REVIEW

The role of purinergic signaling in acupuncture-mediated relief of neuropathic and inflammatory pain

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Abstract

Acupuncture is a traditional medicinal practice in China that has been increasingly recognized in other countries in recent decades. Notably, several reports have demonstrated that acupuncture can effectively aid in pain management. However, the analgesic mechanisms through which acupuncture provides such benefits remain poorly understood. Purinergic signaling, which is mediated by purine nucleotides and purinergic receptors, has been proposed to play a central role in acupuncture analgesia. On the one hand, acupuncture affects the transmission of nociception by increasing adenosine triphosphate dephosphorylation and thereby decreasing downstream P2X3, P2X4, and P2X7 receptors signaling activity, regulating the levels of inflammatory factors, neurotrophic factors, and synapsin I. On the other hand, acupuncture exerts analgesic effects by promoting the production of adenosine, enhancing the expression of downstream adenosine A1 and A2A receptors, and regulating downstream inflammatory factors or synaptic plasticity. Together, this systematic overview of the field provides a sound, evidence-based foundation for future research focused on the application of acupuncture as a means of relieving pain.

Keywords Acupuncture · Analgesia · Inflammatory pain · Neuropathic pain · Purinergic signaling

Abbreviations

EA	Electroacupuncture
Glu	Glutamate
GABA	γ-Aminobutyric acid
SP	Substance P
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ADO	Adenosine
R	Receptor
RA	Rheumatoid arthritis
eATP	Extracellular adenosine triphosphate
NF-κB	Nuclear transcription factor-κB
CCI	Chronic constriction injury
SNI	Spared nerve injury
SNL	Spinal nerve ligation
IBD	Inflammatory bowel disease
TNBS	2,4,6-Trinitrobenzene sulfonic acid
L	Lumbar
CIA	Collagen-induced arthritis
CFA	Complete Freund's adjuvant
MDA	Malondialdehyde
CRP	C-reactive protein
GPx	Glutathione peroxidase
SOD	Superoxide dismutase
GR	Glutathione reductase
AP2M	Adaptor protein-2 complex
CXCR3	CXC chemokine receptor 3
PAP	Prostatic acid phosphatase

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CGRP	Calcitonin gene-related peptide
α,β-meATP	α, β-methylene ATP
STZ	Streptozotocin
DNP	Diabetic neuropathic pain
MAPK	Mitogen-activated protein kinase
TRPV	Transient receptor potential vanilloid
NTPDases	Nucleoside triphosphate diphosphohydrolases
NK-1R	Neurokinin-1 receptor
MCs	Mast cells
CD73	Ecto-5′-nucleotidase
AV-shA1RNA	Short interfering RNA
p	Phosphorylation
cAMP	Cyclic adenosine monophosphate
CREB	Cyclic adenosine monophosphate response element protein
ADA	Adenosine deaminase
IFN-γ	Interferon-γ
IL	Interleukin
TNF-α	Tumor necrosis factor-α
FKN	Fractalkine
CX3CR1	FKN receptor
BDNF	Brain-derived neurotrophic factor
NTF3	Neurotrophic-3
RANKL	NF-κB ligand
PKA	Protein kinase A
PKC	Protein kinase C
PMA	Phorbol 12-myristate 13-acetic acid
ERK	Extracellular regulated protein kinases
SCDH	Spinal cord dorsal horn
DRG	Dorsal root ganglion
HPLC	High-performance liquid chromatography

Introduction

Acupuncture is a form of traditional Chinese medicine first detailed in the "Zhenjiu Jiayi Jing" that has been practiced for over 2500 years. Acupuncture has been recognized as a treatment modality by 113 countries to date, with the World Health Organization listing 43 different diseases and other conditions amenable to acupuncture-mediated treatment, including pain, addiction, nausea and vomiting, bronchitis, asthma, and stroke rehabilitation [1]. Traditional acupuncture consists of the insertion and manual manipulation of needles applied to specific acupoints, whereas electroacupuncture (EA) entails the electrical stimulation of these needles following their insertion [2]. Countless studies have documented improvements in a range of conditions including pain, insomnia, constipation, inflammation, depression, hypertension, and various neurological disorders following acupuncture treatment [3–10]. The ability of acupuncture to treat hyperalgesia has been shown to be associated with

a range of neurotrophic factors, immune factors, dopamine, acetylcholine, glutamate (Glu), γ-aminobutyric acid (GABA), substance P (SP), and cyclic adenosine monophosphate (cAMP) binding elements [11]. Notably, purinergic signaling has been shown to play a role in acupuncture-mediated pain relief [12–24].

In 1971, Burnstock et al. published a report defining purinergic nerves as those that primarily release purine nucleotide neurotransmitters such as adenosine triphosphate (ATP) [25]. The purinergic signaling pathway is shaped by interactions between these purine nucleotides, which include adenine and guanine nucleotides [26–28] and their cognate receptors. Intracellular ATP concentrations generally exceed those of extracellular ATP (eATP). A range of stressors such as mechanical stretching, ischemia, hypoxia, and other stimuli can induce the extracellular release of intracellular ATP stores from normal cells and nerve endings, thus yielding high local concentrations of these purine nucleotides that can activate specific membrane receptors [28], thereby exerting their downstream physiological functions.

Purine nucleotides can produce a range of effects through the activation of specific purinergic receptors, which are broadly classified into the P1 and P2 receptors families (P1Rs and P2Rs) [29–31] based on their pharmacological and biochemical properties [32]. Alternatively, these purinergic receptors are grouped based on the utilized conduction pathway into those that are G protein-coupled receptors (P1Rs and P2YRs) and those that are ligand-gated ion channel receptors (P2XRs) [30]. The metabotropic P1Rs are highly sensitive to adenosine (ADO)-induced activation [30, 32] and include the A1R, A2AR, A2BR, and A3R receptor subtypes. P2Rs consist of both P2XRs and P2YRs [32, 33], the former of which is a family of ATP-gated ion channels (P2X1-7) permeable to cations including Na^+, K^+, and Ca^{2+}. In contrast, the G protein-coupled P2YRs (P2Y1, 2, 4, 6, 11-14) are metabotropic receptors [29, 32, 33].

Purinergic signaling has been influence both neurodevelopmental [34–36] and pathophysiological activity [37], influencing the incidence of psychiatric illnesses [35] and neurological disorders [38–42], while also regulating processes such as immune responses [43–45], inflammation [23, 41, 42, 46], cellular proliferation [47], and differentiation [48]. A report published in 1987 highlighted a potential role for ADO receptors in crude fiber-mediated vibration analgesia [49]. In 1994, Liu et al. demonstrated that the EA-based stimulation of the GB34 (Yanglingquan) and GB39 (Xuanzhong) acupoints results in the prolongation of nociceptive hind limb withdrawal reflex latency, whereas intraperitoneally administered caffeine and theophylline, which function as ADO receptor antagonists, was sufficient to ablate this EA-mediated increase in the nociceptive threshold. Low-intensity EA (1–1.5 mA, 5 Hz) can reportedly cause segmental analgesia, and purinergic signaling may play a

role in this process [50]. More recently, Goldman et al. utilized acupuncture needles that were slowly rotated manually to stimulate the ST36 (Zusanli) acupoint, resulting in the sustained release of high levels of ADO and adenine nucleotides (ATP, adenosine diphosphate (ADP), and AMP), again suggesting a link between peripheral ADO receptor activation and the ability of acupuncture to relieve pain [51]. Building on these results, a growing number of studies have focused on exploring the mechanistic links between purinergic signaling and acupuncture-mediated analgesia [52].

This review has been constructed to primarily focus on changes in purinergic signaling and particular receptor subtypes in the context of the acupuncture-mediated treatment of neuropathic and inflammatory pain, with detailed discussions of what is known regarding the mechanisms underlying these processes. In so doing, we hope to provide a strong scientific foundation for researchers interested in further studies of the mechanisms that mediate acupuncture-associated analgesia.

Review methods

We searched the PubMed database for published studies from March 2012 to May 2022. The key words included ("acupuncture" or "electroacupuncture"), ("neuropathic pain" or "inflammatory pain" or "pain"), and ("purine" or "purinergic signaling" or "adenosine" or "ATP" or "ADP" or "AMP" or "A1" or "A2A" or "A2B" or "A3" or "P2"). Information on the purinergic signaling of acupuncture for neuropathic and inflammatory pain was obtained from the obtained information. The flowchart search process is shown in Fig. 1.

The inclusion criteria were studies: (1) published in English; (2) analgesic mechanism of acupuncture; (3) acupuncture relieves neuropathic pain; (4) acupuncture relieves inflammatory pain; (5) purinergic signaling. Exclusion criteria were (6) conference abstracts, randomized controlled trial, reviews, and meta-analysis studies. Forty-four papers reporting on the role of purinergic signaling in acupuncture-mediated relief of neuropathic and inflammatory pain were reviewed.

Neuropathic pain

The International Association for the Study of Pain recognized neuropathic pain as a form of pain arising as a result of diseases or lesions impacting the somatosensory system [53], including pain hypersensitivity, hyperalgesia, and spontaneous pain. Neuropathic pain is often long-lived and can have a serious negative impact on the physical and psychological well-being of affected individuals [54]. While
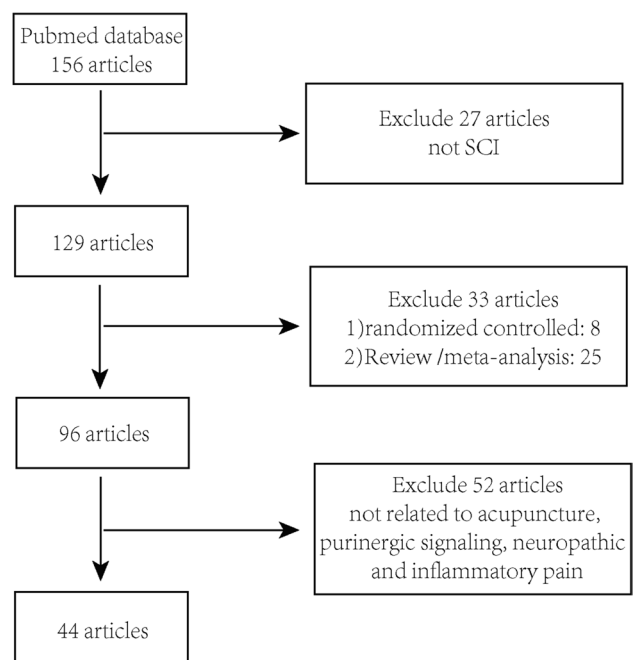

Fig. 1 Flow chart of the search strategy and process

various strategies for the treatment of neuropathic pain have been developed and tested to date, they are generally associated with unsatisfactory efficacy or significant side effects that can be highly disruptive [55]. However, acupuncture provides a means of effectively alleviating this intractable form of pain while minimizing the risk of any adverse reactions [56]. Many animal models have been employed to study neuropathic pain and to evaluate the role of purinergic signaling in the context of acupuncture-mediated analgesia, such as the chronic constrictive injury (CCI), spinal nerve ligation (SNL), spared nerve injury (SNI), diabetic neuropathy pain (DNP), and neck-incision models. Results from these in vivo studies, which have primarily been conducted at the level of the spinal cord, have offered valuable insights that are discussed in greater detail below (Table 1).

Chronic constriction injury

In the CCI model, neuropathic pain is induced via the loose unilateral ligation of the sciatic nerve [80]. Several studies have successfully leveraged the CCI model to provide evidence in support of the importance of purinergic signaling in the acupuncture-mediated alleviation of neuropathic pain. Which, P2X3R, P2X4R, P2X7R, ADO, and A1R play an important role among them.

The study suggested that the application of EA to the GB30 (Huantiao) acupoint was sufficient to significantly reverse the CCI-induced increases in dorsal root ganglion (DRG) eATP levels and adaptor protein-2 complex (AP2M)

Table 1 Purinergic signaling participate in acupuncture attenuation of neuropathic pain

Neuropathic pain models	Subjects	Acupoints	Intervention methods	Parameters used for acupuncture	Mechanisms	Test sites	References
CCI	Mice	GB30	EA	2/100 Hz at 2 mA for 30 min	Spinal dorsal horn: eATP↓, microgliosis↓; DRGs: eATP↓, PAP protein↑, satellite glial cells activation↓, AP2M↓; PAP deficiency affects EA effect	Spinal dorsal horn, DRGs	[57]
CCI	Rats	ST36 GB34	EA	2/100 Hz at 2 mA for 30 min	ATP-activated currents↓, P2X3R↓	DRGs	[58]
CCI	Rats	Jiaji (EX-B2)	EA	2/15 Hz at 1 mA for 20 min	P2X4R↓, OX-42↓	Lumbar spinal cord	[59]
CCI	Rats	GB30	EA	2 Hz at 2 mA for 30 min	P2X4R↓, OX-42↓, IFN-γ↓	Spinal cord	[13]
CCI	Rats	GB30	EA	2 Hz at 2 mA for 30 min	P2X7R↓, IL-1β↓, IL-18↓	Spinal cord	[22]
CCI	Rats	ST36 GB30	EA	2/100 Hz at 2 mA for 30 min	P2X3R↓	Spinal cord, DRGs	[60]
CCI	Rats	ST36 GB34	EA	2 or 15 Hz at 1.5 mA for 30 min	P2X3R↓, pERK1/2↓	Spinal cord	[61]
CCI	Rats	ST36 GB34	EA	2 /100 Hz at 2 mA for 30 min	P2X3R↓, ATP-activated currents↓	DRGs	[62]
CCI	Rats	ST36	EA	2 /100 Hz at 1 mA for 30 min	ADO↑, A1R↑	Spinal cord	[63]
CCI	Rats	ST36	EA	2 /100 Hz at 1 mA for 30 min	ADO↑, CD73↑; CD73 inhibitor affects EA analgesia effect and ADO levels	Spinal cord	[64]
CCI	Rats	ST36 LR3	EA	2/15 Hz at 1 mA for 30 min	astrocyte activation↓, IL-1β↓, IL-6↓, TNF-α↓; A1R antagonist affects EA effect	Spinal cord	[65]
CCI	Rats	ST36 LR3	EA	2/100 Hz at 1 mA for 30 min	A1R↑	Spinal cord	[66]
CCI	Rats	Hua Tuo Jia Ji acupoints	EA	2 or 15 Hz at 2 mA for 15 min	A1R-	SCDH	[67]
SNL	Rats	ST36 BL60	EA	2 Hz at 0.5–1.0–1.5 mA, each lasting for 10 min	P2X3R↓, P2X3R agonist affects EA effect	DRGs	[68]
SNL	Rats	ST36 BL60	EA	2/100 Hz at 1.5 mA for 30 min	P2X4R↓, microglia activation↓, spontaneous excitatory postsynaptic currents↓	Spinal cord	[69]
SNL	Rats	ST36 BL60	EA	2 Hz at 1.5 mA for 30 min	P2X7R↓, IL-1β↓, IL-6↓, TNF-α↓, IL-10↑, Iba1↓, synaptotagmin I↓, BDNF↓, p-p38↓; P2X7R agonist affects EA effect	Spinal cord	[54]
SNL	Rats	ST36 BL60	EA	2/100 Hz at 1.5 mA for 30 min	P2X4R↓, BDNF↓, Iba1↓, GABAAγ2↑, excitatory post-synaptic potential↓,	Spinal cord	[24]

Table 1 (continued)

Neuropathic pain models	Subjects	Acupoints	Intervention methods	Parameters used for acupuncture	Mechanisms	Test sites	References
SNL	Rats	ST36 BL60	EA	2 Hz at 1.5 mA for 30 min	A2AR↑, cAMP↑, PKA↑, p-CREB↑, NTF3↓, NTF4↓, synaptophysin↓, synapsin I↓; A2AR antagonist affects EA effect	Spinal cord	[70]
SNL	Rats	ST36 GB30	EA	≤1 mA, 2 Hz for 30 min	P2X4R-, BDNF↑	Spinal cord	[71]
SNI	Rats	ST36 BL60	EA	2 or 100 Hz at 0.5–1.5–2 mA for 30 min	P2X3R↓	DRGs	[72]
SNI	Rats	ST36 BL60	EA	2 Hz at 1–2 mA for 30 min	P2X3R↓, TRPV1↓	DRGs	[73]
DNP	Rats	ST36 BL60	EA	2 Hz at 1 mA	P2X3R↓; P2X3R agonist affects EA effect	DRGs	[74]
DNP	Rats	ST36 BL60	EA	2 or 100 Hz at 1–2 mA, each lasting for 15 min	P2X3R↓, CGRP↓	DRGs	[75]
DNP	Rats	ST36 BL60	EA	2 Hz at 1–2 mA, each lasting for 15 min	P2X3R↓; P2X3R agonist affects EA effect, PKC activator PMA affects EA effect and ascend P2X3R	DRGs plasma membrane	[76]
DNP	Rats	ST36 BL60	EA	2 Hz at 1 mA for 30 min	P2X4R↓, P2X7R↓	DRGs	[77]
DNP	Rats	BL13 BL20 BL23	Manual acupuncture	20 min	spinal cord: P2X4R↓, OX-42↓; serum: CXCR3↓, IL-1β↓, IL-6↓, TNF-α↓, GSP↓, TG↓, TC↓, LDL-C↓, HDL-C↑	Spinal cord, serum	[78]
Neck-incision	Rats	LI18, LI4-PC6, ST36-GB34	EA	2/100 Hz at 1 mA for 30 min	ATP↓, P2X3R↓, P2X4R↓, P2X7R↓, P2Y12R↓, sFKN↓, CX3CR1↓	Cervical spinal cord	[79]

CCI, chronic constriction injury; *EA*, electroacupuncture; *eATP*, extracellular adenosine triphosphate; *R*, receptor; *DRG*, dorsal root ganglion; *PAP*, prostatic acid phosphatase; *p*, phosphorylation; *AP2M*, adaptor protein-2 complex; *OX-42*, microglia activation marker; *IFN-γ*, interferon-γ; *IL-1β*, interleukin-1β; *IL-18*, interleukin-18; *ERK*, extracellular regulated protein kinases; *ADO*, adenosine; *CD73*, ecto-5′-nucleotidase; *IL-6*, interleukin-6; *TNF-α*, tumor necrosis factor-α; *A1R*, adenosine 1 receptor; *SCDH*, spinal cord dorsal horn; *SNL*, spinal nerve ligation; *IL-10*, interleukin-10; *Iba1*, ionized calcium-binding adapter molecule 1; *BDNF*, brain-derived neurotrophic factor; *p-p38*, phosphorylation of p38; *GABA*, γ-aminobutyric acid; *cAMP*, cyclic adenosine monophosphate; *PKA*, protein kinase A; *CREB*, cyclic adenosine monophosphate response element protein; *NTF3/4*, neurotrophic-3/4; *SNI*, spared nerve injury; *TRPV1*, transient receptor potential vanilloid 1; *CGRP*, calcitonin gene-related peptide; *PKC*, protein kinase C; *PMA*, phorbol 12-myristate 13-acetic acid; *DNP*, diabetic neuropathic pain; *CXCR3*, CXC chemokine receptor 3; *GSP*, glycosylated serum protein; *TG*, triglyceride; *TC*, total cholesterol; *LDL-C*, low-density lipoprotein-cholesterol; *HDL-C*, high-density lipoprotein-cholesterol; *sFKN*, soluble secreted form of fractalkine; *CX3CR1*, FKN receptor

phosphorylation (p) and decreases in DRG prostatic acid phosphatase (PAP) levels. Besides, PAP can catabolize eATP, phosphorylated AP2M can drive the degradation of PAP, and EA can disrupt interactions between PAP and activated AP2M. Notably, reduced PAP expression in the L4-L5(lumbar 4-lumbar 5) DRGs can suppress the therapeutic benefits of EA in this experimental system as a consequence of ascended eATP levels and reduced microgliosis [57]. Moreover, the application of EA to the ST36 and GB34 acupoints can also significantly reduce ATP-activated currents of DRG in CCI model rats [58].

Reported ATP plays a P2XRs-dependent role in the acupuncture-mediated alleviation of neuralgia. Strikingly, EA at the "Jiaji" in CCI model rats was sufficient to increase thermal pain thresholds while lowering spinal cord P2X4R protein levels [59]. In vivo, another research team determined that EA at GB30 was associated with a drop in the excessive levels of interferon-γ (IFN-γ) in the spinal cord, while also noting that P2X4R expression was primarily restricted to microglia in this tissue compartment. In vitro, these authors further determined that IFN-γ signaling can mediate the activation of microglia [13]. Under basal conditions, microglia remain in a relatively quiescent state. However, exposure to pro-inflammatory IFN-γ derived from neurons, glial cells, and infiltrative monocytes can activate these cells [81], and knocking out IFN-γ consequently suppresses microglial activation in the spinal cord while simultaneously relieving tactile abnormalities. Conversely, rats that are intrathecally injected with IFN-γ experienced pronounced tactile pain with concomitant spinal cord P2X4R upregulation. Although such stimulation did not cause pronounced tactile pain in P2X4R knockout rats, the activation of microglia did not decrease [82]. Indeed, EA fails to exert analgesic efficacy or to affect P2X4R-expressing microglia in naïve control rats following intrathecal injection of IFN-γ administration [13]. The ability of EA to reduce P2X4R protein levels expressed by microglia may thus be mediated in part by reductions in IFN-γ expression.

P2X7R is primarily expressed by microglia, and EA has been shown to suppress the activation of microglial P2X7R in the spinal cord of CCI model rats, with a corresponding drop in local interleukin (IL)-18 and IL-1β levels as well as a rise in paw withdrawal threshold and latency values for these animals. Administering A-438079, which is an antagonist of P2X7R, was sufficient to reduce IL-1β expression in these rats, although it failed to impact IL-18 levels [22]. EA-mediated reductions in inflammatory IL-1β production may thus be mediated by the inhibition of microglial P2X7R signaling.

In CCI model rats, EA at ST36 and GB34 has further been shown to suppress P2X3R expression and extracellular regulated protein kinases 1/2 (ERK1/2) phosphorylation in the spinal cord, with the corresponding relief of thermal

and mechanical hyperalgesia in these animals [60, 61]. Conversely, the P2X3R agonists ATP or α,β-meATP increased such hyperalgesia and P2X3R expression, and these levels were lower in response to EA [62]. Intrathecally administering U0126 (a selective ERK1/2 inhibitor) to inhibit ERK1/2 activation also resulted in a significant reduction in spinal P2X3R protein levels in CCI model rats [61], suggesting that EA-mediated reductions in P2X3R expression are at least partially dependent on the phosphorylation of ERK1/2. Moreover, EA treatment can reduce mechanical and thermal hyperalgesia of CCI model rats via inhibit ATP-activated currents and P2X3R expression in DRG [58].

A1R, which is primarily activated by the ATP metabolite ADO, is also associated with the acupuncture-mediated treatment of CCI-induced neuralgia. EA at ST36 can reportedly drive a significant increase in ADO levels and A1R expression in the spinal cord in a rat CCI model system, while the shRNA-mediated knockdown of A1R was sufficient to reverse the analgesic benefits of EA in these CCI model rats without any corresponding changes in sham controls [63]. Moreover, EA has been demonstrated to induce spinal cord ecto-5′-nucleotidase (CD73) upregulation in CCI model rats. Strikingly, the application of APOCP, which is a CD73 inhibitor, was sufficient to suppress EA-associated analgesia and elevated ADO levels in the spinal cord, whereas the A1R agonist CCPA restored the analgesic benefits of EA in these animals [64]. The enzymatic activity of CD73 can lead to the dephosphorylation of AMP, yielding ADO. EA at the ST36 and LR3 (Taichong) acupoints can inhibit satellite glial cell activity and reduce the release of cytokines such as IL-6, IL-1β, and tumor necrosis factor-α (TNF-α), thereby relieving pain. The intrathecal injection of DPCPX, which is an A1R antagonist, overcame EA-associated analgesia and reductions in TNF-α levels [65]. It has been reported that A1R can suppress the release of inflammatory mediators and neurotransmitters including SP and Glu from primary afferent neurons, thus facilitating the presynaptic inhibition of primary afferent neurotransmitters on dorsal horn neurons [83]. Generally speaking, EA appears to drive an increase in ADO concentrations as a result of higher CD73 levels, activating A1R and suppressing the production of inflammatory cytokines such as TNF-α.

Spinal nerve ligation

The SNL model system, which is generally induced by the transection or ligation of the lateral L5 spinal nerve, has been thoroughly characterized to date, revealing a key role for both direct nerve damage and associated neuroinflammatory activity in the incidence of neuropathic pain [84]. Purinergic signaling has been shown to play an important role in SNL-induced neuropathic pain [85–87]. Recent studies have shown that acupuncture can relieve SNL-induced

neuropathic pain by inhibiting the expression of P2X4R and P2X7R in the spinal cord and promoting the expression of A2AR in the spinal cord.

For example, EA at ST36 and BL60 (Kunlun) in SNL model rats can reportedly suppress P2X4R expression at the mRNA level while also reducing the protein levels of P2X4R and P2X7R in the spinal cord, in addition to decreasing the numbers of P2X4R positive microglial [54, 68, 69]. Liang Y et al. demonstrated that EA at these two acupoints was associated with a drop in P2X3R levels not in the injured L5 DRG, but in the healthy L4 DRG, suggesting that EA may primarily preserve the integrity of otherwise intact neurons without affecting those subjected to direct damage [68].

Further research has suggested that the analgesic benefits of EA in SNL model rats may be linked to the inhibition of P2X4R-driven microglial activation and a reduction in substantia gelatinosa neuron excitability [69]. When animals lack P2X4R expression, this reportedly reduces their sensitivity to pain [88]. Treatment with 5-BDBD (a specific P2X4R inhibitor) to inhibit P2X4R can also reportedly suppress brain-derived neurotrophic factor (BDNF) expression while promoting GABAAγ2 upregulation. EA can also reportedly suppress the excitatory postsynaptic potential [24]. P2X4R-mediated microglial activation has also been observed in the context of peripheral nerve injuries, whereas increases in microglial activity can conversely promote P2X4R upregulation [89]. As an ATP-gated cation channel, P2X4R remains inactive under basal conditions. In response to cellular injury, however, the high levels of released eATP induce P2X4R activation and the consequent intake of Na^+ and Ca^{2+} into cells together with K^+ efflux, activating a range of signaling pathways that can induce injury-related responses [90]. EA can thus suppress the efficiency of trans-synaptic excitatory transmission in the context of neuropathy via the inhibition of microglial P2X4R expression, thereby preventing an influx of Na^+ and Ca^{2+} as well as the efflux of K^+, while also suppressing BDNF expression and inducing the upregulation of GABAA γ2.

EA at ST36 and BL60 suppress inflammation in SNL model rats and reduce the severity of hyperalgesia, with decreases in serum IL-1β, IL-6, and TNF-α concentrations, increased serum IL-10 levels, and lower spinal cord levels of the microglial activation marker Iba1, synaptotagmin I, BDNF, and p-p38 levels. These changes coincide with an EA-related decrease in dendritic spine density as compared to model controls, together with fewer synaptic vesicles or synapses exhibiting an abnormally large synaptic space and fewer synaptic vesicles. Injecting these animals with the P2X7R agonist BzATP can attenuate the analgesic effects and inhibit or promote effects for these factors of EA in this model system [54]. When the p-p38 is inhibited using SB203580, this suppresses the release of inflammatory cytokines including IL-1β, IL-6, and TNF-α [91]. The analgesic benefits of EA appear to thus be at least partially attributable to a reduction in P2X7R expression, thereby decreasing p38 activation and suppressing inflammatory cytokine release. Mechanistically, these inflammatory cytokines are key drivers of neuropathic pain, with TNF-α, for example, increasing membrane conductivity and thereby contributing to excessive neuron excitability and hyperalgesia [92]. There is also evidence indicating that low-frequency (2 Hz) EA can exert antinociceptive efficacy in SNL model rats via a spinal microglial IL-10/β-endorphin/μ-opioid receptor pathway [93].

A2AR is an important mediator of the acupuncture-mediated relief of SNL-induced hyperalgesia. EA at ST36 and BL60 promotes the upregulation of A2AR, cAMP, protein kinase A (PKA), and phosphorylation of cyclic adenosine monophosphate response element protein (p-CREB) levels in the spinal cord horn. This coincides with the suppression of neurotrophic factor 3 (NTF3), NTF4, synaptophysin, and synapsin I expression in the spinal cord horn, contributing to the normalization of tissue morphology and the mitigation of synaptic alterations for injured neurons. The injection of the A2AR antagonist SCH58261 can reverse these analgesic and inhibitory benefits of EA treatment [70], while intrathecally injecting the A2AR agonist CGS21680 promotes enhanced IL-10 secretion and the alleviation of neuropathy that can be abrogated via IL-10 antibody treatment [94]. The analgesic effects of A2AR are thus mediated at least in part by IL-10. Adenylate cyclase can also be activated by high ADO concentrations, promoting the production of the multifunctional secondary messenger cAMP [95], in turn activating PKA [70]. These findings suggest a role for EA in the activation of cAMP/PKA signaling, providing a mechanism whereby it modulates inflammatory mediator secretion and mediates neuronal plasticity through the upregulation of A2AR.

Spared nerve injury

In the SNI model of peripheral nerve damage, animals generally undergo the transection or ligation of the common peroneal and tibial nerves while leaving the sural nerve intact, or the transection or ligation of the common peroneal and sural nerves while leaving the tibial nerve intact [96]. A few studies have suggested a role for P2X3R-mediated signaling in this model of neuropathic pain. At 3 days post-surgery, SNI model rats exhibited persistent mechanical allodynia with a significant increase in P2X3R expression and transient receptor potential vanilloid 1 (TRPV1)-positive cells in L4/L5 DRGs [97]. EA at the bilateral ST36 and BL60 acupoints can suppress P2X3R expression on DRG neurons [72], while also reducing the upregulation of TRPV1 on DRG neurons, thus suppressing P2X3R and TRPV1 agonist-induced hyperalgesia [73]. TRPV1 and P2X3R activation promotes a Ca^{2+} influx in the peripheral nervous system

[97]. Peripheral TRPV1 agonist delivery in SNI model rats can also improve the algogenic effects of P2X3R, whereas inhibiting peripheral TRPV1 has no corresponding impact on P2X3R-related algogenic activity [98]. Overall, TRPV1 appears to function in the P2X3R signaling pathway.

Diabetic neuropathic pain

DNP is a highly prevalent and debilitating diabetic complication that affects the quality of life of many diabetic individuals [99]. A streptozotocin (STZ)-induced animal model of diabetes is often used when studying DNP in vivo. Following the injection of STZ, P2X3R upregulation has been reported, and modulating the expression of this purinergic receptor can affect the severity of DNP [100]. However, further research is warranted to clarify the specific role that P2X3R plays in the EA-mediated alleviation of DNP.

In one study, EA at ST36 and BL60 (2 Hz) was associated with the downregulation of P2X3R on L4-L6 DRG neurons [74, 75], with a corresponding decrease in protein kinase C (PKC) phosphorylation [76]. The ability of EA to relieve hypersensitivity has also been shown to be inhibited and enhanced by the P2X3R agonist α, β-me ATP, and the P2X3R antagonist A317491, respectively [74]. The STZ-induced model of diabetes has been shown to promote P2X3R localization to the surface of DRG neurons, thereby enhancing P2X3R-facilitated fast inactivation ATP currents and contributing to the peripheral neuropathy that these model animals ultimately develop [101]. The intraperitoneal injection of phorbol 12-myristate 13-acetate (PMA) to activate PKC can limit the analgesic benefits of EA while increasing P2X3R expression on the plasma membrane of DRG cells [76]. Therefore, the analgesic effect of EA may be achieved by reducing the level of p-PKC, thereby inhibiting the expression of P2X3R. EA at ST36 and BL60 (2 Hz) can also result in a drop in P2X4R and P2X7R expression levels [77]. In a separate study, manual acupuncture was found to decrease spinal cord P2X4R expression while also modulating serum levels of inflammatory mediators including CXC chemokine receptor 3 (CXCR3), TNF-α, IL-6β, IL-2 and influencing lipid metabolism, as evidenced by changes in triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) concentrations [78].

Neck-incision pain

The neck-incision model of neuropathic pain is established by generating a longitudinal incision (~1.5 cm) along the neck midline, after which the bilateral sternal muscles proximal to the thyroid are subjected to repeated blunt stimulation for approximately 30 min using forceps [102]. Studies have demonstrated that acupuncture can relieve neck-incision-associated neuropathy. While a single round of EA at the bilateral LI18 (Futu) or LI4 (Hegu)-PC6 (Neiguan) acupoints can reportedly contribute to the significant augmentation of ATP levels and an increase in P2X7R expression in the upper (C2-C5) spinal cord without any concomitant pain relief, two EA sessions were sufficient to normalize P2X7R, P2X3R, P2X4R, and P2Y12R expression while suppressing the associated hyperalgesia in model animals. ATP levels were decreased following two EA sessions but increased to normal levels after three sessions. EA was also sufficient to suppress neck incision-associated fractalkine (FKN) cleavage while upregulating the FKN receptor CX3CR1 and the phosphorylation of p38 MAPK (mitogen-activated protein kinase). The P2X7R antagonist A-740003 ablated the analgesic benefits of EA [79]. A previous study reported that consistent with evidence that interactions between FKN and CX3CR1 in microglia are mediated by P2X7R, inducing downstream p-p38 MAPK signaling [103, 104]. Following peripheral tissue injuries, large quantities of ATP release drive P2X7R activation. As such, EA can alleviate the pain caused by neck incision in part via the inhibition of an ATP/P2X7R/FKN/CX3CR1/p-p38 signaling axis.

The above data provide strong evidence for the importance of purinergic signaling in the acupuncture-mediated alleviation of neuropathic pain. These effects appear to be mediated through DRG or spinal cord mechanisms. Firstly, on DRG, acupuncture can suppress AP2M phosphorylation and thereby prevent PAP degradation, promoting ATP dephosphorylation and decreasing downstream P2X3R expression (Fig. 2).

Secondly, on the spinal cord, the analgesic effects of acupuncture appear to be mediated through a variety of pathways. For example, acupuncture can decrease the ATP AP2M phosphorylation and thereby decrease downstream P2XR signaling activity. P2X3R signaling mechanisms dependent on p-PKC and ERK1/2, while P2X7R can alleviate neuralgia via FKN/CX3CR1/p-p38 signaling and the consequent suppression of the production of inflammatory mediators including IL-1β, IL-6, and TNF-α. Moreover, P2X7R is capable of controlling BDNF and synaptotagmin I levels in response to acupuncture, contributing to the relief of aberrant synaptic changes. Acupuncture-related signaling through P2X4R is under the regulatory control of IFN-γ, inhibiting the intake of Na⁺ and Ca²⁺ into cells together with K⁺ efflux, resulting in the downstream regulation of BDNF and GABAAγ2 signaling activity and levels of IL-1β and TNF-α, and the inhibition of excitatory synaptic transmission related to neuropathy. The acupuncture-associated activation of CD73 also contributes to an increase in ADO concentrations, facilitating the relief of pain via the A1R/TNF-α and A2AR/cAMP/CREB/PKA pathways. Acupuncture can also control IL-10, NTF3/4, synaptophysin, and
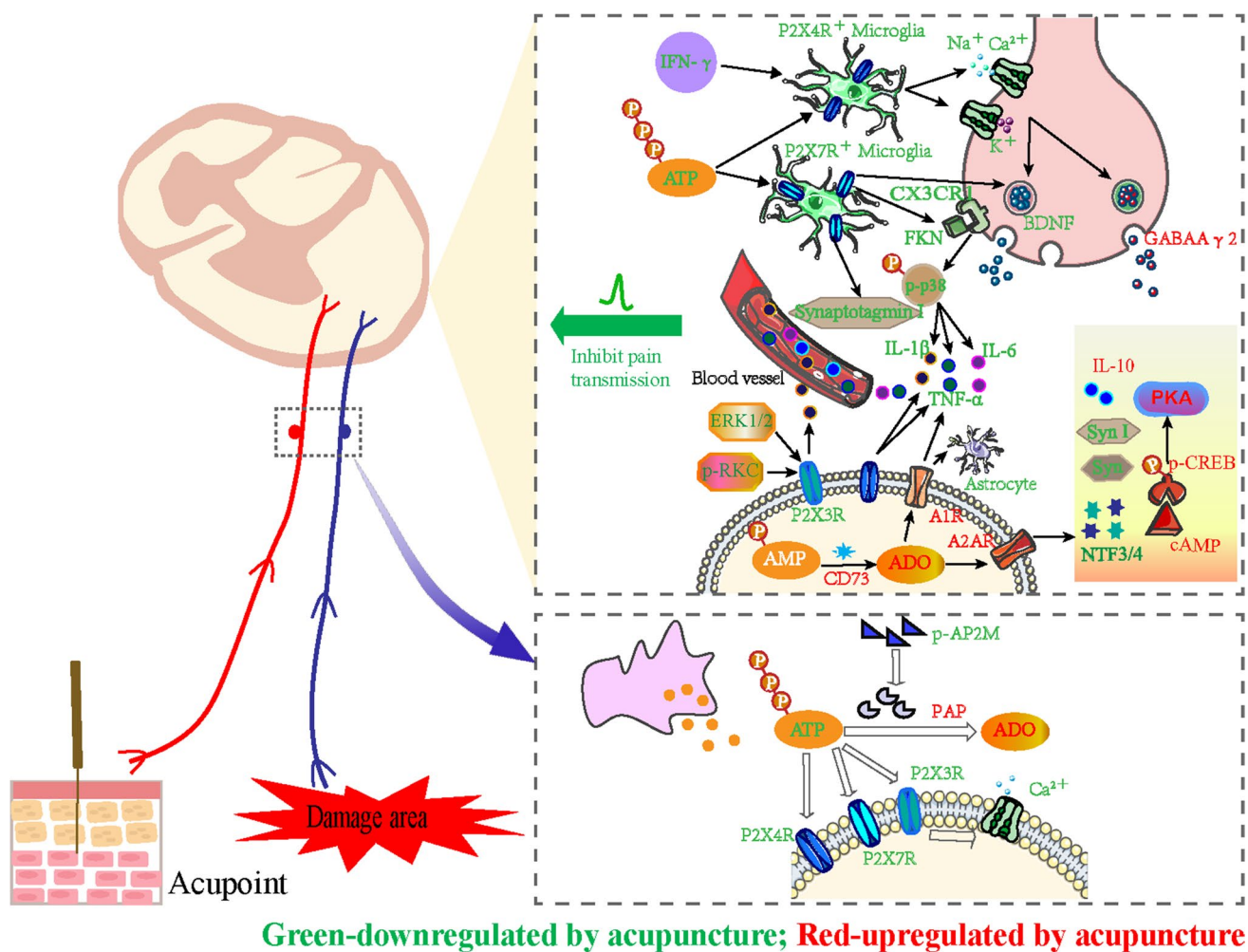
Fig. 2 The mechanisms whereby purinergic signaling participate in acupuncture attenuation of neuropathic pain. PAP, prostatic acid phosphatase; ATP, adenosine triphosphate; AMP, adenosine monophosphate; CD73, ecto-5′-nucleotidase; ADO, adenosine; IFN-γ, interferon-γ; FKN, fractalkine; CX3CR1, CX3C chemokine receptor 1; ERK, extracellular regulated protein kinases; PKC, protein kinase C; A2AR, adenosine 2A receptor; A1R, adenosine 1 receptor; TRPV1, transient receptor potential vanilloid 1; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; IL-10, interleukin-10; cAMP, cyclic adenosine monophosphate; CREB, cyclic adenosine monophosphate response element protein; NTF3/4, neurotrophic-3/4; PKA, protein kinase A; IL-6, interleukin-6; GABA, γ-aminobutyric acid; BDNF, brain-derived neurotrophic factor; Syn, synaptophysin; Syn I, synapsin I

synapsin I via A2AR-dependent mechanisms, alleviating synaptic changes and tissue abnormalities associated with neuronal damage (Fig. 2). P2Y12R has also been tentatively linked to the acupuncture-mediated relief of neuropathy. Relatively few studies to date have focused on other P1Rs and P2Rs, and additional mechanistic research will thus be vital to confirm and expand upon these results.

Inflammatory pain

Inflammatory pain is the result of immunological responses to deleterious stimuli, and it is driven by the release of inflammatory mediators that activate pain receptors in the nervous system [105]. Such pain is a common clinical finding, and it is associated with a diverse array of chronic and acute diseases [15], adversely impacting patient quality of life. At present, a range of treatments for such pain have been developed, but they can often cause disruptive side effects. In contrast, acupuncture can alleviate such pain with a lower risk of adverse events as has been demonstrated in multiple experimental models of inflammatory pain and related diseases [106]. These include the complete Freund's adjuvant (CFA), carrageenan, formalin, or collagenase-induced models, as well as 2,4,6-trinitrobenzene sulfonic acid (TNBS)- or dextran sodium sulphate-induced inflammatory bowel disease (IBD) (Table 2). The CFA-induced arthritis model has thus far been the most widely used when studying the mechanisms through which acupuncture can

Table 2 Purinergic signaling participate in acupuncture attenuation of inflammatory pain

Inflammatory pain models	Subjects	Acupoints	Intervention methods	Parameters used for acupuncture	Mechanisms	Test sites	References
RA	Human	ST36 HT7 ST25 LR3 GB25 SI3 GB34 SI4 LI11 LI4 SP6 SP9	Laser acupuncture	904 nm, beam brea of 1cm², power 100 mW, power density 100 mW/cm², energy dosage 4 J, energy density 4 J/cm², irradiation time 40 s, and frequency 100,000 Hz	ATP↑, IL-6↓, MDA↓	Plasma	[107]
RA	Human	ST36 LR8 SP9 PC6	Laser acupuncture	808 nm wavelength, continuous wave, a power density of 100 mW cm², energy density of 7.5 J cm², and the irradiation period of 60 s	ATP↑, CRP↓, IL-6↓, MDA↓	Plasma	[108]
RA	Human	TE5 LI11 DU14 SP6 LR3 ST36 GB34 LI4	Laser acupuncture	904nm, 100mW power output, 1 min irradiation time, beam area of 1 cm², total energy per point 6J, energy density 6J/cm², irradiance 0.1 W/cm², frequency 10000 Hz, duty-cycle 100%	ATP↑, SOD↑, GR↑, GSH↑, GPx↓, catalase activities↑, MDA↓, CRP↓, ESR↓, IL-6↓, serum nitrate and nitrite↓	Plasma	[109]
CFA	Rats	ST36	Manual acupuncture	30 s of lifting–thrusting and 30 s of twisting, lasting for 20 min	eATP↑, TRPV4 antagonist, and NTPDase inhibitor affect acupuncture analgesia effect and eATP levels	ST36	[110]
CFA	Rats	ST36	Manual acupuncture	30 s of lifting–thrusting and 30 s of twisting, lasting for 20 min	eATP↓, NTPDase -2/3↑	DRGs, sciatic nerve	[111]
CFA	Rats	ST36 BL60	EA	100 Hz at 0.5–1–1.5 mA, each lasting for 10 min	P2X3R↓, P2X3R agonist affect EA effect	DRGs, SCDH	[15]
CFA	Rats	ST36 BL60	EA	0.5–1.0–1.5 mA, 2 Hz, 100 Hz or 2/100 Hz, 20 min, 30 min or 45 min	P2X3R↓, TRPV1↓, P2X3R and TRPV1 agonist affect EA effect	DRGs	[112]
CFA	Rats	ST36 BL60	EA	2/100 Hz at 0.5–1–1.5 mA, each lasting for 10 min	ST36: ADO↑; DRGs: SP↓, CD68↓, NK-1R↓, TNF-α↓, IL-1β↓, IL-6↓; SP receptor and A1R antagonist affect EA effect	ST36, DRGs	[3]
CFA	Mice	ST36	Manual acupuncture	30s of lifting–thrusting, needle retention for 30 s, 30 s of twisting, and needle retention for 30s, total 30 min	ADO↑, degranulation of mast cells↑, histamine H1 antagonist affects acupuncture effect, β-endorphin (cerebrospinal fluid)↑	ST36	[113]
CFA	Rats	ST36 BL60	EA	100 Hz at 1–2 mA for 30 min	P2X3R↓, TRPV1↓	DRGs	[73]
CIA	Rats	ST36 SP6	EA	2 Hz at 2.0–2.5 mV for 30 min	peripheral blood: ADO↑, TNF-α↓, ADA↓, CD73↑; synovial tissue: CD73↓, ADA↓	Peripheral blood, synovial tissue	[114]
CIA	Mice	ST36 SP6	EA	2 Hz at 0.07 mA for 30 min	A2AR antagonist affects EA effect; blood: TNF-α↓	Blood	[115]

Table 2 (continued)

Inflammatory pain models	Subjects	Acupoints	Intervention methods	Parameters used for acupuncture	Mechanisms	Test sites	References
CIA	Rats	ST36 SP6	EA	2 Hz at 2.0–2.5 mV for 30 min	Peripheral blood: TNF-α↓, PKA↓, RANKL↓, ERK1/2↓, NF-κB↓; ankle joints: PKA↑, ERK1/2↑; A2AR antagonist affects EA effect	Peripheral blood, ankle joints	[116]
IBD	Mice	BL25	EA	2 Hz at 1 mA for 30 min	A1R↑, A2AR↑, A3R↑, A2BR↓, SP↓, IL-1β↓; A1R, A2AR, A3R and A2BR antagonist affects EA effect	Intestinal tissue	[117]

RA, rheumatoid arthritis; *ATP*, adenosine triphosphate; *IL-6*, interleukin-6; *MDA*, malondialdehyde; *CRP*, C-reactive protein; *SOD*, superoxide dismutas; *GR*, glutathione reductase; *GSH*, glutathione; *GPx*, glutathione peroxidase; *ESR*, erythrocyte sedimentation rate; *CFA*, complete Freund's adjuvant; *eATP*, extracellular adenosine triphosphate; *TRPV*, transient receptor potential vanilloid; *DRG*, dorsal root ganglion; *SCDH*, spinal cord dorsal horn; *ADO*, adenosine; *NTPDase-2/3*, nucleoside triphosphate diphosphohydrolase 2/3; *SP*, substance P; *NK-1R*, neurokinin-1 receptor; *TNF-α*, tumor necrosis factor-α; *IL-1β*, interleukin-1β; *IL-6*, interleukin-6; *A1R*, adenosine 1 receptor; *CIA*, collagen-induced arthritis; *ADA*, adenosine deaminase; *CD73*, ecto-5′-nucleotidase; *A2AR*, adenosine 2A receptor; *PKA*, protein kinase A; *RANKL*, NF-κB ligand ; *NF-kB*, nuclear transcription factor-κB; *ERK*, extracellular regulated protein kinases; *IBD*, inflammatory bowel disease; *A3R*, adenosine 3 receptor; *A2BR*, adenosine 2B receptor

alleviate inflammatory pain, as the model is easy to establish and results in pathological symptoms consistent with those of humans suffering from rheumatoid arthritis (RA), providing a system in which acupuncture can provide long-lived beneficial analgesia.

Rheumatoid arthritis

RA is a form of inflammatory autoimmune disease of the joints that causes progressive joint damage and eventual disability [118, 119]. The efficacy of laser acupuncture as a treatment for RA has been linked to ATP in a study by Adly et al. who analyzed samples from 30 RA patients and observed significant increases in ATP and antioxidant marker levels following laser acupuncture treatment, with corresponding reductions in IL-6 and malondialdehyde (MDA) levels and improvements in joint mobility [107]. Laser acupuncture can also reportedly suppress a range of other inflammatory and oxidative stress-related mediators including serum C-reactive protein (CRP) levels [108], the erythrocyte sedimentation rate (ESR), and glutathione peroxidase (GPx) activity while increasing the plasma activity levels of superoxide dismutase (SOD), catalase, glutathione reductase (GR), and glutathione (GSH) [109]. Based on these results, laser acupuncture appears to provide an effective means of reducing RA-related inflammatory pain through processes related to increases in plasma eATP concentrations and the consequent suppression of inflammation and oxidative stress.

Adjuvant-induced arthritis

The subcutaneous injection of CFA in mammals causes a range of pathological symptoms proximal to the injection site including pain, swelling, inflammatory cell infiltration, proliferative activity, and the erosion of bone tissue [120, 121]. Purinergic receptor signaling has been demonstrated to be central to the establishment of CFA-associated inflammatory pain [122], suggesting that such signaling is also likely to be involved in the acupuncture-mediated relief of such pain. In CFA-treated rats, significant increases in levels of ATP, ADP, AMP, and ADO were detected at the ST36 acupoint by high-performance liquid chromatography (HPLC) at 30 min post-moxibustion relative to baseline levels, with ATP levels peaking at 60 min while the other metabolites reached peak concentrations at 30 min, followed by gradual declines [123]. Consistent results were also observed in mice following the manual rotation of acupuncture needles inserted at ST36 [26]. Similar purine dynamics were also evident in microdialysate samples collected from human volunteers following acupuncture at ST36, although these purine concentrations failed to rise if the needle was inserted without subsequent rotation [27]. These results thus offer

support for the role of purinergic signaling in the acupuncture-mediated relief of inflammatory pain.

Manual acupuncture has also been reported to facilitate eATP accumulation at the target acupoint, whereas this accumulation and associated analgesic effects can be abrogated when using a selective TRPV4 channel antagonist. The transient accumulation of eATP during manual acupuncture treatment was also promoted by the non-specific nucleoside triphosphate diphosphohydrolase (NTPDase) inhibitors ARL67156, with the concomitant ablation of the analgesic benefits of such treatment [110]. Members of the NTPDase (CD39) family are key enzymes responsible for nucleotide hydrolysis, thereby degrading ATP to produce ADP and AMP [124]. Manual acupuncture-associated analgesia has been shown to alter the mRNA-level expression of NTPDase-2 and NTPDase-3 [111]. Manual acupuncture has also been shown to normalize excessive DRG and sciatic nerve eATP levels in CFA model rats [111]. The directionality of this regulatory activity is in contrast to that observed for manual acupuncture in the above-mentioned studies, potentially owing to the differences in the target analytical timepoint, which followed the first acupuncture of mice in the former study [110], but was performed after second acupuncture in the latter [111]. This may have contributed to differences in the dynamics of eATP hydrolysis, although further research will be essential to test this possibility.

After its release from cells, eATP primarily activates P2X3R present on the surface of nociceptive primary sensory neurons, particularly on neurons of small and intermediate size [125, 126]. Significant increases in DRG P2X3R and TRPV1 expression are evident in the context of inflammatory pain [97, 127], and the concomitant release of ATP from inflammatory and injured cells can thus drive the activation of the P2X3R ion channels in the membrane of these DRG neurons, thereby inducing nociceptor activation and transmitting pain-related signals [128]. EA (100 Hz) at ST36 in CFA model rats contributes to a decrease in P2X3R protein levels and P2X3R-positive neuron numbers in the DRG and spinal cord dorsal(SCDH) horn compartments, with a corresponding drop in TRPV1 protein levels and TRPV1-positive neuron numbers in the DRG. Treating these rats with agonists of P2X3R or TRPV1 was sufficient to significantly attenuate or potentiate, respectively, the analgesic benefits of EA [15, 112]. Peripheral P2X3R agonist delivery in CFA model rats can also improve the algogenic effects of TRPV1, whereas inhibiting peripheral P2X3 receptors has no corresponding impact on TRPV1-related algogenic activity. In contrast, inhibiting peripheral TRPV1 is sufficient to reverse the algogenic effect of P2X3R in a rat model of CFA-induced spontaneous pain [98]. Overall, TRPV1 appears to function in a pathway downstream of P2X3R. A reduction in TRPV1 channel sensitivity and openness can reduce the

influx of Na^+ and K^+ into cells, inhibiting pain transmission [11]. On DRG, acupuncture may thus alleviate pain signaling via inhibit the ATP/P2X3R axis in part through the activation of TRPV4 channels and the promotion of the hydrolysis of eATP. However, in the spinal cord, acupuncture can down-regulate P2X3R expression, but there is a lack of evidence on how it regulates P2X3R and its upstream and downstream to alleviate CFA-induced inflammatory pain.

ADO also plays an important role in the acupuncture-mediated relief of inflammatory pain. A pronounced increase in local ADO levels, as measured by HPLC, was observed following EA at the bilateral ST36 and BL60 acupoints. Such treatment also results in the downregulation of DRG mRNA and protein levels of SP, neurokinin-1 receptor (NK-1R), CD68, TNF-α, IL-1β, and IL-6 in CFA model rats [3]. The binding of SP to NK-1R can exacerbate the release of inflammatory cytokines including TNF-α, IL-1β, and IL-6 [129], which function as direct inducers of inflammatory pain. The transection of the dorsal nerve root abrogated the analgesic effects of EA while promoting the upregulation of SP and NK-1R, and downstream inflammatory signaling mediators. These analgesic effects were also suppressed by treatment with antagonists of A1R or the SP receptor, with A1R antagonist treatment also resulting in SP and NK-1R upregulation [3]. Fujita T et al. determined that the analgesic effects of acupuncture can be ablated by the oral administration of the A1R antagonist caffeine, whereas the cessation of caffeine intake can restore the efficacy of such treatment [130]. This same response is not evident in mice that lack the expression of A1R [26]. The intraperitoneal or acupoint-specific delivery of the A1R agonist CCPA can reportedly recapitulate the beneficial effects of EA in a mouse inflammatory pain model, alleviating the thermal hyperalgesia that these animals experience. Injecting the A1R antagonist rolofylline can, conversely, suppress the anti-nociceptive effects of EA [131]. In mice, manual acupuncture promotes mast cells (MCs) activation and degranulation through the mechanosensitive TRPV2 channel expressed on the membrane of these cells, thus resulting in an acupoint-localized increase in ADO concentrations [113]. MCs are key immune cells that are responsive to diverse stimuli, and their degranulation results in the release of a range of cytokines that can shape both appropriate physiological responses and deleterious pathological conditions. The local injection of sodium tryptophate at ST36 to prevent MCs degranulation in mice undergoing manual acupuncture treatment has been shown to lower ADO concentrations proximal to this acupoint as compared to control animals that did not receive this injection [113]. Acupuncture may thus induce mast cell activation and ADO release in a TRPV2-dependent manner, whereupon ADO can bind to A1R and regulate the production of SP and downstream inflammatory signaling mediators.

Collagen-induced arthritis

The collagen-induced arthritis (CIA) model of RA is induced by intradermally immunizing animals using heterologous collagen-II, producing a disease that is genetically, pathologically, and immunologically similar to human RA [132, 133]. Both ADO and its A2AR receptor are reportedly integral to the acupuncture-mediated alleviation of CIA. For example, EA at the ST36 and SP6 (Sanyinjiao) acupoints for 21 days in CIA model rats is associated with significant increases in peripheral blood ADO concentrations and mRNA levels of the CD73 relative to control animals, with corresponding reductions in peripheral blood adenosine deaminase (ADA) and TNF-α mRNA levels. Importantly, EA treatment in these animals was associated with improved joint histology including reduced synovial hyperplasia, joint fusion, and inflammatory cell infiltration [114]. ADO functions as a key messenger in anti-inflammatory and other paracrine signal transduction pathways throughout the body in a range of tissue compartments and organs. CD73 and ADA activity levels play a major role in governing ADO concentrations, with CD73 dephosphorylating AMP to yield ADO [134], whereas ADA deaminates ADO to inosine and thus reduces extracellular ADO concentrations [135]. By inhibiting ADO deaminase activity, pentostatin can prolong the analgesic benefits of acupuncture [51].

ADO-mediated receptor activation can modulate the production of inflammatory factors and alleviate inflammatory pain [136]. Relative to CIA model mice treated with EA, CIA model controls and EA-treated mice that were also administered the A2AR antagonist SCH58261 exhibited more severe bone erosion, arthritis, and inflammatory cell infiltration [115]. Strikingly, such A2AR antagonist treatment completely eliminates the anti-inflammatory effects of EA. EA at ST36 and SP6 also reportedly reduces serum TNF-α, PKA, ERK1/2, nuclear transcription factor-κB (NF-κB), and receptor activator of NF-κB ligand (RANKL) levels, while SCH58261 reverses these EA-related changes [116]. A2AR can interact with ADO and activate PKA, thus promoting immunosuppressive cell biogenesis and local tissue infiltration, thereby shaping anti-inflammatory activity [52].

Inflammatory bowel disease

IBD refers to two major forms of chronic inflammatory diseases of the gastrointestinal tract, including ulcerative colitis (UC) and Crohn's disease (CD), both of which often exhibit a relapsing disease course in patients [137, 138]. Pain is among the first symptoms reported at the onset or exacerbation of IBD in 50–70% of patients [139]. Purinergic signaling has been reported to play a central role in the pathogenesis of IBD [140], and several clinical trials to date have demonstrated that acupuncture can effectively alleviate UC- and CD-associated pain in patients [141]. As such, further research is warranted to clarify the roles that purinergic signaling pathways play in this acupuncture-mediated treatment of IBD-related pain.

Following a 7-day EA treatment period, significant increases in colon A1R, A2AR, and A3R protein levels, a drop in colon A2BR protein levels, and the alleviation of visceral pain and disease-related symptoms were observed in a TNBS-induced mouse model of IBD. The analgesic benefits of EA in this model system, however, were abrogated by treatment with A1R, A2AR, or A3R antagonists, while A1R, A2AR, A3R, or A2BR antagonists were able to inhibit or potentiate the benefits of EA with respect to reductions of intestinal SP and IL-1β expression [117]. A3R agonists can also facilitate a pronounced drop in pro-inflammatory mediator levels [142], while A2BR plays a key role in pro-inflammatory signaling in intestinal epithelial cells [143].

The above results suggest that purinergic signaling mediators including ATP, P2X3R, ADO, A1R, and A2AR play important roles in the acupuncture-mediated relief of inflammatory pain. Localized in the acupoint area, TRPV2 and TRPV4 present on the surface of immune cells and nerve endings can be activated in response to acupuncture, whereupon ATP and ADO can be released into the extracellular milieu in part through mast cell degranulation. CD39 and CD73 can dephosphorylate ATP to yield ADO. ADO can also activate A1R and interfere with SP binding to NK-1R in the DRG, in turn decreasing the secretion of pro-inflammatory cytokines including TNF-α, IL-1β, IL-6, and CD68 (Fig. 3).

In the DRG, ATP is dephosphorylated under the action of CD39, decreasing P2X3R activation and inhibiting downstream TRPV1 signaling. Reductions in the sensitivity and openness of these TRPV1 channels can in turn modulate nociceptive transmission as a consequence of decreased Na$^+$ and K$^+$ influx into cells (Fig. 3).

In the peripheral blood, acupuncture also induces the release of ATP into the plasma, exerting antioxidant effects and regulating other inflammation- and oxidative stress-related factors (including SOD, catalase, GR, GSH, CRP, MDA, GPx, ESR, and serum nitrate levels). The specific mechanisms linking these changes to ATP, however, remain to be studied in detail. In addition, acupuncture can also increase CD73 and suppress ADA activity, thereby promoting ADO production and disrupting ADO hydrolysis. While the peripheral blood TNF-α, PKA, ERK1/2, RANKL, and NF-κB concentrations are also decreased in response to ADO-A2AR binding interactions (Fig. 3). While A2BR may also be associated with the alleviation of inflammatory pain, little research to date has focused on this receptor or A3R, and more work will thus be vital to clarify their mechanistic importance in the context of acupuncture analgesia.
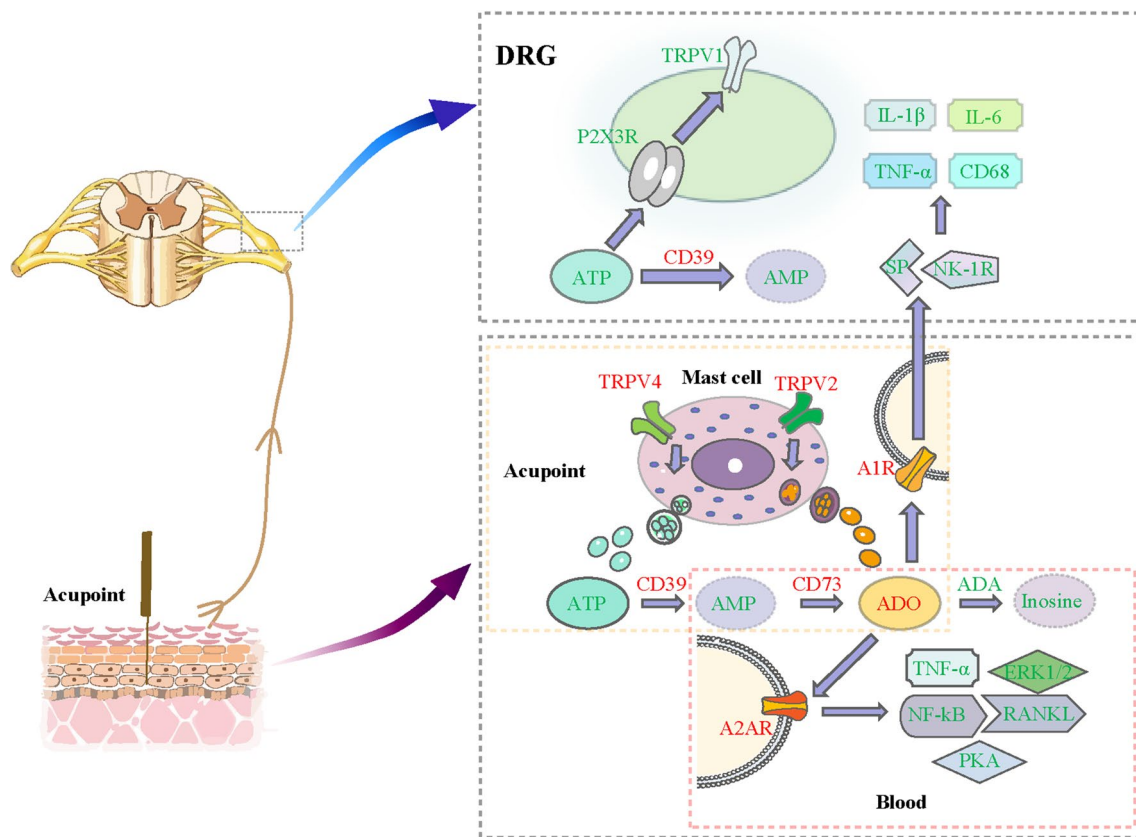
Green-downregulated by acupuncture; Red-upregulated by acupuncture

Fig. 3 The mechanisms whereby purinergic signaling participate in acupuncture attenuation of inflammatory pain. DRG, dorsal root ganglion; TRPV1, transient receptor potential vanilloid 1; ATP, adenosine triphosphate; ADO, adenosine; A1R, adenosine 1 receptor; SP, substance P; NK-1R, neurokinin-1 receptor; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; IL-6, interleukin-6; ERK, extracellular regulated protein kinases; NF-kB, nuclear transcription factor-κB; RANKL, NF-κB ligand; PKA, protein kinase A; A2AR, adenosine 2A receptor; CD39, ectonucleoside triphosphate diphosphohydrolase-1; AMP, adenosine monophosphate; CD73, ecto-5′-nucleotidase; ADA, adenosine deaminase; TRPV4, transient receptor potential vanilloid 4; TRPV2, transient receptor potential vanilloid 2

Conclusion and perspectives

The present systematic overview of research focused on the analgesic effects of acupuncture highlights the key role that purinergic signaling plays in this context. In addition to serving as an essential intracellular energy source, ATP can also function as a purinergic signaling mediator. Cellular damage can deplete local energy reserves and induce pathological effects. Acupuncture can promote an increase in local eATP concentrations and the subsequent enzymatic dephosphorylation of ATP to yield ADO, thus reducing ATP binding to the P2X3R, P2X4R, and P2X7R receptors while regulating ADO binding to P1Rs including A1R, A2AR, A2BR, and A3R. These changes modulate associated inflammatory signaling, neurotransmitter release, and anion/cation influx and efflux, thus inhibiting neuronal excitability and minimizing synaptic variability. These beneficial effects, in turn, suppress inflammation and apoptotic nerve cell death,

instead favoring neuronal regeneration, ultimately contributing to analgesic benefits.

While these results highlight many promising avenues for ongoing research, they are subject to certain limitations. For one, the vast majority of these studies have focused on animal model systems rather than human patients, and it thus remains to be determined as to whether these results can be translated to a clinical setting. Secondly, these studies largely focused on specific purine nucleotides and receptors (ATP, ADO, P2X3R, P2X4R, P2X7R, A1R, and A2R). Whether other purinergic mediators including AMP, ADP, P2YRs, and A3R play a role in the acupuncture-mediated relief of pain has yet to be established. The time-dependent changes in the levels of ATP and metabolites thereof during acupuncture treatment also remain to be fully clarified. This is particularly important given that ATP can rapidly undergo catabolic processing to yield ADO, and the effects of ATP and ADO on pain

are in direct opposition to one another. At present, detecting real-time changes in the levels of these metabolites remains extremely challenging owing to technical limitations. Third, the studies discussed herein have largely focused on neuropathic or inflammatory pain, whereas little corresponding research has been performed for degenerative diseases including depression, Parkinson's disease, and Alzheimer's disease. More research clarifying the acupuncture-specific pathways upstream and downstream of purinergic signaling will also be invaluable as a means of better contextualizing the analgesic benefits of this form of alternative medical treatment. Finally, the animal models used to conduct these studies are all subject to certain limitations such as short-term hyperalgesia, and many studies solely focused on the protective benefits of acupuncture during the early stages of pain. More research focused on the efficacy of acupuncture as a treatment for chronic pain is thus warranted, such as chronic low back pain, chronic cancer pain, chronic visceral pain, and chronic migraine.

Overall, this review provides a strong and up-to-date overview of the key role that purinergic signaling plays in the acupuncture-mediated treatment of pain. Further efforts to expand on these results will highlight new opportunities for treating patients suffering from hyperalgesia through the application of acupuncture in combination with appropriate medications, thereby better helping to control and eliminate pain. While the research conducted to date is very promising, the scope of many of these studies was relatively narrow, underscoring the importance of further in-depth analyses focused on better elucidating how purinergic signaling shapes the analgesic benefits of different types of acupuncture.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Manuscript is approved by all authors for publication.

Competing interests The authors declare no competing interests.

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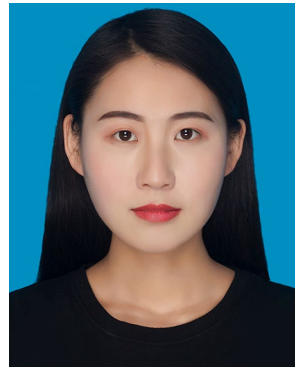
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