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INVITED REVIEW

Peripheral receptors and neuromediators involved in the antihyperalgesic effects of acupuncture: a state-of-the-art review

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Abstract

The present study aims to describe state-of-the-art of preclinical studies that have investigated peripheral receptors and neuromediators involved in the antihyperalgesic effects of acupuncture. The PubMed, Scopus, and Web of Science databases were searched using the integrative review method. Preclinical articles that involved the study of peripheral receptors and neuromediators on the pain control effects of acupuncture in rats or mice were selected using a predefined search strategy. From this search, 456 articles were found, and 29 of them met the inclusion criteria of the study. The selected articles addressed the following peripheral receptors: opioid ($n = 9$), adenosine ($n = 5$), cannabinoid ($n = 5$), transient receptor potential vanilloid (TRPV) ($n = 3$), histamine ($n = 2$), adrenergic ($n = 1$), muscarinic ($n = 1$), corticotrophin-releasing factor (CRF) ($n = 2$), IL-1 ($n = 1$), and endothelin ($n = 1$) receptors. The peripheral neuromediators correlated with the peripheral pain control effect were as follows: opioid peptides ($n = 4$), adenosine ($n = 3$), histamine ($n = 1$), substance P ($n = 1$) calcitonin gene-related peptide (CGRP) ($n = 1$), anandamide ($n = 1$), nitric oxide ($n = 1$), and norepinephrine ($n = 1$). This review summarizes the methods used to investigate the peripheral effects of acupuncture and discusses the main findings on each family of receptors and neuromediators. Ten families of peripheral receptors and 8 types of neuromediators were correlated with the antihyperalgesic effects of acupuncture in preclinical studies. Considering the benefits of a better understanding of the role of peripheral receptors and neuromediators in the context pain management, the findings of the present study highlight the importance of deepening the exploration of the peripheral mechanisms of acupuncture.

Keywords Acupuncture · Electroacupuncture · Pain · Hyperalgesia · Peripheral receptors · Peripheral neuromediators acupuncture-induced analgesia · Rat · Mouse

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Introduction

Pain is a common symptom found in different medical conditions and has detrimental impacts ranging from the individual patient level to the healthcare system level. Many areas of interest have been studied in the field of pain research to address different aspects of pain. Considering the taxonomy of pain, the term hyperalgesia has been proposed to summarize all types of increased pain sensitivity [30, 45]. In this context, the control of hyperalgesia triggered by different interventions (pharmacological and nonpharmacological), especially in the field of basic science research, is described as the "antihyperalgesic effect" [15, 17].

Pain control is modulated by complex network receptors and neuromediators that operate at the level of the central nervous system (CNS), represented by the spinal cord and

the brain, and at level of the peripheral nervous system, composed of sensory neurons [31]. External agents acting on these nervous system pathways may potentially change the pain signaling process and, depending on multifactorial variables, they can induce maladaptive plasticity states [47]. According to the predominant mechanism involved (nociceptive, neuropathic or nociplastic), different approaches can be used to promote an antihyperalgesic treatment [47].

Considering the nonpharmacological options for pain management, acupuncture is a therapeutic approach that has been increasingly used in conventional mainstream medicine [28]. Acupuncture is recommended by the World Health Organization for the treatment of a wide list of health problems [41], and several clinical trials and systematic reviews have demonstrated the efficacy of acupuncture to manage pain conditions [62].

The chain of events triggered by acupuncture begins with the introduction of fine needles into different regions of the body, called acupoints [44]. Through manual or electrical stimulation (electroacupuncture) of the acupuncture needles, several neural and neuroactive components distributed in the peripheral somatic tissues surrounding the inserted needle are modulated [43, 73]. Such modulation triggers peripheral, spinal, and supraspinal effects that promote the amplification of endogenous physiologic mechanisms capable of restoring homeostasis, controlling pathologic processes [43, 72].

Multiple studies have compiled the neurobiological effects of acupuncture regarding the spinal and supraspinal mechanisms [72], which are currently considered the main components of its analgesic action. In addition to the central mechanism of acupuncture, clinical and preclinical studies have highlighted the importance of peripheral receptors and neuromediators as essential elements of the antihyperalgesic effects of acupuncture [19, 31, 59].

Peripheral antihyperalgesic effects can be triggered exogenously through local drug administration [2] and endogenously by neural and neuroactive components present in the injured tissue [53, 54, 73]. Several studies have demonstrated the modulation of immune cell activity in the somatic peripheral tissue after acupuncture needling [69], and many neuromediators involved in acupuncture-mediated analgesia are being mapped, such as opioids, cannabinoids, adenosine, and the transient potential receptor vanilloid [72].

Efforts to understand the endogenous responses that modulate pain are crucial to optimize therapeutic possibilities and minimize treatment side effects [2, 77]. In this context, studies investigating the peripheral effects of acupuncture needling can play an important role in mapping the peripheral physiological responses to somatic tissue injury and understanding how to potentiate endogenous mechanisms related to pain control. Considering that the current scientific literature lacks a concise compilation, this article aims to describe state-of-the-art basic science preclinical studies that have investigated peripheral receptors and neuromediators involved in the locally occurring phenomena related to antihyperalgesic effects of acupuncture at the acupoint region and in the area of injured peripheral somatic tissue.

Methods

The present study is an integrative review. It addresses the results of previous research with the objective of evaluating the accumulated knowledge on the subject in a systematically and organized way to identify the main current findings and possible gaps to guide future studies [52]. Integrative reviews have been recently used in the context of the basic science field and demonstrated to be a useful approach to concisely perform a literature review process [13, 14].

The articles published until November 2019 were identified by searching the following three databases: PubMed, Scopus and Web of Science. According to the acupuncture-related terms used in previous research, the search strategy adopted in the present study was as follows: (acupuncture or electroacupuncture or electroacupuncture or electroacupuncture) AND mice or mouse or rat AND receptor or neuromediator or neuromodulator AND (periph*) [40]. The objective of the abovementioned search strategy was to find basic science papers involving rats or mice that investigated the influence of the acupuncture stimuli in peripheral receptors or neuromediators involved in the locally occurring phenomena related to antihyperalgesic effects at two main regions: (1) the acupoint region and (2) the area of injured peripheral somatic tissue. To restrict the discussion only to locally occurring phenomena, papers investigating other peripheral components, such as the dorsal root ganglion and visceral tissue, were excluded. We found that these variables could extend the review to multiple research methods, and other neurobiological mechanisms would be better addressed in other specific reviews. For these reasons, we focused this review on locally occurring phenomena at the level of the peripheral somatic tissue in the abovementioned two regions.

The selection of papers was performed by two authors based on the reading of the titles and abstracts of the articles. The following inclusion criteria were used for full reading of the articles: (1) primary basic science studies related to acupuncture; (2) studies conducted in rats or mice; (3) investigation of peripheral receptors at the level of the acupoint or injured peripheral somatic tissue; and (4) full text published in English. Furthermore, after reading the reference list of the articles initially selected, we manually reviewed the references with titles that indicated a possible relationship with the theme of the review. Thus, the abstracts of the articles found by a manual search, and if they matched the inclusion criteria of the study, they were also added for full reading.

In the initial search, 464 articles were found; 456 articles were related to the search with the descriptors in the databases, and 7 were manually selected. After reading the abstracts, 180 duplicate documents and another 242 were excluded according to the following criteria: article written in language other than English ($n = 21$); article not related to acupuncture ($n = 26$); article that used bee venom injection at acupuncture points ($n = 16$); article that was not a primary study involving animals ($n = 3$); or article that did not investigate receptors or neuromediators located in the peripheral nervous system or somatic tissue ($n = 176$). Of the 41 articles selected for full reading, 12 additional articles were excluded. Among these, 8 did not address peripheral or neuromediators/receptors at the level of the acupoint or at the level of injured peripheral somatic tissue (these investigated receptors in the following structures: dorsal root ganglion $n = 2$; postganglionic neurons $n = 1$; spinal cord neurons ($n = 3$), visceral tissue ($n = 2$)). Finally, four articles were excluded because they investigated receptors using systemic injection of antagonist drugs that had no selectivity for peripheral receptors (Fig. 1).

After finishing this initial review process, 29 articles were included for analysis in the present study, and the following data were extracted: first author; year of publication; age, sex and species of animal; experimental pain model; type of acupuncture; acupuncture stimulation parameters; acupoints investigated; receptors or neuromediators investigated; method for studying receptors or neuromediators; location of receptors or neuromediators; and study conclusions. These records were initially collected by one author and then reviewed by another author. Finally, any divergences were discussed together to form a consensus about what information would be included in the study.

Results

General findings

Table 1 lists the characteristics of the included studies, which addressed the following families of peripheral receptors: opioid ($n = 9$) [4, 5, 12, 27, 48, 55, 58, 64, 69], adenosine ($n = 5$) [18–20, 22, 27], cannabinoid ($n = 5$) [7, 55, 56, 67, 70], transient receptor potential vanilloid (TRPV) ($n = 3$) [1, 20, 65], histamine ($n = 2$) [20, 21], corticotrophin-releasing factor (CRF) ($n = 2$) [12, 69], adrenergic ($n = 1$) [28], muscarinic ($n = 1$) [8], interleukin 1 (IL-1) ($n = 1$) [49], and endothelin ($n = 1$) receptors [35]. The types peripheral neuromediators found were as follows: opioid peptides ($n = 4$) [12, 55, 63, 69], adenosine ($n = 3$) [19, 20, 22], histamine ($n = 1$) [21], substance P ($n = 1$) [24], calcitonin gene-related peptide (CGRP) ($n = 1$) [24], anandamide ($n = 1$) [7], nitric oxide ($n = 1$) [32], and norepinephrine ($n = 1$) [6]. Among the studies included, 20 used electroacupuncture (EA), seven used manual acupuncture (MA), two studies did not use stimuli in the acupoints (the acupoints were identified by electrical resistance for further verification of the presence of specific

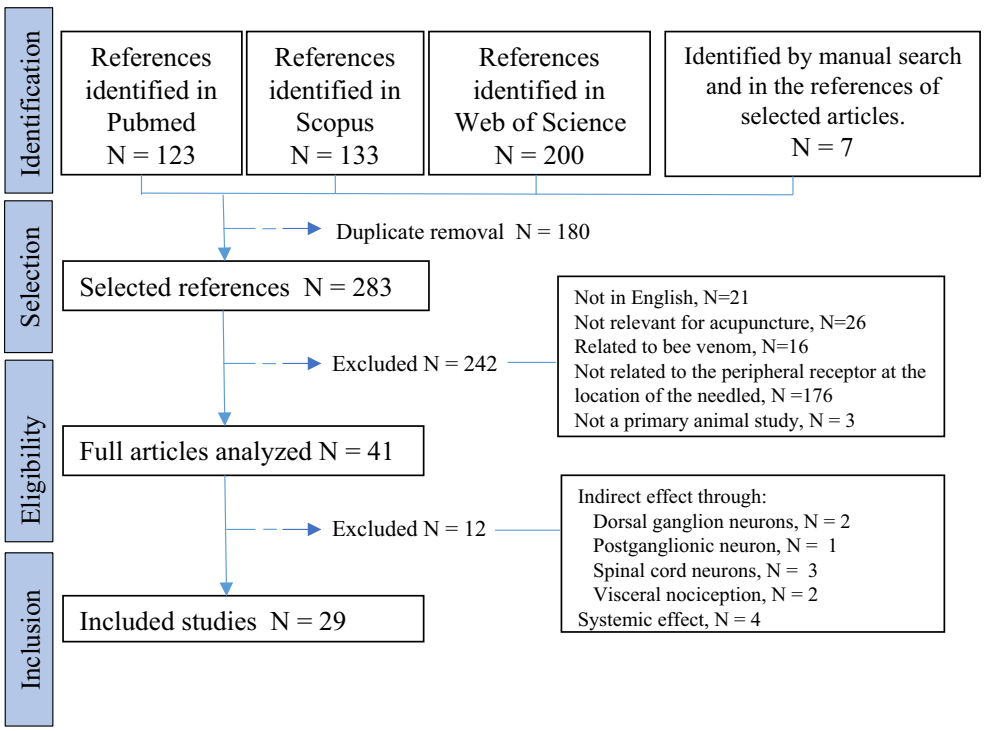

Fig. 1 Flow diagram of the study selection process

Table 1 Compilation of data from the 30 preclinical articles regarding peripheral acupuncture receptors

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation's method	Location of the peripheral receptor/ neuromediator	Conclusions
Opioid receptor -Ceccherelli, 2002 [4]	-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 opioids receptors -Taguchi, 2010 [58]	-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 (opioids 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 non-selective peripheral opioid receptor antagonist and the μ, δ and κ opioid receptor antagonists.
Opioid receptor/β-endorphin -Zhang, 2005 [70]	-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.	Opioids 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, 2003 [48]	-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 (non-selective opioid receptor antagonist)	-EA ipsilateral to injured paw (3 Hz, 0.1 ms, 60 min.). The intensity ranged from 2–3 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.
Opioid receptor/β-endorphin -Fang, 2013 [12]	-CFA in the rat paw. -Male Wistar rat. -Age not informed	-Intraplantar injection (0.05 mL) of non-selective 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 opioids 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 3 opioid receptor subtypes may also be involved.
Opioid receptor, δ opioid receptor -Wang, 2013 [65]	-CFA in the rat paw. -Male Wistar rat. -Age not informed	-Intraplantar injection of a non-selective 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 hours, indicating anti-inflammatory effects. Sustained late mechanical

Table 1 (continued)

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation's method	Location of the peripheral receptor/ neuromediator	Conclusions
					antinociception was mediated by peripheral opioid receptors and was suppressed by intraplantar application of non-selective and a = δ opioid receptors antagonists.
Opioids peptides: β-endorphin, meta-enkephalin, dynorphin A -Wang, 2014 [64]	-CFA induced hind paw inflammation 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	Opioids 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, 2018 [5]	-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.
CB2 cannabinoid receptor; μ-opioid receptor/ β-endorphin	-CFA in the rat paw. -Male Sprague-Dawley adult rat.	-Intraplantar injection of selective RCB2 agonist (AM1241, 1 mg/kg) and antagonist (AM630, 150 μg/kg)	-EA ipsilateral to the injured paw (30 min once a day, 2 Hz, 1 mA, 0.1 ms) -GB30 and GB34	CB2R on infiltrated keratinocytes, macrophages and T lymphocytes. Levels of μ-opioid receptor in inflamed skin tissues.	By activating CB2Rs, EA significantly increases the mRNA level of POMC, the protein level of β-endorphin, and the percentage

Table 1 (continued)

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation's method	Location of the peripheral receptor/ neuromediator	Conclusions
-Su, 2011 [55]		-Intraplantar injection of the irreversible and selective μ-opioid receptor antagonist (β-FNA (250 μg/kg). -POMC mRNA levels in inflamed paw tissue (CRP) -β-endorphin protein level in inflamed paw tissue (WB) -RNAm and μ-opioid-1 receptor protein levels in inflamed tissue (PCR and WB) -β-endorphin levels expressed in keratinocytes and macrophages of inflamed tissue (immunofluorescence).			of keratinocytes, macrophages, and T-lymphocytes expressing β-endorphin in the inflamed skin tissues.
CB2 cannabinoid receptor -Zhang, 2010 [71]	-CFA in the rat paw. -Male Sprague-Dawley adult rat.	-CB2R mRNA and protein levels in inflamed tissue (PCR and WB) -CB2R distribution in keratinocytes and immune cells in inflamed tissue (immunofluorescence)	-EA ipsilateral to the injured paw (30 min, 2 or 100 Hz, 0.1 ms pulse) -GB30 and GB34	CB2R on keratinocytes and immune cells recruited at the inflamed site.	EA at 2 or 100 Hz treatment upregulates CB2R expression in the inflamed skin tissue.
CB2 cannabinoid receptor/-anandamide -Chen, 2009 [7]	-CFA in the rat paw. -Male Sprague-Dawley adult rat.	-Intraplantar injection of CB1R (AM251, 30 μg) and CB2R (AM630, 30 μg) antagonist -Anandamide concentration in inflamed tissue by HPLC	-EA ipsilateral to the injured paw (30 min, 2 or 100 Hz, 0.1 ms pulse) -GB30 and GB34	CB2R on keratinocytes and immune cells recruited at the inflamed site.	EA at 2 and 100 Hz increased anandamide levels in inflamed tissue, which is capable of activating CB2R and contributing to the local antinociceptive effect of EA.
CB2 cannabinoid receptor -Su, 2012 [56]	-CFA in the rat paw. -Male Sprague-Dawley adult rat.	-Intraplantar injection of CB2R agonist (AM1241, 1 mg/kg) and CB2 receptor specific antagonist (AM630, 150 μg/kg) -PCR to identify mRNA and WB levels to determine IL-1b protein levels, IL-6 and TNFalpha in inflamed tissue.	-EA ipsilateral to the injured paw (30 min, 2 Hz, 0,1 ms) -GB30 e GB34	CB2R on keratinocytes and immune cells, including mast cells, macrophages, B and T lymphocytes, and NK cells.	CB2R in keratinocytes and immune cells at the site of the injured paw contribute to the EA effect on the reduction of pain behavior and proinflammatory cytokines at the local level.
CB2 cannabinoid receptor -Yuan, 2018 [68]	-Intraarticular injection of MIA into the left knee joint of mice. -Female C57BL/6 mice	-Immunofluorescence labeling -CB2R knockout mice -Histological evaluation (Safranin O-Fast Green staining protocol/fluorescence microscope)	-EA ipsilateral to the injured knee (30 min, 2 Hz, 1 mA and 0,1 ms, once every other day for 4 weeks) -Ex-LE4 and ST35	CB2R in the menisci of KOA mice and in the synovial fibroblasts.	EA reduced the expression of IL-1β by activating the CB2R, thus inhibiting the chronic pain in the mouse of KOA.

Table 1 (continued)

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation's method	Location of the peripheral receptor/ neuromediator	Conclusions
A1 adenosine receptor/adenosine -Goldman, 2010 [19]	-8 weeks old -CFA in the mice paw -SNI -C57BL/6 J male mice. -8–14 weeks old	-Acupoint microdialysis method -KO model: A1R and A2R -A1 receptor agonist acupoint injection (CCPA, 0.1 mM, 20 μl)	-MA ipsilateral to the injury (30 min duration with rotation every 5 min) -ST36	A1 receptors at nociceptive neuron fibers of the superficial fibular nerve (near point ST36).	Local A1 receptors are involved in acupuncture-mediated analgesia.
A1 adenosine receptor/adenosine -Hurt, 2012 [22]	CFA injected under the glabrous skin to inflame one hind paw -SNI -Male C57BL/6 mice -2–6 months in age	-Prostatic acid phosphatase (hPAP) acupoint injection (an enzyme that increases extracellular adenosine bioavailability) -A1R agonist (CPA/5 nmol) acupoint injection. -A1R receptor antagonist (CPX/1 mg/kg) acupoint injection. -Knockout animals for the A1R receptor. -Immunohistochemistry.	-BL 40 injected with PAP (enzyme) or CPA (A1 agonist) ipsilateral to the injured site.	A1R in nociceptive neurons and in nociceptive axon terminals that surrounds the acupoint.	PAP inhibits nociception via na A1R and PLC-dependent mechanism in the periphery. Injection of hPAP into the BL 40 acupuncture point had nociceptive effects that lasted substantially longer than acupuncture.
A2A adenosine receptor -Li, 2015 [26]	-Synovitis in collagen induced arthritis (CIA) in mice. -Male C57BL/B6 mice. -4–5 weeks of age	-A2AR antagonist intraperitoneal injection (SCH58261, 5 mg/kg) -X-ray for morphological analysis of joint bone (on day 60). ELISA: determination of plasma TNF-α level. -Histological analysis of pathological changes in joint tissue (immunohistochemistry of A2AR expression and hematoxylin-eosin staining).	-EA ipsilateral to the injured knee (30 min every 24 h for 14 days, 2 Hz, 0.07 mA, 0.3 ms) -ST36 and SP6	A2A receptors located on the synovial membrane.	EA significantly reduced pathological scores, TNF-α levels, and bone damage scores on X-ray. The anti-inflammatory effects of EA were reversed by coadministration of the A2A antagonist.
A1 adenosine and opioid receptor -Liao, 2017 [27]	-CFA in the mice paw. -C57BL/6 male mice. -Aged 8 to 12 weeks.	-A1 agonist (CPA, 0.1 mg/kg in 10 μl saline) acupoint injection and A1 antagonist (Rolophilin, 3 mg/kg in 10 μl saline) acupoint injection. -Mu opioid receptor (endomorphin 1) acupoint injection.	-EA (square pulses of 15 to 100 μs duration, 2 Hz, 1 mA) -ST36	A1 receptors in peripheral nociceptive neurons.	EA and adenosine A1 receptor agonist injection into ST36 reduces pain behavior. The μ opioid agonist injected into the acupoint does not reduce this behavior. Blocking peripheral opioid receptors with systemic injection of naloxone methiodide abolished acupuncture analgesia.
A1 adenosine receptor -Fujita, 2017 [18]	-Intra-articular administration of MIA and CFA into	-Non-selective adenosine receptor antagonist acupoint injection (5 μl caffeine).	-MA ipsilatateral to the injured site (20 min/rotated for 1 min every 3–4 min) -ST36	The A1 receptors responsible for mediating analgesia are located in the vicinity of the acupuncture point.	Small amounts of caffeine may block the analgesic effects of acupuncture. Manual acupuncture needling do not alter the gene

Table 1 (continued)

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation's method	Location of the peripheral receptor/ neuromediator	Conclusions
	the left hind limb ankle joint. -C57BL/6 male and female mice -Aged 8–12 weeks	-A1 receptor gene expression in ST36 acupuncture tissues by real-time PCR -Plasma caffeine concentrations were quantified using an ELISA kit.			expression of A1 receptor at the acupuncture point site.
TRPV1 receptor -Wu, 2014 [66]	-CFA in the rat paw. -ICR mice, sex not informed. -Aged 8 to 12 weeks	-Acupoint injection of TRPV1 (capsaicin), TRPV4 (GSK1016790A) and ASIC3 (acidic saline solutions pH 5, 4, and 3) agonists. -Expression of TRPV1, TRPV4 and ASIC3 in the different anatomical layers of tissue at ST36 and sham (Western blotting) -Distribution pattern of TRPV1, TRPV4 and ASIC3 in tissue layers at ST36 (Immunofluorescence) -Components for calcium wave propagation (Western blotting)	-MA unilateral (30 min 2 rotations every 5 min) -ST36	TRPV1, TRPV4 and ASIC3 receptors are expressed in neural fibers and non-neural cells (skeletal muscle and probably fibroblasts) in different layers of the ST36 point (but not in a non-acupoint in the gluteus maximus muscle)	TRPV1 (but not TRPV4 and ASIC3) can act as an acupuncture response channel by detecting physical acupuncture stimulation and conducting signaling via calcium wave
TRPV1 receptor -Abraham, 2011 [1]	-Healthy rats. -Male Sprague-Dawley rats. -4–5 months old.	-Immunostaining in the area of the acupoint with antibodies against TRPV1. -Technique of co-localization immunohistochemistry of TRPV1 and neuronal nitric oxide synthase.	-EA unilateral (2 sessions of 20 min separated by an interval of 80 min, 1 mA, duration of 1 ms to 3 pulses/s). -BL40	TRPV1 expression in sensory cutaneous nerves, mast cells and epithelial cells. In the dermis, TRPV1 is expressed in nerve fibers and connective tissue cells.	The high expression of TRPV1 receptors in subepidermal nerve fibers and their positive regulation after EA stimulation may mediate signal transduction at the central level. Expression of this receptor in subepidermal connective cells may lead to local effects of EA.
-Muscarinic receptor -Chung, 2011 [8]	-Carrageenan in the rat paw. -Male Sprague-Dawley rats. -Age not informed	-Intraplantar injection of muscarinic receptor antagonist (atropine methylbromide, 2 mg/kg in 0.2 ml of saline).	-EA (duration 45 min, 4 Hz with 0.45 ms duration, 0.7–1.0 mA) -Area corresponding to the cymba conchae in humans.	Muscarinic receptors at the site of inflammation.	Local muscarinic receptors are involved in the effects of atrial acupuncture in controlling edema and mechanical hyperalgesia.
Alpha receptor -Liu, 2015 [28]	-Healthy rats -Males and females Wistar rats. -Age not informed	-Injection into the acupoint´s dermis of the agonist (phenylephrine 40–60 μl, 0.125 mg/ml), and the antagonist of the alpha receptor (regitin 40–60 μl, 0.1 mg/ml).	-MA (rotation about 1 Hz for 10 min/ST36). -MA (Rotation about 1 Hz for 5 min/no acupuncture on the lower back of rats along the scapular midline)	Alpha receptor on the erector pilli muscles of the skin located along the pilomotor line.	Alpha receptor is related to the transmission of acupuncture and analgesia signals through this mediated signal.

Table 1 (continued)

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation's method	Location of the peripheral receptor/ neuromediator	Conclusions
		-Skin incision along the sympathetic substance line near the acupuncture point. -Catecholamine levels in the skin after HPLC (microdialysis) determination.			
CRF and IL-1 receptors -Sekido, 2004 [49]	-Carrageenan in the rat paw. -Male Sprague-Dawley rats. -Age not informed	Intraplantar injection of the selective α-helical CRF antagonist CRF (2.5, 3.7 and 5.0 ng) and recombinant IL-1 receptor antagonist, IL-1ra (25, 50 and 100 ng).	-EA (60 min, 3 Hz, 0.1 ms per pulse) -ST36	Peripheral CRF and IL-1 receptors on immune cells at the site of inflammation.	EA may be able to release CRF and IL-1 from immune cells that trigger the release of opioid peptides in inflamed tissue. Such peptides activate peripheral opioid receptors, reducing neuronal excitability or the release of proinflammatory neuropeptides and thus inhibiting pain.
Histamine receptor: H1 -Huang, 2012 [21]	-CFA in the left knee of mice. -Male Sprague-Dawley rats. -Age not informed	-Acupoint injection of histamine (50 μL), disodium cromolyn (20 μL) and H1 receptor antagonist (cremastine 50 μL) -Microscopic Analysis	-MA/30 min/handling for 30 s and 30 s interval -ST36	Distinct subgroup of C fibers (excited by histamine), histamine sensitive central projection neurons	Mechanical stimulation of acupuncture promotes degranulation of mast cells, which release histamine, through H1 receptor fiber subgroups are activated and modulate the sensation of pain at central level.
H1 histamine receptor, TRPV2 receptor and A1 adenosine receptor -Huang, 2018 [20]	-CFA in the left knee of mice. --Male Sprague-Dawley rats. -Age not informed	-TRPV2 gene knockout model -Acupoint microdialysis method and HPLC -Acupoint injection of H1 receptor agonist (50 μL 2-pyridineethanamine dihydrochloride) -Acupoint injection of H1 receptor antagonist (50 μL chlorprophenpyridaminemaleat-e) -Acupoint injection of A1 receptor agonist CCPA (20 μL) -Sodium cromolyn acupoint injection (20 μL) -Tissue sectioning and mast cell staining -Microscope -Detection of beta endorphin by ELISA	-MA/30 min and 20 min: 30 s lift, 30 s hold, 30 s twist and 30 s hold -ST36 on the left leg	H1 histamine receptor, A1 adenosine receptors and TRPV2 receptor at the acupoint site.	Mast cells are activated by acupuncture through TRPV2 receptors and are central in transducing mechanical stimulation into H1 or A1 receptor activation, triggering the effects of acupuncture.

Table 1 (continued)

Receptor/ neuromediator, author and year	Animal model/sex/ age	Method of investigating the peripheral effect of acupuncture	Acupoints and stimulation's method	Location of the peripheral receptor/ neuromediator	Conclusions
Endothelin receptor: ETB -Mazzardo-Martins, 2018 [35]	-Hind mice paw ischemia/-reperfusion (CPIP model). -Female Swiss mice -Age not informed.	-Peripheral administration (intraplantar) of the ETb antagonist (B1-788/10 nmol) -Peripheral administration (intraplantar) of the ETb agonist (SRTX 56c/30 pmol) -WB	-EA (20 min/F1 = 2 Hz, 0.7 ms pulse with, 5 s of stimulation; F2 = 10 Hz, 0.2 ms pulse with, 5 s of stimulation) with alternating polarities -ST36 and SP6	Peripheral ETb receptors are expressed in endotelial cells, smooth muscle cells, in macrophages and keratinocytes.	Suggest that EA's analgesic effect is synergic with ETB receptor activation in the periphery, as well as central (spinal cord) ETB receptor blockade.
Substance P and calcitonin gene related peptide (CGRP) -Kashiba, 1991 [24]	-Non-injured Wistar rats -Male and female adult rats	-Double staining immunohistochemical demonstration of SP and CGRP (anti-SP monoclonal antibody and anti-CGRP polyclonal antibody)	EA (30 min/10 Hz/100 μs) the palm of left forelimb	SP and CGRP-like immunoreactive fibers from small nerve bundles located on the skin of the EA stimulated forelimb paw	These results suggest that EA induces release of SP and CGRP from peripheral terminals of primary sensory neurons.
Nitric oxide -Ma 2003 [32]	-Non-injured Sprague-Dawley rats. -Male adult (5–6 months) rats	-Western blots using polyclonal anti-nNOS and anti-endothelial nitric oxide synthase (eNOS) antibody in the skin tissues. -Skin concentrations of nitric oxide were quantified using chemiluminescence	Acupoint/meridian detection by electric resistance (no stimulation was applied) -Meridian regions from PC 2 to 6, BL 36 to 57, CV 3 to 22, and GV 2 to 14	Nitric oxide at samples of the skin meridian/acupoint regions	Nitric oxide expression is consistently higher in the skin acupoints/meridians associated with low electric resistance.
Norepinephrine -Chen, 2006 [6]	-Non-injured Sprague-Dawley rats. -Male adult rats.	-Enzyme immunoassay (EIA) of skin tissue homogenate -Thin layer chromatography (TLC) of the acupoint skin	Acupoint detection by electric resistance (no stimulation was applied) -BL56, PC6, and GV6	Norepinephrine at samples of skin acupoint region	Skin norepinephrine synthesis/release in acupoints/meridians is increased in skin acupoints.

Abbreviation list: *CFA*, Freund complete adjuvant; *MA*, manual acupuncture; *EA*, electroacupuncture; *CRF*, corticotropin releasing factor; *PCR*, polymerase chain reaction; *WB*, Western blotting; *HPLC*, high performance liquid chromatography; *KO*, knockout; *CIA*, collagen induced arthritis; *ASIC*, acid-sensing ion channels; *NLX*, naloxone; *NTI*, naltrindole; *POMC*, proopiomelanocortin; *NK*, natural killer; *IL-1*, interleukin 1; *TRPV*, vanilloid transient potential; *CTOP*, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-ThrNH2; *Nor-BNI*, nor-binaltorphimine; *CCPA*, 2-chloro-N(6)-cyclopentyladenosine; *MSU*, crystallization of monosodium urate; *CPIP model*, chronic post-ischemia pain; *SP*, substance P; *MIA*, monosodium iodoacetate; *KOA*, knee osteoarthritis; *CPX*, 8-cyclopentyl-1,3-dipropylxanthine; *SNI*, spared nerve injury; *CPA*, N6-cyclopentyladenosine; *CGRP*, calcitonin gene-related peptide; *CBR*, cannabinoid receptor; *nNOS*, nitric oxide synthase; *ICR mice*, is a strain of albino mice originating in SWISS

local neuromediators at the acupoint level), and one study used only acupoint injection of an agonist drug or enzyme.

The EA frequencies used in the studies were as follows: 2 Hz ($n = 6$), 2–100 Hz ($n = 4$), 3 Hz ($n = 4$), 10 Hz ($n = 2$), 100 Hz ($n = 2$), 4 Hz ($n = 1$), 5 Hz ($n = 1$), and 30 Hz ($n = 1$). The most common acupoints selected were: ST36 ($n = 15$), GB30 ($n = 8$), GB34 ($n = 4$), SP6 ($n = 2$), BL60 ($n = 2$), BL40 ($n = 2$), auriculotherapy (cymba conchae) ($n = 1$), Ex LE4 ($n = 1$) and ST35 ($N = 1$). Some articles described the study of meridian regions (PC2 to PC6, BL36 to BL57, CV3 to CV22, GV2 to GV14, BL56, PC6, and GV6) ($n = 2$) and body areas (the palm of the left forelimb) ($n = 1$). A summary of the abovementioned acupoints and meridian regions represented in the analogous anatomic regions of the human body are presented in Fig. 2. Only four studies evaluated healthy animals; the others used the following pain models. Intraplantar injection models included complete Freund adjuvant (CFA) ($n = 15$), carrageenan ($n = 4$), and capsaicin ($n = 2$) models. Intra-articular injection models included CFA ($n = 3$) and monosodium iodoacetate injection (MIA) ($n = 2$) and crystallization of monosodium urate (MSU) ($n = 1$) models. Peripheral nerve lesion model included the spared nerve injury (SNI) ($n = 2$). Other models used included collagen-induced arthritis ($n = 1$) and ischemia/reperfusion of the paw (CPIP) ($n = 1$).

The study methods used in the analysis of peripheral receptors or mediators included the following: local injection of specific receptors antagonistic drugs ($n = 19$) and agonists drugs ($n = 10$), western blotting (WB) ($n = 6$), polymerase chain reaction (PCR) ($n = 5$), immunofluorescence ($n = 5$), histological analysis of the tissue by microscopy ($n = 5$), immunohistochemistry ($n = 7$), use of knockout animals ($n = 4$), ELISA ($n = 3$), antibody use ($n = 3$), high-performance liquid chromatography (HPLC) ($n = 5$), enzyme injection ($n = 1$), membrane stabilizer injection ($n = 2$), oral intake of the antagonist ($n = 1$), immunolabeling ($n = 1$), radioimmunoassay ($n = 1$), skin incision ($n = 11$) and enzyme immunoassay (EIA) ($n = 1$). The research methods of each peripheral receptor and neuromediator are described in detail in Table 1.

Opioid receptors, beta-endorphin, enkephalin, and dynorphin

In the beginning of acupuncture research in the 1970s, only central opioid mechanisms were considered to account for to the analgesic effects of acupuncture [2, 75]. However, with the discovery that opioid receptors are also peripherally expressed in nociceptive C fibers and non-neuronal tissues, the peripheral role of these receptors started to be investigated in the acupuncture field [2].

Several studies strengthened the knowledge of the relationship between the antihyperalgesic effect mediated by acupuncture and the peripheral opioid system [31, 36, 69, 72, 73]. Through intraplantar preadministration of naloxone methiodide (an antagonist restricted to peripheral opioid receptors), Zhang et al. (2005) [69] and Fang et al. (2013) [12] observed the prevention of the antihyperalgesic effect of EA treatment in a pain model of inflammation induced by CFA. Furthermore, through the radioimmunoassay technique, Fang et al. (2013) [12] verified an increase in the levels of beta-

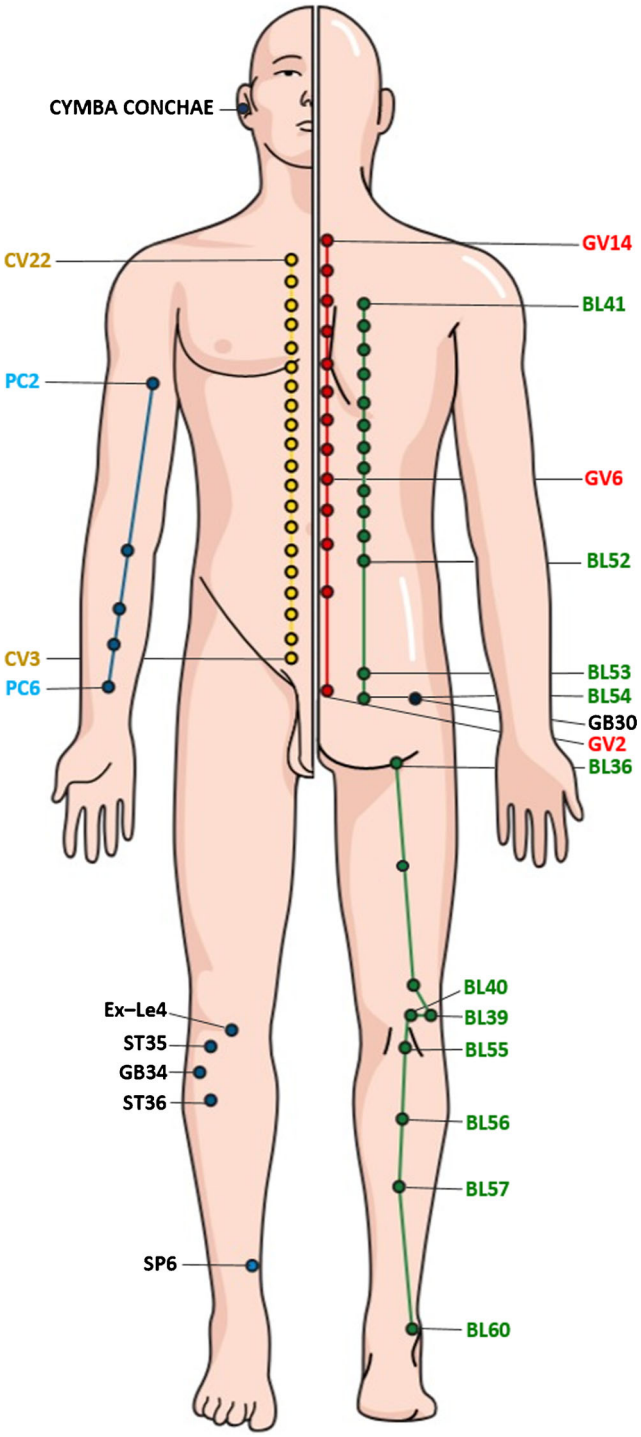

Fig. 2 Acupoints and meridian regions found in the basic science studies of this review represented in their analogue anatomic regions

endorphin, an endogenous opioid neuromediator, in inflamed rat paws after EA. In addition, intraplantar injection of beta-endorphin mimicked the effect of EA in reducing the nociceptive behavior of animals [12, 69].

Another article, using the inflammatory pain model of carrageenan paw injection, found that EA-mediated analgesia could not be blocked using intravenous (systemic) doses of μ, δ and κ receptor antagonists [58]. On the other hand, these same antagonists administered via the intraplantar route at the inflamed paw abolished the antihyperalgesic effect of EA [58]. These results suggest that the μ, δ and κ opioid receptors located at peripheral nerve endings and non-neural tissue are correlated with the EA effect.

In a gout model induced by MSU (crystallization of monosodium urate) injected into the animals' ankles, Chai et al. (2018) [5] also observed that intra-articular antagonist injection of μ and κ opioid receptors (but not systemic injection) abolished the antihyperalgesic effect of acupuncture. This same effect was not observed with the use of a δ opioid receptor antagonist [5]. In this same study, the effect of 2/10 Hz EA was found to be comparable to that of indomethacin (a nonsteroidal anti-inflammatory drug) in improving the behavior of pain and thermal hyperalgesia. In addition, EA was correlated with increased levels of beta-endorphin in the inflamed skin tissue of the ankle [5]. Furthermore, Wang et al. (2014) [63] demonstrated that EA-induced antihyperalgesia in peripheral somatic tissue not only involved beta-endorphin but was also correlated with local enkephalin and dynorphin participation [63].

Taken together, these research findings suggest that peripheral opioids receptors and neuromediators play essential roles in acupuncture analgesia. The mechanism is probably related to the desensitization of peripheral sensory nerves through activation of peripheral opioid receptors, which can block the release of proinflammatory cytokines produced by immune cells, such as polymorphonuclear leukocytes and mononuclear cells [72]. Other mechanisms that may be correlated with increased opioid release in the inflamed area through acupuncture occur via activation of sympathetic nerve fibers, which stimulate the migration of immune cells containing opioids, and via COX-2 inhibition, which positively regulates endocannabinoid metabolism, with a consequent increase in local opioid levels [72].

Cannabinoid receptors and anandamide

The study of the endocannabinoid system and the selective regulation of its receptors can help to expand the therapeutic perspective in the context of pain regulation [38, 42]. Two main receptors are related to the biological effects of cannabinoids, CB1 (CB1R) and CB2 (CB2R), which have distinct cell distribution and signaling [2]. The first, identified in peripheral nerves, spinal neurons and abundant in brain areas, is considered a synaptic modulator. On the other hand, CB2R is expressed predominantly in immune cells, and its activation has potentially less systemic side effects, such as hypomobility and catalepsy, than those that are associated with CB1R activation [2]. CB1R and CB2R can be activated by cannabis-derived, synthetic agonists and by endogenous cannabinoids produced in mammalian tissues, such as N-arachidonoyl ethanolamine (anandamide) and 2-arachidonoyl glycerol [10, 37, 42, 57].

The participation of cannabinoid receptors in the antihyperalgesic effect mediated by acupuncture was evaluated in the included studies based on the administration of agonists or antagonists in CFA-induced inflammation models and in knockout animals for the receptor in a knee osteoarthritis model.

It was demonstrated that 2 and 100 Hz EA can increase protein levels and CB2R mRNA in the inflamed tissue, quantified by western blotting and PCR, respectively [70]. Another study found that EA increased the levels of anandamide in inflamed tissue compared with the sham group [7]. Moreover, a CB2 receptor antagonist pretreatment decreased the antihyperalgesic effect of EA [7]. On the other hand, pretreatment with a selective antagonist of the CB1R did not lead to a change in analgesia [7]. In the context of non-neural cells, EA increased the number of keratinocytes, macrophages and T lymphocytes reactive to CB2R in inflamed tissue samples [70].

Another study found that EA treatment increased the number of fibroblasts containing CB2R in the menisci of rats with knee osteoarthritis [67]. Furthermore, Yuan et al. (2018) [67] identified decreased expression of IL-1β-positive cells in the menisci of rats with osteoarthritis treated with EA. In addition, EA had no effect on reducing the expression of IL-1β-positive cells in a knockout group for CB2R. In the same study, EA improved the parameters of hyperalgesia (thermal and mechanical), the evaluation score of cartilage changes and the weight supported by animals with osteoarthritis, while in knockout animals for CB2R, these effects were not observed [67]. According to Yang et al. (2018) [67], EA can reduce the expression of IL-1β by activating CB2R, which inhibits pain behavior in rat osteoarthritis models.

To investigate the mechanisms by which the activation of CB2R contributes to pain control, Su et al. (2012) [56] verified that AM1241 (an agonist selective for CB2R) and EA significantly reduced levels of IL-1β, IL-6 and TNF in inflamed tissue. In another article from the same research group, intraplantar AM1241 and EA improved thermal hyperalgesia and mechanical allodynia [55]. Additionally, crosstalk between the opioid and cannabinoid systems mediated by acupuncture was proposed by the authors with the observation that EA increased protein levels of beta-endorphin (reversed with a μ-opioid selective receptor antagonist) and increased levels of pro-opiomelanocortin (POMC) (attenuated by

pretreatment with AM630, a selective antagonist for CB2R) at the inflamed tissue site [55].

Collectively, studies involving cannabinoid receptors and related neuromediators indicate that at the level of inflamed tissue, acupuncture increases the level of anandamide, potentiating the stimulus to CB2R for opioid production, which is able to block the release of proinflammatory cytokines. In addition, acupuncture induces greater expression of CB2R and endogenous opioids in keratinocytes and infiltrative immune cells at the injured site of peripheral somatic tissue [72].

Adenosine receptors and adenosine

Adenosine receptors are coupled to G proteins and are classified into 4 subtypes: A1 (A1R), A2A, A2B and A3 [2, 77]. Among the mechanisms by which adenosine is released into the extracellular space, hydrolysis of ATP by ectonucleotidase is included [2, 77]. Due to the frequent release of such metabolites after tissue stimulation [11, 46], it has been suggested that these effects could be related to acupuncture.

Of the activated receptors in the periphery, only A1R has antinociceptive effects [46, 77]. In associated studies, A1Rs were located in peripheral nociceptive neurons [27] and sensory afferents of ascending nerves [19]. The attenuation of hyperalgesia and pain behavior after injection of an A1R agonist at the ST36 acupoint was demonstrated in both inflammatory and neuropathic pain models. In contrast, manual acupuncture [19] was ineffective when performed at the ST36 acupoint contralateral to the lesion and in A1R knockout animals. In another study, the injection of a selective A1R agonist into the BL40 acupoint induced antihyperalgesic effects but did not affect thermal sensitivity on the contralateral side and had no effect on the ipsilateral side in A1R knockout animals [22]. Therefore, these studies reinforce the importance of local activation of A1R to obtain the antihyperalgesic effect of acupuncture in these models [19, 22].

Goldman et al. (2010) [19] observed higher adenosine concentrations than other nucleotide concentrations in the ST36 acupoint using a microdialysis method. Based on these findings, Hurt and Zylka (2012) [22] suggested the possibility of administering prostatic acid phosphatase (hPAP), an enzyme that increases extracellular adenosine bioavailability, in the acupoint, with the aims of inhibiting nociception independent of the introduction of needles and extending the duration of the antihyperalgesic effect. The results showed that local peripheral acupoint injection of hPAP inhibits thermal hyperalgesia in models of chronic pain induced by CFA and spared nerve injury (SNI) for up to 7 days after the drug administration (with a duration almost 100 times longer than the effect of manual acupuncture) [22]. In knockout animals for the A1 receptor, there was no change in hyperalgesia after local injection of hPAP [22]. Furthermore, local AMP injection amplified the magnitude of the antihyperalgesic effect in mice previously treated with hPAP, indicating an effect dependent on the availability of this substrate [22].

In agreement with the results of previous studies [22, 39], Fujita et al. (2017) [18] observed that local acupoint injection of caffeine (nonselective antagonist A1 and A2) blocked the antihyperalgesic effect of acupuncture [18]. According to present knowledge, these data suggest that caffeine consumption has the potential to reduce the antihyperalgesic effect of acupuncture [18, 22].

In another study using a collagen-induced synovitis model (experimental model that mimics rheumatoid arthritis), EA was found to promote an anti-inflammatory effect (and chondroprotective effect) by activating A2A receptors present in the synovial membrane and inflammatory cells in the joint [26]. This anti-inflammatory effect was reversed after intraperitoneal injection of an A2A antagonist [26]. It is worth mentioning that in the study published by Goldman et al. (2010) [19], no correlation was found between the A2A receptors and the antihyperalgesic effect of manual acupuncture, thus showing that there may be differences in the activation of peripheral receptors related to the stimulus type (manual acupuncture or EA) or by the type of injury studied (inflamed paw or synovitis).

Transient potential receptor vanilloid

TRPV ion channels are a family of ligand-dependent ion channels that function as molecular detectors for physical stimuli. All the channels of the TRPV family subtypes (TRPV1, TRPV2, TRPV3 and TRPV4) are expressed in nociceptive neurons, where they act as signal transducers for mechanical, thermal and chemical stimuli [9]. TRPV1, also known as the capsaicin receptor, was cloned in 1997 from rat dorsal root ganglia (DRGs) [3]. TRPV1 is a polymodal receptor because it does not respond only to the active ingredient in pepper but also to noxious heat (≥ 43 °C), low pH (protons) [3], and several other agents [23, 33, 34, 50, 66, 76]. A total absence of behavioral and physiological responses to capsaicin and partial reduction in responses to noxious heat have been observed in TRPV1-deficient mice, but no alterations have been observed in responses to noxious mechanical stimuli [23, 76].

In the acupuncture literature, Abraham et al. (2011) [1], observed a higher number of subepidermal nerve fibers expressing TRPV1 in sections of skin containing BL40 acupoint than in nonacupoint control skin using an immunolabeling method. It was demonstrated that EA was able to upregulate TRPV1 receptor expression in acupoints and that this effect was less pronounced than stimulation in nonacupoint EA-stimulated areas. In addition, subepidermal nerve fibers showed the colocalization of TRPV1 with peripherine, a marker for C and A-δ fibers [1].

To demonstrate how physical stimulation is turned into neural signaling, Wu et al. (2014) [65] mapped different receptors present in acupoint regions and investigated if mechanosensitive channels were associated with the conversion of needling stimuli and proposed possible sensing pathways linking channel activation to neural signaling. Through western blotting, the authors identified a greater density of receptors in the anatomical layers of the ST36 acupoint than in a nonacupoint region. Abundances of TRPV1, TRPV4 and the ionic acid detection channel 3 (ASIC3) were found in the acupoint area [65] but not in the nonacupoint area. In the ST36 acupoint region, an expression of components of calcium wave propagation was also observed. The authors proposed that TRPV1 might act as an acupuncture-reactive channel by sensing physical stimulation from acupuncture needling and conducting this signal to sensory neurons [65]. In another set of experiments, it was demonstrated that acupoints injected with capsaicin, an agonist of TRPV1 receptor, reproduced the analgesic effects of acupuncture. On the other hand, this effect was not observed with the acupoint injection of TRPV4 or ASIC3 agonists [65]. Considering other vanilloid receptors, one study found that TRPV2 receptors were associated with acupuncture-induced antihyperalgesia by interacting with mast cell activity. This will be further discussed together with the histamine receptors [20].

Adrenergic receptors and norepinephrine

The adrenergic receptors are a family of G protein-coupled receptors that mediate diverse effects at virtually all sites throughout the body [2]. These receptors have been extensively studied by a variety of techniques and are subdivided into three major categories: the α1-adrenoceptors, α2-adrenoceptors, and β-adrenoceptors [2]. Peripheral adrenergic receptors that can influence pain signaling are located on sensory nerves, postganglionic sympathetic nerve fibers, and immune cells [2]. Endogenous ligands of peripheral adrenergic receptors are the catecholamines epinephrine and norepinephrine, which are released from the adrenal medulla and the postganglionic sympathetic nerve fibers [2].

In previous studies, the distribution of iodine-125-catecholamine along the skin of rats was mapped by macroradiography with marked tyrosine [29]. These experiments demonstrated symmetrical lines containing dense noradrenergic fibers innervating the arrector pili muscles (AP muscles) that traverse across the body, forming the so-called sympathetic substance lines (SSLs) [29]. Supposing a possible correlation between these SSLs with the acupuncture meridian paths, Liu et al. (2015) [28] investigated whether the transmission of acupuncture stimuli can occur through sympathetic activity and contraction of the AP muscles (which form a visible skin reaction called a “pilomotor line”). In this study, it was observed that both manual acupuncture and acupoint injection of phenylephrine, an alpha receptor agonist, produced an antinociceptive effect (measured by the tail flick test) and a pilomotor line reaction (recorded by a camera) [28]. These effects were not observed when the skin was incised along the SLLs, or regitin (an alpha receptor antagonist) was injected into the SSLs. Moreover, the antinociceptive effect of acupuncture was not blocked when incisions were made more than 5 mm away from the SSL path related to the stimulated acupoint [28]. The results of the study suggest that acupuncture antinociceptive signaling occurs along the skin, more specifically through the SSLs, by means of adrenergic alpha receptors present in the AP muscles [28]. In the context of neuromediators related to the adrenergic system, one study found that the increased levels of norepinephrine at the skin of acupoint regions are correlated to nitric oxide release [32], and this will be detailed in the final session of this discussion.

Muscarinic receptors

Muscarinic acetylcholine receptors (mAChRs) have five different subtypes that have been described in literature (M1 to M5). In particular, the subtypes M2 (M2R) and M4 are known to participate in central analgesia [2, 72]. Peripheral actions are mainly attributed to M2R found in skin nociceptors. Experimental studies have shown that stimulation of peripheral M2R can promote antinociceptive effect by reducing the levels of proinflammatory peptides [2] .

In the context of acupuncture, Chung et al. (2011) [8] investigated the role of peripheral mAChRs in the antihyperalgesic and anti-inflammatory effects of EA. The study showed that EA at the cymba conchae reduced mechanical hyperalgesia and edema in an animal model of paw inflammation. In addition, the intraplantar injection of atropine methyl bromide, a peripheral muscarinic receptor antagonist, reversed the effects EA [8]. Furthermore, the use of intraplantar injection of naloxone (an opioid antagonist) could not reverse the effect of EA. The authors of the study suggested that the electrostimulation of the cymba conchae, an auricular area innervated by the vagus nerve, may trigger different effects than body EA by activating a vagal cholinergic response rather than an opioid response [8].

There is experimental evidence that both EA at ST36 and transcutaneous nerve stimulation at the auricular branch of the vagus nerve can modulate immune responses of systemic inflammation (sepsis) [61, 74]. However, further studies need to better explore if these same mechanisms are correlated with the modulation of peripheral mAChRs in areas of somatic tissue injury.

Corticotropin-releasing factor and interleukin 1 neuromediators

Corticotropin-releasing factor and interleukin 1 are released by immune cells in inflamed tissues and appear to be involved in EA-mediated analgesia [49]. In an animal model induced by carrageenan paw injection, an increase in the pressure threshold in the paw after EA persisting for 24 h was recorded [49]. This effect was blocked dose-dependently by the intraplantar administration of alpha-helical CRF antagonists and an IL-1 receptor antagonist; however, there was no change in the nociceptive parameters when these same antagonists were applied intravenously [49]. These authors suggested that the release of CRF and IL-1 promoted by EA may trigger increased levels of opioid peptides in the inflamed area that interact with peripheral opioid receptors to reduce pain behavior [49].

Substance P and calcitonin gene-related peptide neuromediators

Substance P and CGRP are neuromediators previously investigated in acupuncture studies [31]. In this context, several studies noted the release of substance P (SP) and CGRP from the peripheral terminals of primary sensory neurons after local administration of histamine [60] and capsaicin [16]. Furthermore, it was observed that EA induces small diameter primary sensory afferents (fibers C or A-δ) to release SP and CGRP from their peripheral terminals, which correlates with local vasodilation and peripheral sensory fiber excitability [24].

H1 receptor and histamine neuromediators

Other important receptors and neuromediators investigated in the area of acupuncture are the histamine receptor, expressed in the distal terminal of primary sensory neuron, and the mediators released by mast cell degranulation. In previous studies, it was demonstrated that local mast cell degranulation is correlated with the effect of peripheral acupuncture needling [20]. In addition, some authors have found that TRPV2 receptors seem to be associated with acupuncture-induced mast cell degranulation [20, 68]. Based on in vitro experiments and in vivo experiments involving TRPV2 knockout animals, it has been hypothesized that mechanical stimulation produced by acupuncture needling can activate TRPV2 receptors present in the mast cells [20, 68]. The TRPV2 activation produces mast cell degranulation resulting in the release of several neuromediators that, in the context of the antihyperalgesic effects of acupuncture, interact mainly with histamine and adenosine receptors [20].

Interestingly, mast cell degranulation and activation of A1 adenosine receptors seem to play essential roles in the peripheral effects of acupuncture needling in animal models. A recent study demonstrated the antihyperalgesic effect of A1 agonist injection into an acupoint was completely blocked by a previous acupoint injection of cromolyn sodium (drug that inhibits mast cell degranulation) [20]. Moreover, using a microanalysis method, it was verified that cromolyn sodium acupoint injection prevented the enhanced adenosine levels at the acupoint region found after acupuncture needling. This experiment adds an important finding to the previous acupuncture literature, which suggests that the local increased adenosine levels at the acupoint region (related to acupuncture analgesia induced by A1R activation) is triggered (or dependent) on mast cell degranulation [20].

Regarding histamine receptors, several studies found that histamine or H1 agonist injected into acupoints can induce the antihyperalgesic effects of acupuncture [20, 21]. Moreover, the use of H1 antagonists inhibits the antihyperalgesic effects of manual acupuncture needling [21]. As H1 receptors are expressed in primary afferent neurons but not in mast cells, and the H1 receptor seems to be activated by local histamine produced by local mast cell degranulation and by the axonal reflex triggered by the acupuncture needling.

Nitric oxide neuromediator

Another neuromediator studied in the peripheral effect of acupuncture is nitric oxide (NO). The presence of this neuromediator has already been demonstrated at the site of acupuncture points in both clinical and experimental studies [31]. In this context, Ma (2003) [32] reported the highest concentration of NO in the skin of meridian regions and acupuncture points (detected by lower electrical resistance) compared to other areas of the body of rats. NO also appears to be related to an effect of greater norepinephrine synthesis in the regions of acupuncture points compared to other regions of the body [6], which has been shown to be correlated with the antihyperalgesic effect of acupuncture [28].

Endothelin receptor

Finally, it was observed in a recent study that peripheral endothelin receptors were associated with EA effects [35]. This study showed that EA-induced antihyperalgesia was abolished by intraplantar injection of an endothelin B receptor ($ET\text{-}_B$) antagonist into the paws of mice submitted to the chronic postischemia pain model [35]. Moreover, it was found that the peripheral $ET\text{-}_B$ expression, as measured by western blot, was increased after EA treatment. The antihyperalgesic effect of acupuncture found in their study could be related to increased endogenous opioid release triggered by $ET\text{-}_B$ receptors expressed in the keratinocytes at the injured site [35].

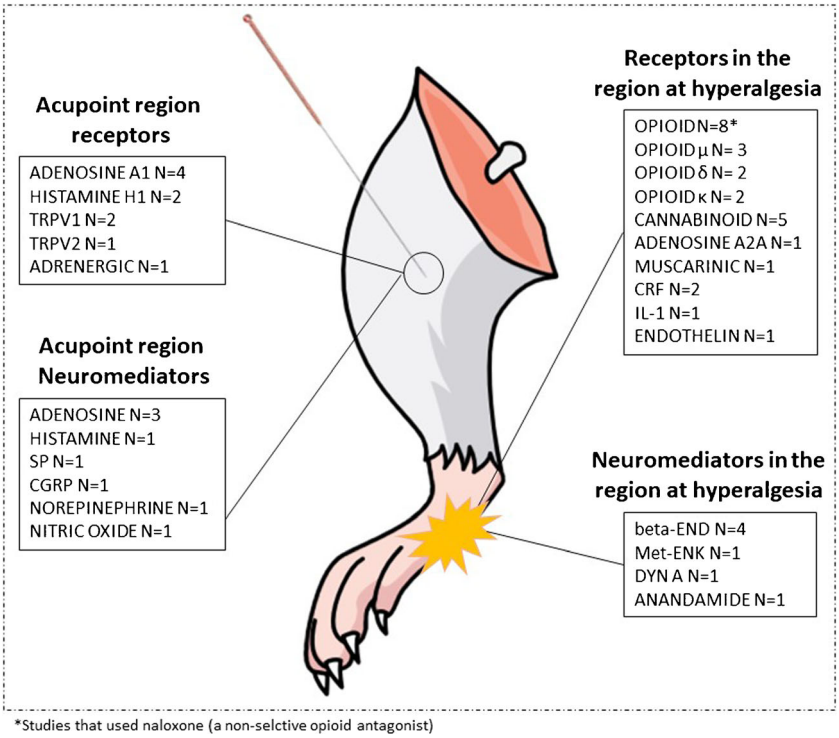

Fig. 3 Number of studies that investigated the role of peripheral receptors and neuromediators in the acupoint region and in the region of hyperalgesia

Additional considerations of acupuncture pain control mechanisms and study limitations

The study of the neurobiology of acupuncture in the past 50 years has demonstrated that acupuncture stimuli can potentiate pain control related to peripheral and central components of the nervous system [72, 73]. These effects are linked to a complex interaction of various endogenous mechanisms that also involve the modulation of immune system cells and other non-neural components [73]. In this context, the present review contributes to the current acupuncture literature by mapping and elaborating data from basic science studies that investigated peripheral receptors and neuromediators involved in acupuncture-induced antihyperalgesia. This can help basic science researchers to access state-of-the-art data from the current literature in terms of different methods and research findings regarding the investigation of the beneficial effects of acupuncture. In addition, the present review can support clinical acupuncture research findings from a mechanistic perspective and bring new insights to the design of future clinical studies with the scope of testing the peripheral effects of acupuncture.

Overall, it is evident that peripheral signaling mediated by several neuromediators and receptors are an essential part of pain control related to acupuncture needling. A relevant issue to address, which has been overlooked in past studies, is that the peripheral effects of acupuncture needling on neuromodulators and receptors are different when comparing the acupoint region and the injured somatic tissue region. This observation seems to be an obvious assumption; however, we did not find any previous articles that clearly addressed this issue. On the other hand, most studies generalized the findings and did not precisely discuss the rationale or implications of selecting different acupoint regions to investigate their effects on specific peripheral pain models.

The present study shows that, in the acupoint region, adenosine, histamine, TRPV and adrenergic receptors play key roles in the signaling of acupuncture needling. Moreover, adenosine, histamine, SP, CGRP, nitric oxide and norepinephrine were the neuromodulators released in the acupoint region that influenced acupuncture-induced antihyperalgesia (Fig. 3). Notably, most of the receptors and neuromodulators described are related to excitatory signaling to peripheral nervous system. Conversely, adenosine and adenosine A1 receptors in the acupoint region lead to local inhibitory signaling from nociceptive stimuli. Because of this dual role of opposing excitatory and inhibitory mechanisms triggering antihyperalgesic effects, some authors suggest that the term acupuncture stimulation should be renamed to “acupuncture blockade” [51].

Based on previous studies focused on the neurobiology of acupuncture [72, 73], it is likely that local excitatory neuromodulators/receptors are transducing the physical stimuli of acupuncture to the periphery to potentiate the modulation of spinal and supraspinal endogenous pain control mechanisms. Compiling the studies that found the inhibitory correlations [18, 19, 22, 59], this effect is restricted to the area that

received the acupuncture needling (no effects observed on contralateral needling). In this case, the local acupuncture effect probably inhibits the transmission of a nociceptive stimuli originating from a distal area in relation to the acupoint via activation of A1 receptors. It not clear in the current literature if A1 receptors are consistently present in the trunk (or axons) of peripheral nerves (A1 receptors are usually expressed in distal terminals of C fiber neurons); however, some authors have suggested that adenosine receptors can modulate the axonal excitability of unmyelinated C fibers [25]. Further studies need to be properly designed to better clarify this "dual role" of peripheral signaling at the acupoint region.

Moving forward in the analysis of neuromediators and receptors at the injured somatic tissue region, the present study shows that opioid, cannabinoid, adenosine, muscarinic, CRF, IL-1 and endothelin receptors are correlated with acupuncture-induced antihyperalgesia. The neuromediators found to interact with some of these receptors at the injured site are the following: beta-endorphin, meta-enkephalin, dynorphin and anandamide (Fig. 2). Most of these neuromodulators and receptors have been discussed in previous studies to integrate a list of several components involved in local endogenous mechanisms of pain control [71]. In this context, basic science acupuncture studies have demonstrated potentiation of these pain control mechanisms by enhancing the local release of neuromediators that inhibit nociceptive signaling [36]. The interaction of the abovementioned neuromediators with receptors that are present at the distal terminal of pain-transmitting neurons and in local immune cells can promote direct inhibition of transduction of pain at the distal end of sensory nerves or an indirect effect by reducing the local release of proinflammatory cytokines (peripheral desensitization) [71, 72]. It is not

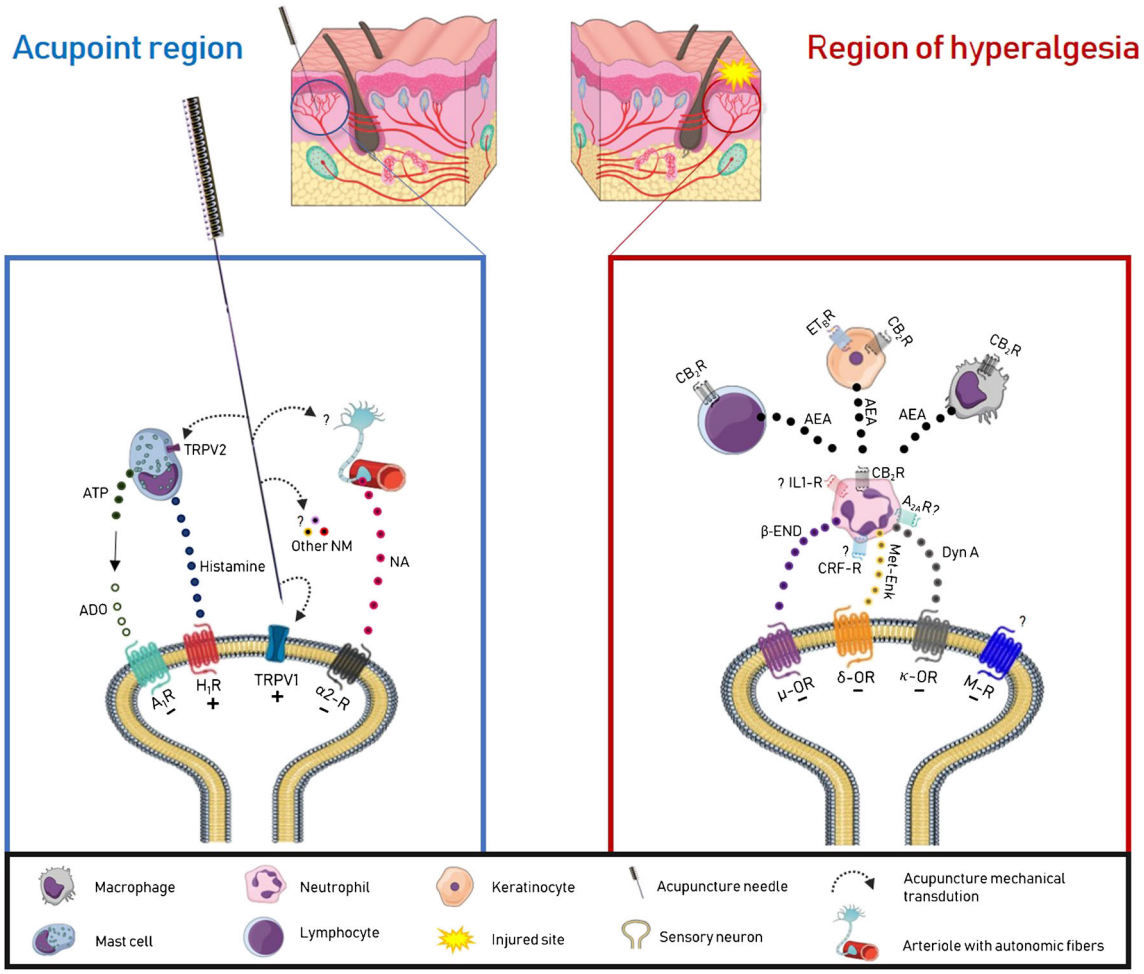

Fig. 4 Schematic illustration representing interactions related to the locally occurring phenomena triggered by the acupuncture stimulation in the acupoint region and in the region of hyperalgesia. (?) Mechanisms of signal transduction not clarified by the studies included in the review. TRVP2, transient receptor potential vanilloid type 2; ATP, adenosine triphosphate; ADO, adenosine, A1R, adenosine A1 receptor; H1R, histamine H1 receptor; TRPV1, transient receptor potential vanilloid type 1; NA; noradrenaline; α2-R alpha2-adrenergic receptor; CB2R, cannabinoid receptor type 2; ETBR, endothelin receptor type B; AEA, anandamide; IL1-R, interleukin-1 receptor; CRF-R, corticotropin-releasing hormone receptor; A2AR, adenosine A2 receptor; β-END, beta-endorphin; Met-Enk, met-enkephalin; Dyn A, dynorphin A; μ-OR, mu-opioid receptor; δ-OR, delta opioid receptor, κ-OR, kappa opioid receptor, M-R, muscarinic receptor. Other NM (neuromediators) includes: nitric oxide, substance P and calcitonin gene-related peptide. + excitatory signaling. – inhibitory signaling

clear how acupuncture needling can enhance the presence of neuromediators at the local injured tissue. We can hypothesize that these effects are related to antidromic nerve signaling induced by acupuncture needling and/or spinal/supraspinal mechanisms linked to crosstalk between the immune and nervous systems. For further clarification, these hypotheses need to be tested in future studies. A visual representation of the possible interactions of different components related to the locally occurring phenomena triggered by acupuncture stimulation is illustrated in Fig. 4.

Another interesting topic uncovered by this review is that the investigation of peripheral receptors/neuromediators can give unanticipated insights into the development of new pain relief drugs. Peripheral administration of low doses of drugs can potentiate (with fewer side effects than systemic drugs) the peripheral mechanisms already mapped by acupuncture researchers. Moreover, a better understanding of the peripheral effects of acupuncture can help to support the rationale of studies that address the association of medications and acupuncture to increase the duration of analgesia or to analyze the potential exogenous substances that can inhibit the effects of acupuncture, such as blocking A1R receptors after caffeine ingestion, already demonstrated in some studies [18, 22, 39, 77].

Our study has, however, some limitations related to the selected eligibility criteria. Only articles published in the English language and retrieved from three well-recognized scientific databases were selected. This limited the search to publications in the mainstream scientific literature accessed in Western countries. Future studies should expand the search to East Asian databases and other languages to include possible new studies and findings in the context of the peripheral effects of acupuncture. Another limitation was the selection of studies conducted only involving mice or rats. Although mice and rats are the principal animals used in basic science, other animals, such as rabbits, guinea pigs and ferrets, are also used in experimental research. This could possibly limit the identification of additional data. Furthermore, all 29 studies included in the review have their own limitations, especially in regard to the following: (1) nondetailed description of the acupuncture treatment procedures; (2) no report if the experiment evaluators were blinded during outcome measurement; and (3) lack of use of a standardized format to report the research, such as the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Conclusion

The peripheral antihyperalgesic effects of acupuncture are correlated with ten families of receptors, including the following: opioid, adenosine, cannabinoid, TRPV, histamine, adrenergic, muscarinic, CRF, IL-1, and endothelin receptors. Eight types of neuromediators are also correlated with these peripheral effects of acupuncture: opioid peptides, adenosine, histamine, SP, CGRP, anandamide, nitric oxide and norepinephrine.

The described peripheral effects of acupuncture involve pharmacological approaches of injecting specific receptor agonist or antagonist drugs into the acupuncture point or at the peripheral injured tissue. Neuromediators were injected into the acupoint region to test if this could mimic the antihyperalgesic effects of acupuncture and, using HLPC technique, increased levels of some neuromediators were found at the acupoint region or at the peripheral injured tissue after acupuncture treatment. Several histological and immunological methods as well as multiple pain induction models and acupoint stimulus parameters were utilized in the study of the peripheral effects of acupuncture.

Considering the potential benefits of a better understanding of the role peripheral receptors and neuromediators in the context of pain management, the findings of the present study highlight the importance of more in depth future studies exploring the peripheral mechanisms of acupuncture.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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