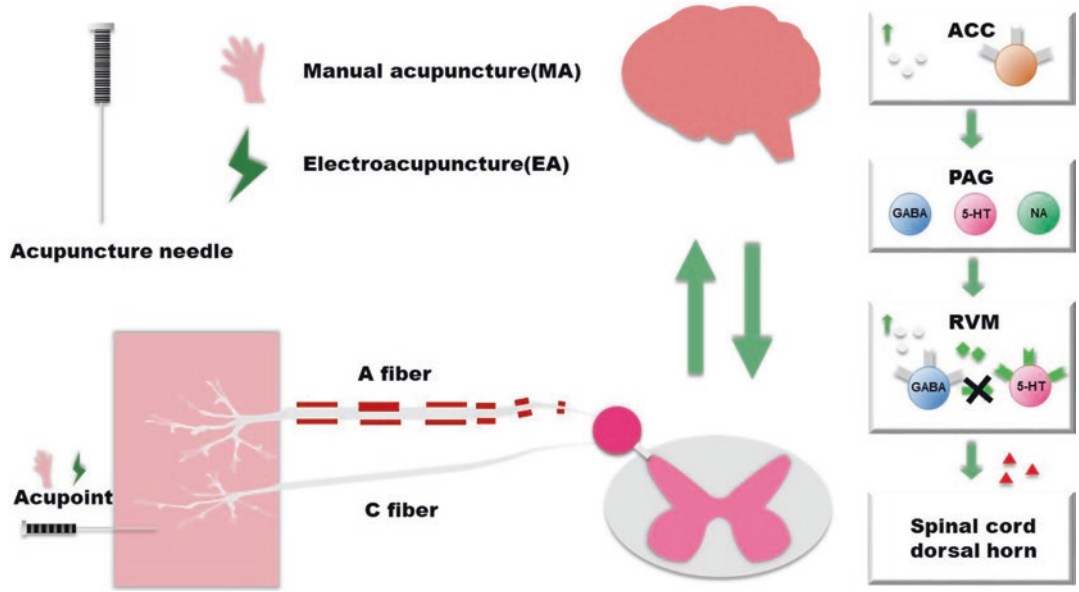

Fig. 2 Putative mechanisms underlying acupuncture-induced analgesia. Acupuncture-induced analgesia is a comprehensive effect that starts by activation of acupoints, which have special anatomic structures, and the acupuncture-induced signals are transmitted to the spinal cord and to the relevant areas of the brain. The descending pain modulation system, including the anterior cingulated cortex (ACC), the periaqueductal gray (PAG), and the rostral ventromedial medulla (RVM), are ultimately activated to relieve pain. (Chen et al. 2020)

(Hu et al. 2016), which indicate that LC is an important part in the descending noradrenergic pathways involved in EA analgesia (Fig. 2).

3.2.3 Endogenous Nociceptive Modulation System: Descending Facilitatory System

The descending facilitatory system, which mainly contains the anterior cingulate cortex (ACC), the NRM, the nucleus reticularis gigantocellularis, and the SDH, is a top-down pain modulation pathway independent of the descending inhibitory system, which shares several nuclei. The descending facilitatory pathways project along the ventrolateral funiculi onto the SDH. Chronic pain is mostly sustained by facilitatory influences (Frank et al. 2002). While 5-HT is an important neurotransmitter in the descending inhibitory system, it plays a key role in the descending facilitatory system as well.

A study reveals that the 5-HT3 receptor antagonist applied to the spinal cord induced tonic facilitation of noxious punctate mechanical stimulation in sham rats, while it inhibited neuronal responses to lower intensity punctate mechanical stimuli and noxious heat-evoked responses in SNL rats (Patel and Dickerson 2018), which indicate that 5-HT3 receptor contributes facilitation effect in neuropathic state.

3.2.4 Role of Spinal Glial Cells and Cytokines in Acupuncture Analgesia

Glial cells, including microglia, astrocytes, and oligodendrocytes, surround neurons and contribute to pain hypersensitivity when activated in pathological states, and acupuncture has been widely reported to play an analgesic role by regulating the function of spinal cord glial cells (Ji et al. 2016). Electroacupuncture (EA) is widely believed to inhibit the activation of astrocytes and microglia induced by nerve injury significantly (Wang et al. 2018; Liang et al. 2016). The most immediate evidence is the expression of spinal microglial marker OX-42, and astrocytic marker glial fibrillary acidic protein (GFAP) was reduced by EA. In addition, the levels of matrix metalloproteinase-2 (MMP-2), MMP-9, tumor necrosis

factor α (TNF-α), and interleukin-1β (IL-1β) were decreased after EA (Gim et al. 2011). Besides, the inhibitory effect of acupuncture on microglia activation including the reduction of microglia oxygen free radicals and P38 mitogen-activated protein kinase (p38 MAPK) and phosphorylation of extracellular signal-regulated kinase (ERK) was also observed in a spared nerve injury in a rat model, and the TNF-α, IL-1β, cyclocxygenase-2 (COX-2), interleukin-6 (IL-6), and prostaglandin 2 (PGE2) in the spinal cord were also decreased after EA (Cha et al. 2012; Ji et al. 2017). As p38 MAPK is a key signaling molecules in microglial activation, a newly reported study shows that the inactivation of the p38 MAPK pathway by EA might be related to chemokine CX3CL1, which plays an important role in neuroinflammation. It has been found that EA can downregulate the expression of CX3CL1 in neurons (Li et al. 2019a, b). In a mouse model of post-incision pain, pretreatment of EA effectively prevented pain, and IL-10 in spinal astrocytes was critical for the analgesia of EA and central sensitization (Dai et al. 2019). Acupuncture has also been reported to inhibit the activation of spinal astrocytes and the upregulation of TNF-α through adenosine A1 receptors (Zhang et al. 2018). Acupuncture can also inhibit the activation of C-Jun N-terminal kinase (JNK) and mediate analgesia (Lee et al. 2013) (Fig. 3).

3.3 Sympathetic and Parasympathetic Nervous System in Acupuncture Anti-inflammatory Effects

Inflammation is a defensive response of the body to stimulation, and its basic pathological changes mainly include local tissue metamorphism,

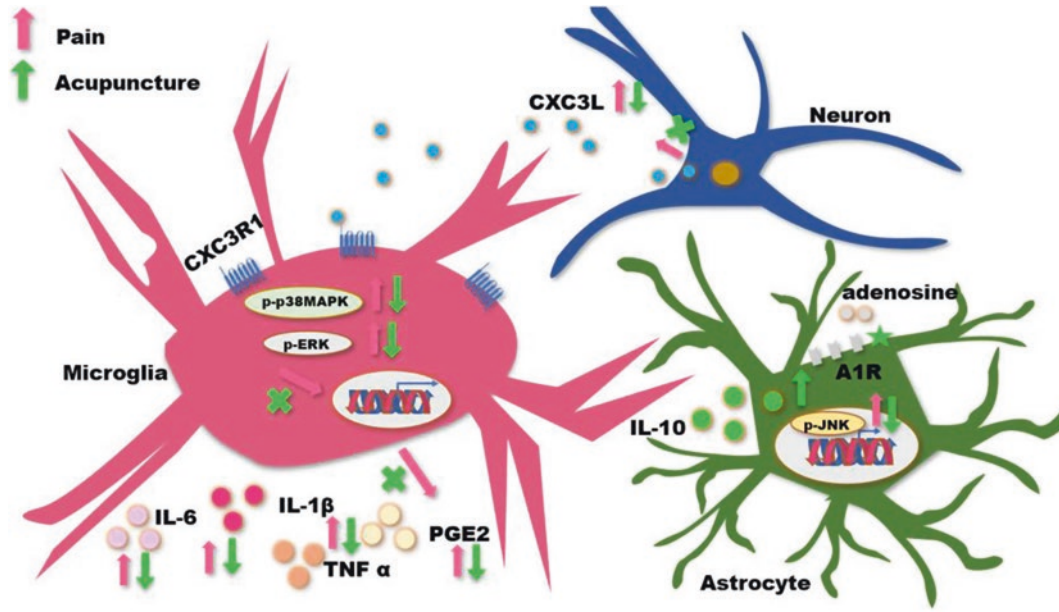

Fig. 3 Role of spinal glial cells in acupuncture-induced analgesia. Role of spinal glial cells in acupuncture-induced analgesia. EA has analgesic effects by interrupting spinal glial cell activation. Painful states like nerve injury or inflammation induce nociceptors to secrete glial modulators, and this results in the activation of microglia and astrocytes in the spinal dorsal horn. Microglia and astrocytes then secrete neuromodulators like IL-1β, IL-6, TNF α, and prostaglandin E2 (PGE2) to maintain chronic pain. Acupuncture can inhibit glial cell activation by downregulating chemokine CX3CL1 and increasing anti-inflammatory cytokine IL-10. Acupuncture analgesia has two phases, the immediate phase and the long-term phase. In the immediate phase, acupuncture mainly inhibits microglial activation by suppressing the p38MAPK and ERK pathways. In the long-term phase, acupuncture mainly inhibits astrocyte activation by blocking the C-Jun N-terminal kinase signaling pathway. (Chen et al. 2020)

exudation, and proliferation. In general, inflammation is beneficial and is an automatic defense response of the human body, but sometimes insufficient inflammatory response can easily cause infection, while excessive or long-lasting inflammatory reaction can easily lead to and/or enhance chronic pain in some diseases including rheumatoid arthritis and osteoarthritis.

In the past, it was generally believed that the anti-inflammatory response in the body was mainly realized by cellular immunity and humoral immunity, which lasted for a long time and acted slowly, until Borovikova et al. put forward the concept of vagus nerve-mediated cholinergic anti-inflammatory pathway for the first time, which provided a new train of thought and theoretical basis for the study of the mechanism of nervous system regulation on inflammation (Borovikova et al. 2000). Recent advances in neuroimmunology have suggested that the autonomic nerve system (ANS) is one of the key pathways in neuroimmunoregulatory networks, and it has a definite inhibitory or exciting effect on multiple systems of the human body. The balance between the two branches of ANS (sympathetic and parasympathetic) plays an important role in directing the inflammatory response toward proinflammatory or anti-inflammatory outcomes (Song et al. 2012).

As an important part of Chinese traditional medicine, acupuncture can regulate the visceral function by stimulating the local acupoints. This theory of meridian-viscera correlation is the earliest somatic visceral-related theory in the world (Ma 2020). In this regulation process, a large number of clinical and animal experiments have shown that acupuncture stimulation can effectively regulate systemic inflammation and is closely related to the function of ANS. The specific effect will be affected by acupuncture points, acupuncture intensity, disease status, and so on.

3.3.1 Acupuncture Anti-inflammation and Parasympathetic Nervous System

As we all know, the vagus nerve accounts for 70% of the parasympathetic regulation of internal organs, so it is the bridge between brain and visceral function (Park and Namgung 2018). At the beginning of the twenty-first century, Borovikova and others pioneered the concept of vagus nerve activity regulating inflammatory response (Borovikova et al. 2000), and they found that direct electrical stimulation of the cervical vagus nerve inhibited the synthesis of TNF in the liver and decreased the peak value of serum TNF, while acetylcholine significantly inhibited the release of cytokines (IL-1β, IL-6, and IL-18) released by macrophages stimulated by lipopolysaccharides (LPS) but did not inhibit the release of anti-inflammatory cytokine IL-10. Subsequent studies have shown that inflammatory cytokines produced by peripheral organs can activate the afferent part of the vagus nerve and send synaptic connections through the nucleus of the solitary tract (NTS) to the dorsal nucleus of the vagus nerve (DMN) in the brainstem (Tracey 2002). Stimulation of the efferent nerve of the vagus nerve can activate splenic sympathetic neurons in the celiac ganglion to release norepinephrine and activate acetylcholine release (Ulloa et al. 2017), and it binds to α7-nicotinic acetylcholine receptors on macrophages, lymphocytes, and other nonneuronal cells, which inhibited the release of inflammatory cytokines through NF-κB activation while stimulating the STAT3 pathway, thus achieving the purpose of anti-inflammation (de Jonge et al. 2005).

The 361 acupoints in humans are basically located near the neuronal networks (Vida et al. 2011). When acupuncture induces mechanical stimulation at the neuromuscular junction and causes the local release of neuroregulators, it can simulate vagus nerve stimulation to a certain extent. Taking the Zusanli acupoint (ST36) stimulation as an example, ST36 mechanically stimulates connective tissue to activate the sciatic nerve, which in turn activates the NTS through the paraventricular trigeminal nucleus region of the medulla oblongata, then activating the efferent pathways of the parasympathetic nervous system from the NTS to DMN (Pavlov et al. 2003). C-Fos immunohistochemical staining (Fang et al. 2017) proves that ST26 stimulation plays an important role in the activation of the NTS neurons

and the input of afferent vagus nerve. In addition, in the animal sepsis model with a lethal dose of LPS, electroacupuncture preconditioning had a significant survival-promoting effect on lethal LPS rats, while vagotomy abolished the anti-inflammatory and animal survival effects of EA, indicating that EA activated the vagus nerve efferent circuit (Fang et al. 2017). Electroacupuncture at ST36 has also been found to prevent inflammation and lung tissue injury in severely burned rats by stimulating the vagus nerve (Song et al. 2015). Definitely, cholinergic anti-inflammatory mechanism can also be induced by other acupoints. Electroacupuncture at the Baihui acupoint (GV20) and the Dazhui acupoint (GV14) can activate the dorsal motor nucleus of the vagus nerve by c-Fos immunohistochemistry, which can reduce brain injury, apoptosis, and inflammation (Chi et al. 2018). It can be seen that vagus nerve activity is a main regulating factor of EA regulating inflammation.

3.3.2 Acupuncture Anti-inflammatory Effects and Sympathetic Nervous System

While the vagus nerve has been a primary target mediating neuroimmune reaction in many studies, a potential role of sympathetic nerve activity has also been proposed (Park and Namgung 2018). Studies have shown that bilateral section of the splanchnic sympathetic nerves before LPS treatment resulted in a fivefold increase in the plasma TNF-α response, but bilateral vagotomy had no effect. This suggests that celiac ganglion neurons innervated by visceral sympathetic nerves may be responsible for anti-inflammation (Martelli et al. 2014). Sympathetic-mediated anti-inflammatory immune response is partly achieved by acting on different immune cells. For instance, the extraintestinal sympathetic nerve is activated in distal bacterial infection and releases norepinephrine, which integrates with β2 adrenergic receptor macrophages in the muscular layer of gastric mucosa. β2 adrenergic receptor signaling mediates macrophage polarization upon bacterial infection activating protective phenotype of the gastrointestinal tract (Gabanyi et al. 2016). Besides, sympathetic splenic nerves can control inflammation in experimental sepsis by activating T lymphocytes to inhibit the production of spleen TNF-α but not by interacting directly with macrophages (Vida et al. 2011). Study has shown that EA could decrease splenic lymphocytes apoptosis via inhibiting Fas protein expression, consequently preventing deleterious immunological changes in the postoperative state (Wang et al. 2005).

Another point of view shows that the effect of the SNS is bimodal, enhancing or depressing levels of proinflammatory and anti-inflammatory cytokines depending on the time point of immune system activation. In antigen-dependent arthritis (CIA) models, the sympathetic nerve stimulation (SNS) supports inflammation during the asymptomatic phase of CIA, whereas it inhibits inflammation during the chronic symptomatic phase (Härle et al. 2005). This phenomenon may be explained that during inflammation, the decrease of local nerve fiber density may lead to the decrease of local neurotransmitters and the expression of adrenergic receptor subtypes in immune cells is transferred to α-adrenergic receptor. Norepinephrine activates α-adrenoceptor at low concentration and plays a corresponding proinflammatory effect (Nance and Sanders 2007). The effects of norepinephrine at high concentrations are mediated by the classical β2-adrenoceptor-cAMP-protein kinase A (PKA) pathway, thus inhibiting the release of proinflammatory factors (LaJevic et al. 2011). This may be due to the differences in the number of target cells, the state of activation, and the expression of adrenoceptor subtypes and intracellular signaling pathways (Pongratz and Straub 2013).

The anti-inflammatory effects of acupuncture on the activation of sympathetic nervous system, including systemic or local catecholamine release, are closely related to the activation site and electroacupuncture frequency. Studies have shown that in the model of peripheral inflammation, low-frequency (1 Hz) EA of ST36 can lead to local release of catecholamines from sympathetic postganglionic nerve endings, thus acting on β-adrenergic receptors on immune cells to inhibit inflammation (Kim et al. 2007). On the

contrary, high-frequency (120 Hz) EA of ST36 can induce the release of systemic norepinephrine through the preganglionic nerve of the adrenal medulla for anti-inflammation (Kim et al. 2008, 2015). In addition, not only the frequency of EA but also the intensity of EA has heterogeneity in the drive of sympathetic reflex. Recent studies of endotoxin systemic inflammation have shown that low-intensity ES (0.5 mA) at the ST36 acupoint of the hind limb drives the vagus nerve-adrenal axis, producing anti-inflammatory effects that depend on neuropeptide Y (NPY+) adrenal chromaffin cells. However, higher stimulation intensity (1–3 mA) is needed to drive spinal cord sympathetic reflex, whether at ST36 or abdominal ST25, activating NPY+ spleen norepinephrine neurons. The final outcome of proinflammatory or anti-inflammatory is determined by the state of the disease (Liu et al. 2020a, b). To sum up, it is not difficult to see that acupuncture has complex and multidimensional effects on neuroimmune regulation.

4 Conclusion

Great progress has been achieved in recent years in explaining the basic mechanisms of acupuncture; however, the complexity of acupuncture is far from being fully understood. For example, when talking about EA, the commonly used frequencies are 2 Hz, 15 Hz, 2/15 Hz, and 100 Hz. The release of some transmitters such as endogenous opioid has been shown to be frequency dependent, but the mechanisms behind this remain to be elucidated. On the other hand, the mechanism underlying the cumulative effect of EA on chronic pain still remains unclear. Furthermore, with the fast development of neuroscience especially the optogenetic technique to manipulate neural activity, concrete neural-circuit mechanism can be demonstrated in the near future.

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